#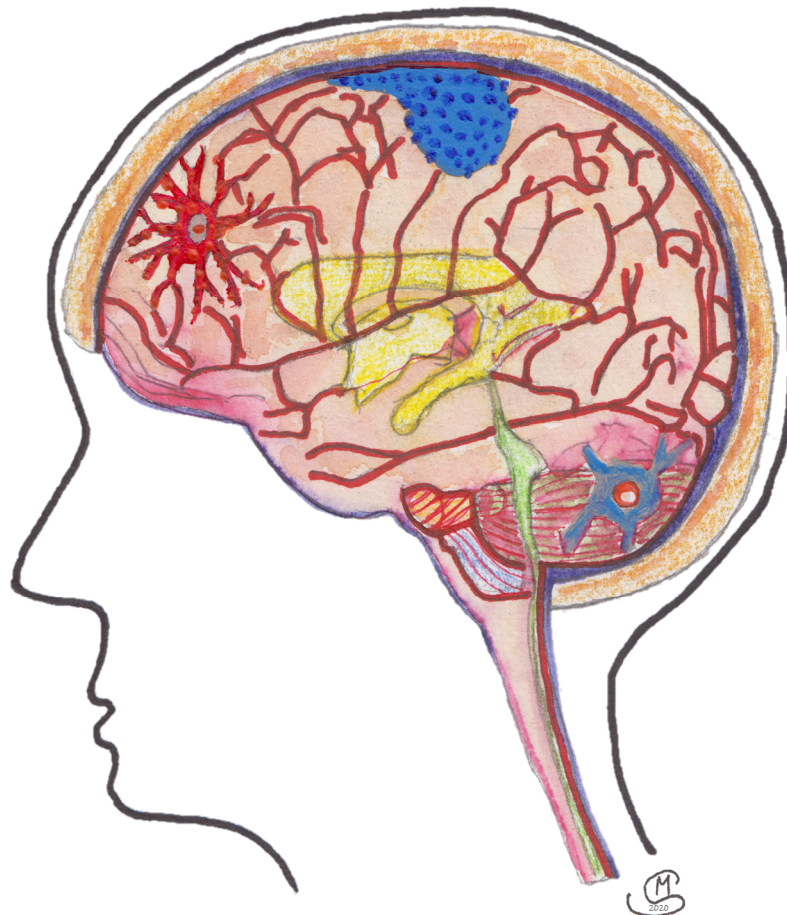 The immunopeptidomic landscape of intracranial neoplasias – Target identification for immunotherapy of glioblastoma, medulloblastoma, and meningioma

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# The immunopeptidomic landscape of intracranial neoplasias – Target identification for immunotherapy of glioblastoma, medulloblastoma, and meningioma

# Das Immunpeptidom intrakranieller Neoplasien – Identifizierung von Zielen für die Immuntherapie von Glioblastomen, Medulloblastomen und Meningeomen

**Dissertation**

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*für meine Eltern*

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# Preface

During the course of my PhD thesis, I had the possibility and the pleasure to work on various projects dealing with the exploration of the human leukocyte antigen (HLA) peptidome of primary human samples originating predominantly from different solid tumor entities. However, this thesis can only provide a deeper insight into a few of these studies. I decided to present my results on the immunopeptidomic landscape of intracranial neoplasias, namely glioblastoma, medulloblastoma, and meningioma as well as to include a meta-analysis comparing these three brain tumors originating from different cell types.

A detailed introduction into the biology of antigen presentation on HLA molecules as well as the tumor entities covered by this thesis is provided in **CHAPTER 1**. Further, the ingenious and ground-breaking principle of cancer immunotherapy is introduced supplemented with information on previous and current immunotherapeutic approaches targeting glioblastoma, medulloblastoma, or meningioma. **CHAPTER 2** focuses on the insights gained by multi-omics analyses including whole exome and RNA sequencing in combination with immunopeptidomics of samples of a large cohort of glioblastoma patients. The availability of autologous primary and recurrent tumors of several patients allows investigating antigen presentation, mutation, and RNA expression dynamics during the course of disease progression. Results from studying the HLA peptidome of medulloblastomas and meningiomas including label-free quantitation of relative HLA ligand abundances on meningeal tumors as compared with autologous dura are presented in **CHAPTER 3** and **CHAPTER 4**. A comparison of these three intracranial neoplasias of different cellular origin is provided in **CHAPTER 5** in the form of a meta-analysis. The implementation of a mix of ten heavy isotope-labeled synthetic peptides as retention time standard is described as technical excursus in **CHAPTER 6**. This peptide mix is a tool to not only monitor mass spectrometric performance, but also to compare different systems and to perform e.g. data-independent acquisition with subsequent normalization of retention times between measurements. A general discussion and summary of the most important findings as well as an outlook beyond this thesis are provided in **CHAPTER 7**.

I am convinced that investigating the entirety of naturally presented HLA ligands is a cornerstone to state-of-the-art patient-tailored immunotherapies. It was a pleasure to get into the fascinating techniques of immunopeptidomics and mass spectrometry and I highly appreciate the previous work of colleagues in this field establishing methods, workflows, and bioinformatic tools on the basis of which I conducted my studies.

# Publications

### Research articles

Neidert MC, Kowalewski DJ, Silginer M, Kapolou K, Backert L, <u>Freudenmann LK</u>, Peper JK, Marcu A, Wang SS, Walz JS, Wolpert F, Rammensee HG, Henschler R, Lamszus K, Westphal M, Roth P, Regli L, Stevanović S, Weller M, Eisele G. The natural HLA ligandome of glioblastoma stem-like cells: antigen discovery for T cell-based immunotherapy. *Acta Neuropathol.* 2018;135(6):923-938.

Löffler MW, Mohr C, Bichmann L, <u>Freudenmann LK</u>, Walzer M, Schroeder CM, Trautwein N, Hilke FJ, Zinser RS, Mühlenbruch L, Kowalewski DJ, Schuster H, Sturm M, Matthes J, Riess O, Czemmel S, Nahnsen S, Königsrainer I, Thiel K, Nadalin S, Beckert S, Bösmüller H, Fend F, Velic A, Maček B, Haen SP, Buonaguro L, Kohlbacher O, Stevanović S, Königsrainer A, Rammensee HG. Multi-omics discovery of exome-derived neoantigens in hepatocellular carcinoma. *Genome Med.* 2019;11(1):28.

<u>Freudenmann LK</u>, Mayer C, Rodemann HP, Dittmann K. Reduced exosomal L-Plastin is responsible for radiation-induced bystander effect. *Exp Cell Res.* 2019;383(1):111498.

Marcu A, Bichmann L, Kuchenbecker L, Backert L, Kowalewski DJ, <u>Freudenmann LK</u>, Löffler MW, Lübke M, Walz JS, Velz J, Moch H, Regli L, Silginer M, Weller M, Schlosser A, Kohlbacher O, Stevanović S, Rammensee H-G, Neidert MC. The HLA Ligand Atlas. A resource of natural HLA ligands presented on benign tissues. *bioRxiv.* 2019:778944.

### Review articles

<u>Freudenmann LK</u>, Marcu A, Stevanović S. Mapping the tumour human leukocyte antigen (HLA) ligandome by mass spectrometry. *Immunology.* 2018;154(3):331-345.

# Summary

Intracranial neoplasias account for more than 80,000 new cases in the US every year representing the eighth most common tumors in adults older than 40 years and even the most frequent pediatric tumor entity. They comprise malignancies of the brain parenchyma such as glioblastoma or medulloblastoma as well as meningeal neoplasms. Throughout the last decades, the outcome of intracranial neoplasias has remained poor calling for intensive research to establish novel therapies. Especially recurrent and non-resectable tumors are characterized by a profound lack of evidence-based therapeutic options, while irradiation of the developing brain in children causes severe long-term sequelae. With the development and successful administration of checkpoint antibodies, a new era of multimodal anti-tumor therapies has dawned. Patients suffering from intracranial neoplasias have, however, not clinically benefited from the recent advancements. The response to checkpoint blockade, in particular, positively correlates with mutational burden, which is low in brain tumors. This also reasons why neo-antigens have not yet been confirmed to be presented on HLA molecules of brain tumor cells. In a recent clinical trial, the administration of a multi-peptide vaccine targeting non-mutated HLA ligands has elicited remarkable immune responses in primary glioblastoma. Although we succeeded in proving the first two neo-antigenic HLA ligands in glioblastoma, we are convinced that non-mutated antigens will coin peptide-specific immunotherapeutic efforts for intracranial neoplasias with low mutational burden.

To define candidate targets for cancer immunotherapy, a large-scale study of the HLA class I and II peptidomes of glioblastoma (n=62), medulloblastoma (n=28), and meningioma (n=33) was performed. We defined a novel set of antigens and peptides characterized by natural, frequent, and tumor-exclusive HLA presentation on native human tumor tissue, whereby established tumor-associated and cancer-testis antigens did not fulfill these criteria. Tumor exclusivity was assessed by comparison with a reference immunopeptidome dataset acquired from approximately 400 benign human tissues covering 30 different organs. For glioblastoma, an additional comparison of primary and recurrent tumors was performed delineating not only dynamics of antigen presentation during disease progression, but also enabling further restriction of candidate targets to those robustly presented at both disease conditions. The implementation of a mix of ten synthetic heavy isotope-labeled peptides as retention time standard will guide future immunopeptidomic studies fostering innovative mass spectrometric data acquisition strategies and quantitative approaches with enhanced identification and reproducibility rates.

In conclusion, we investigated the immunopeptidomic landscape of glioblastoma, medulloblastoma, and meningioma in an unprecedented depth unveiling a large novel set of entity-specific tumor-associated antigens and peptides. Being naturally, frequently, and tumor-exclusively presented on HLA class I or II molecules, these represent prime candidates to deliver the power of T cells *via* antigen-specific immunotherapies to intracranial neoplasias. This may contribute to meet the urgent demand for alternatives to radiotherapy in the treatment of pediatric brain tumors as well as for therapeutic regimens to manage non-resectable neoplasms and disease recurrence.

# Zusammenfassung

Jedes Jahr werden in den Vereinigten Staaten mehr als 80.000 neue Fälle intrakranieller Neoplasien, welche die achthäufigste Tumorart bei Erwachsenen über 40 Jahren und sogar die häufigste Tumorerkrankung im Kindesalter sind, diagnostiziert. Dazu zählen Neubildungen des Hirnparenchyms wie Glioblastome oder Medulloblastome und Tumore der Hirnhäute. Während der letzten Jahrzehnte blieb die Prognose intrakranieller Neoplasien schlecht, was intensive Forschung zur Etablierung neuer Therapien unabdingbar macht. Besonders für rekurrente und nicht resezierbare Tumore gibt es keine evidenzbasierte Behandlung, wohingegen die Bestrahlung des kindlichen sich entwickelnden Gehirns schwere Langzeitschäden verursacht. Die Entwicklung und erfolgreiche Anwendung von Immuncheckpoint-Antikörpern hat eine neue Ära multi-modaler Tumortherapien eingeläutet. Patienten mit intrakraniellen Neoplasien haben von den jüngsten Fortschritten klinisch gesehen jedoch nicht profitiert. Besonders das Ansprechen auf Immuncheckpoint-Inhibitoren korreliert positiv mit der Mutationslast, welche in Hirntumoren niedrig ist. Das erklärt unter anderem auch, weshalb es bislang nicht möglich war, die HLA-Präsentation von Neo-Antigenen auf Hirntumorzellen nachzuweisen. In einer kürzlich durchgeführten klinischen Studie rief die Anwendung eines Multi-Peptidimpfstoffs, der sich gegen nicht-mutierte HLA-Liganden richtet, beachtliche Immunantworten in Patienten mit primärem Glioblastom hervor. Obwohl wir den Nachweis für die ersten beiden mutierten HLA-Liganden im Glioblastom erbringen konnten, sind wir davon überzeugt, dass nicht-mutierte Antigene peptid-spezifische immuntherapeutische Ansätze für intrakraniellen Neoplasien mit geringer Mutationslast prägen werden.

Um mögliche Zielantigene für Immuntherapien zu bestimmen, wurde eine groß angelegte Untersuchung des HLA Klasse I- und II-Peptidoms in Glioblastomen (N=62), Medulloblastomen (N=28) und Meningeomen (N=33) durchgeführt. Wir haben eine neuartige Zusammenstellung von Antigenen und Peptiden definiert, welche sich durch natürliche, häufige und tumor-exklusive HLA-Präsentation auf nativem humanem Tumorgewebe auszeichnen – etablierte tumor-assoziierte und Cancer-Testis ("Tumor-Hoden") Antigene erfüllten diese Kriterien dagegen nicht. Die Bestimmung von Tumor-Exklusivität erfolgte durch den Abgleich mit einem Referenzdatensatz, welcher Immunpeptidome von circa 400 benignen humanen Geweben aus 30 verschiedenen Organen beinhaltet. Bei Glioblastomen wurden zudem primäre und rekurrente Tumore verglichen, was nicht nur Dynamiken der Antigenpräsentation im Laufe des Fortschreitens der Erkrankung aufdeckte, sondern bot auch die Möglichkeit, eine Beschränkung auf jene möglichen Zielantigene vorzunehmen, welche in beiden Krankheitsstadien robust präsentiert sind. Die Implementierung einer Mischung aus zehn isotopen-markierten synthetischen Peptiden als Retentionszeitstandard wird zukünftige Studien des Immunpeptidoms leiten, was innovative massenspektrometrische Datenaufzeichnungsstrategien und quantitative Ansätze mit verbesserten Identifikations- und Reproduzierbarkeitsraten unterstützen wird.

**Zusammenfassung**

Zusammenfassend haben wir das Immunpeptidom des Glioblastoms, des Medulloblastoms sowie des Meningeoms in einer beispiellosen Tiefe untersucht, was die Zusammenstellung einer umfangreichen und neuartigen Liste tumor-assoziierter Antigene und Peptide ermöglichte. Da diese natürlich, häufig und tumor-exklusiv auf HLA Klasse I- oder II-Molekülen präsentiert sind, stellen diese erstklassige Kandidaten dar, um intrakranielle Neoplasien durch antigen-spezifische Immuntherapien vermittelt durch T-Zellen anzugreifen. Das könnte dazu beitragen, der dringenden Forderung nach Alternativen zur Strahlentherapie bei der Behandlung kindlicher Hirntumoren sowie nach Leitlinien für die Behandlung nicht resezierbarer und rekurrenter Neoplasien nachzukommen.

# CHAPTER 1

# Introduction

# 1 Intracranial neoplasias

## 1.1 Incidence and classification

In the United States (US), neoplasms of the central nervous system (CNS) are the most common pediatric tumors (0-14 years), the third most frequent cancer among people from 15-39 years, and the eighth most common tumors in adults older than 40 years. From 2012-2016, 405,740 new cases of primary brain and CNS tumors were reported. Among these, malignant neoplasias (30.2%) were less than half as common as benign ones (69.8%). 5-year relative survival rates accounted for 35.8% for malignant and 91.5% for benign CNS tumors, respectively. Meningioma was the most frequent non-malignant brain and CNS tumor (53.3% of non-malignant / 37.6% of all neoplasms), whereas glioblastoma represented the most common malignant CNS tumor (48.3% of malignant / 14.6% of all neoplasms) (Table 1).[1]

**Table 1. Incidence and survival rates of three selected intracranial neoplasms in the US (2012-2016).** Information were obtained from Ostrom *et al.*[1] * Median age at diagnosis reported for embryonal tumors, from which medulloblastomas constitute 64%. Missing data are marked as not available (n.a.).

| | Glioblastoma | Medulloblastoma | Meningioma | | |
| --- | --- | --- | --- | --- | --- |
| | | | Total | Malignant | Non-malignant |
| **Proportion of all brain and CNS tumors** | **14.6%** | **0.6%** | **37.6%** | **0.4%** | **37.2%** |
| **5-year incidence** | **59,164** | **2,234** | **152,756** | **1,774** | **150,982** |
| 0-14 | 503 | 1,475 | 293 | 23 | 270 |
| 15-39 | 2,713 | 596 | 9,260 | 118 | 9,142 |
| 40+ | 55,948 | 163 | 143,203 | 1,633 | 141,570 |
| **Average annual incidence** | **11,833** | **446** | **30,551** | **355** | **30,196** |
| 0-14 | 101 | 295 | 59 | 5 | 54 |
| 15-39 | 543 | 119 | 1,852 | 24 | 1,828 |
| 40+ | 11,190 | 33 | 28,641 | 327 | 28,314 |
| **Median age at diagnosis** | **65** | **8*** | **66** | **65** | **66** |
| **Incidence rate male : female ratio** | **1.58** | **1.66** (age 0-14) | n.a. | **0.89** | **0.43** |
| **5-year relative survival** | **6.8%** | **73.7%** | **n.a.** | **68.2%** | **88.0%** |
| 0-14 | 21.8% | 72.3% | n.a. | 75.3% | 95.7% |
| 15-39 | 26.2% | 78.5% | n.a. | 84.1% | 97.0% |
| 40+ | 5.5% | 66.2% | n.a. | 66.6% | 87.3% |

Besides distinguishing benign and malignant tumors, primary CNS neoplasias are grouped into glial and non-glial tumors according to their cell type of origin (Figure 1).[2] Gliomas arise from glial cells (astrocytes, oligodendrocytes, ependymal cells, and microglia), non-neuronal cells in the CNS that are supportive to neurons. Astrocytes fulfill homeostatic functions by regulating water and ion levels, modulating neurotransmission in tripartite synapses, and contributing to the formation of the blood-brain barrier. The insulating myelin sheath of axons is formed by oligodendrocytes and allows rapid conduction of electrical impulses. Microglia represent brain-resident phagocytic immune cells, whereas ependymal cells line the ventricular system, the central canal of the spinal cord as well as the choroid plexus, and produce cerebrospinal fluid (CSF).[3,4] Glial intracranial neoplasias, namely astrocytoma, oligodendroglioma, and ependymoma can either grow in a diffuse or non-diffuse manner, whereby diffuse growth goes along with deep infiltration of surrounding benign brain tissue. Non-gliomas originate from hematopoietic / lymphoid cells (CNS lymphoma), the meninges (meningioma), or embryonal neuroepithelial / cerebellar granule neural precursor cells (medulloblastoma).[1,2,5,6]

In 2016, grading of intracranial neoplasms as recommended by the World Health Organization (WHO) was for the first time not only based on histological and clinical, but also on molecular patterns which are of major importance for sub-classification of tumor types. Glioblastomas that have progressed from anaplastic astrocytoma or low-grade glioma, designated as secondary glioblastomas, have mutations of the isocitrate dehydrogenase (IDH) 1/2 genes (IDH$^{mut}$), whereas primary glioblastomas have non-mutated / wild-type (WT) IDH1/2 genes in most cases (IDH$^{WT}$).[2] Medulloblastomas (MB) are subdivided into four groups by means of expression and mutational profiles. In the first group, the Wingless and Int-1 (Wnt) pathway has a key role (WNT-activated; 11%), whereas the second group is characterized by strong activation of the Sonic Hedgehog (SHH) pathway (SHH-activated; 30%). Within this group, tumors with mutated and WT tumor suppressor p53 (TP53) are further distinguished from each other (SHH-activated/TP53$^{mut}$; SHH-activated/TP53$^{WT}$). Group 3 (25-28%) and Group 4 (35%) medulloblastomas are both non-WNT/non-SHH with dominant signaling through the photoreceptor/gamma-aminobutyric acid (GABA-)ergic or the neuronal/glutamatergic pathway instead (Figure 1).[2,7,8]



**DIFFUSE**

**Astrocytoma:**
Low-grade astrocytoma (II)
Anaplastic astrocytoma (III)
**Glioblastoma IDH$^{WT}$ / IDH$^{mut}$ (IV)**

**Oligodendroglioma:**
Low-grade oligodendroglioma (II)
Anaplastic oligodendroglioma (III)

**NON-DIFFUSE**

**Astrocytoma:**
Pilocytic / subependymal giant cell astrocytoma (I)
Pleomorphic xanthoastrocytoma (II)
Anaplastic pleomorphic xanthoastrocytoma (III)

**Ependymoma:**
Sub- / myxopapillary ependymoma (I)
Ependymoma (II-III)
Anaplastic ependymoma (III)

**GLIOMAS**

Astrocytoma

Oligodendroglioma

Ependymoma

**NON-GLIOMAS**

Meningioma

Medulloblastoma

Primary CNS lymphoma

**Meningioma:**
Benign meningioma (I)
Atypical meningioma (II)
Anaplastic / malignant meningioma (III)

**Medulloblastoma:**
WNT (IV)
SHH/TP53$^{WT}$ (IV)
SHH/TP53$^{mut}$ (IV)
Non-WNT/non-SHH: Group 3 and 4 (IV)

**Figure 1. Overview of glial- and non-glial brain tumors.** WHO grades are indicated in Roman numerals for selected CNS neoplasms. Tumor entities that are part of the subsequent experimental section are color-marked. Image adapted from Malhotra *et al.*[9] and supplemented with information from Louis *et al.*[2]

## 1.2 Glioblastoma

### Etiology, common mutations, and epigenetic features

Glioblastoma is the most frequent and most malignant primary intracranial neoplasia. It mainly affects adults at a median age of 65 years and is characterized by the poorest 5-year relative survival rate of 6.8% among all brain tumors (Table 1). It constitutes 57.3% of all gliomas and only little is known about its etiology.[1] Hereditary cancer syndromes including Lynch or Li-Fraumeni syndrome, neurofibromatosis type 1 and 2, Turcot's syndrome (brain tumor-polyposis syndrome), Gorlin syndrome (nevoid basal cell carcinoma syndrome), and Cowden's

disease contribute to only 5% of glioblastoma cases.[10-16] While (therapeutic) ionizing radiation represents a well-known risk factor, the neurocarcinogenic effect of polycyclic aromatic hydrocarbons, *N*-nitroso compounds, or other chemicals is still under investigation.[10-12,17-19] Likewise, the association of glioblastoma with simian virus 40 (SV40) and different human herpes viruses (HHV) such as human cytomegalovirus (HCMV) in glioblastoma is controversially discussed.[10,20-23] Heterozygous IDH1/2 mutations (IDH1: predominantly R132H (90%),[2,24-26] less frequently R132C and R132G;[24] IDH2: mostly R172K[26-29]) occur in only 5% of primary or *de novo* glioblastomas, whereas these non-synonymous mutations are characteristic of secondary glioblastoma (approximately 80%).[10,24,30] Determination of the IDH-status is part of routine clinical diagnostics, since IDH-mutant tumors are associated with better prognosis (median overall survival after surgery and radiochemotherapy 31 *versus* 15 months).[2,13] Non-mutated IDH catalyzes the reaction of isocitrate into α-ketoglutarate (α-KG), which is subsequently converted into D-2-hydroxyglutarate (D2HG) by mutant IDH exhibiting neo-enzymatic activity. D2HG acts as an oncometabolite by competitive inhibition of α-KG-dependent enzymes affecting collagen synthesis, cell signaling, and epigenetic regulation.[10,26] Secondary glioblastomas occurring in younger patients are relatively rare (5-10% of all glioblastomas) and subsequent information therefore focuses on classical *de novo* glioblastoma.[2,10,31-33]

The core pathways driving glioblastoma comprise enhanced phosphatidylinositol-3-OH kinase (PI3K) signaling, impaired activation of the Rb and p53 pathways, and uncontrolled growth factor signaling (Figure 2).[10,33-35] Amplification of the epidermal growth factor receptor (EGFR), the cyclin-dependent kinase 4 (CDK4), and the p53 inhibitor mouse double minute 2 homolog (MDM2) are common genetic events in glioblastoma.[10,12,32-34,36] The truncated epidermal growth factor receptor variant III (EGFRvIII) is a gain-of-function mutation resulting in constant activation of the EGFR pathway and is present in around 30% of newly diagnosed glioblastomas.[10,12,33,34,37] Homozygous deletion of neurofibromin-1 (NF1), phosphatase and tensin homolog (PTEN), the cyclin-dependent kinase inhibitor 2A (CDKN2A / p16$^{Ink4a}$), or the cyclin-dependent kinase 4 inhibitor 2B (CDKN2B / p15$^{Ink4b}$) cause a loss of Ras, PI3K, or CDK4 inhibition, respectively. Besides p16$^{Ink4a}$, CDKN2A encodes the p53 activator alternative open reading frame (ARF / p14$^{ARF}$), which is lacking in case of CDKN2A deletion.[10,12,32-34,36] Impaired p53 and Rb signaling can also be due to p53 and Rb mutations. PI3K mutations and deletions of PTEN as well as CDKN2A and Rb mutations seem to be mutually exclusive.[10,24,31,34,38] PIK3CA mutations can either render the p110α catalytic subunit of the PI3K complex constitutively active or reduce its affinity to the inhibitor p85α.[10,34,39-41] Figure 2 visualizes the frequencies of these mutations as well as the role of mutated proteins in the pathways driving glioblastoma. Common chromosomal aberrations comprise gain of chromosome 7, 19, and 20, loss of chromosome 10, 13, and Y as well as rearrangements affecting chromosome 12q.[24,42-46]

**A**
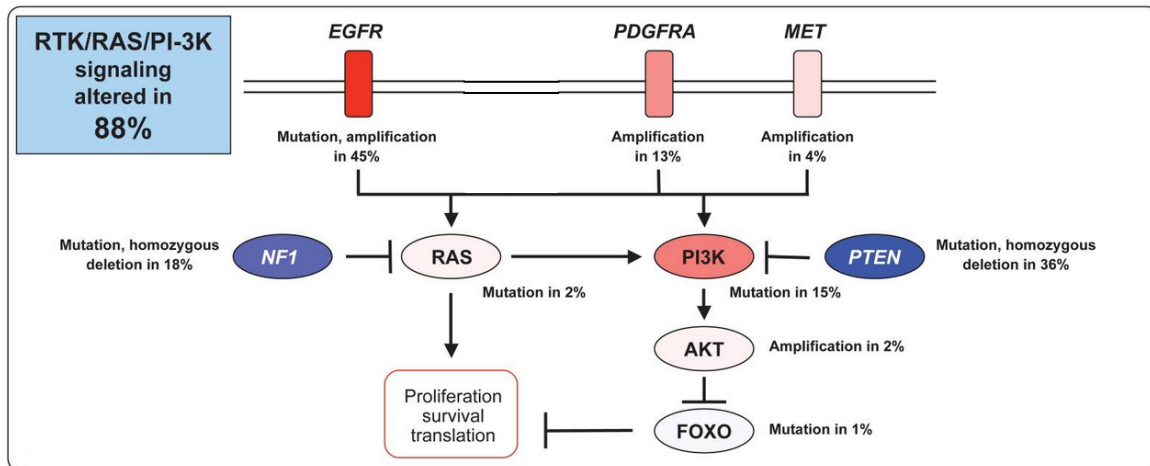


**B**
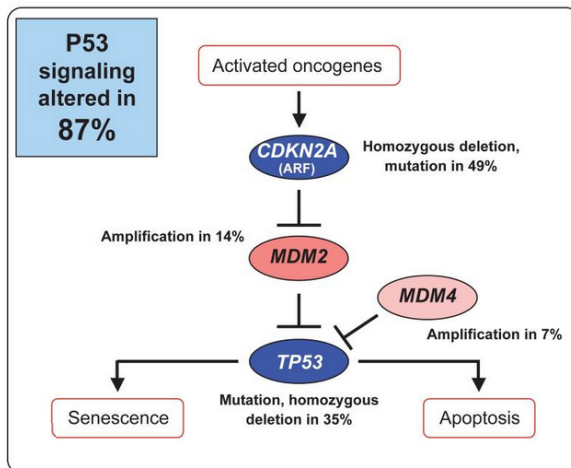


**C**
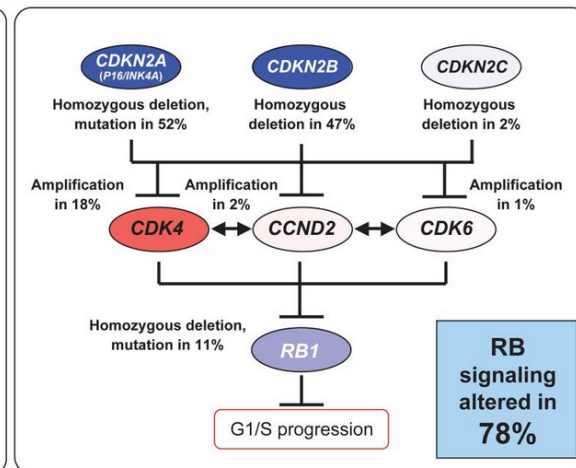


**Figure 2. Pathways driving glioblastoma.** Color-coded frequencies of gain-of-function (red) and loss-of-function mutations (blue) in glioblastoma patients. **(A) Increased Ras and PI3K signaling.** Loss of NF1 or PTEN, EGFR amplification, and PI3K mutations occur in many patients. **(B) Impaired senescence and apoptosis induction *via* p53.** In glioblastoma, TP53 or CDKN2A loss-of-function mutations and amplification of MDM2 are frequent genomic events. **(C) Reduced Rb signaling.** CDK4 gene amplification as well as CDKN2A, CDKN2B, or Rb loss can lead to uncontrolled progression of the cell cycle. Image adapted from McLendon *et al.*[34] and corrected with information from McLendon *et al.*[47].

Mutant IDH-derived D2HG competitively inhibits tet methylcytosine dioxygenase 2 (TET2). As a consequence, demethylation of DNA is impaired creating the so-called glioma CpG island methylator phenotype (G-CIMP).[10,26,48,49] G-CIMP is associated with better prognosis of glioblastoma patients.[10,50,51] Promoter methylation is a common epigenetic mechanism to silence gene transcription. DNA methylation of the $O^6$-methylguanine-DNA methyltransferase (MGMT) promoter region is observed in 35% of glioblastoma patients. Alkylating chemotherapeutics such as temozolomide (TMZ) cause $O^6$-alkylated guanine nucleobases. Repair of this DNA damage is mediated by MGMT, whereby decreased MGMT levels owing to promoter methylation can improve the efficacy of alkylating chemotherapies.[10,13,27,52-57] The MGMT-status of glioblastoma patients is routinely determined in clinical practice.[13] Recently, primary IDH^WT glioblastomas have been found to exhibit distinct DNA methylation patterns laying the foundation for annotation to the following epigenetic subclasses: mesenchymal,

midline, MYCN, RTK I, RTK II, RTK III, H3.3 G34 mutant, and infantile hemispheric glioma. The latter are observed in infants with supratentorial localization and at a median age of 0 years, whereby molecular characteristics have so far not been specified. Patients at a median age of 11 years with supratentorial or posterior fossa tumors are often affected by glioblastomas of the MYCN subtype with characteristic MYCN oncogene amplifications. Midline-type glioblastomas predominantly affect younger patients (median age of 13 years), are localized in the cerebellum, thalamus, or spine and have various copy number alterations on gene level (e.g. CDKN2A/B loss, platelet-derived growth factor receptor alpha (PDGFRα) amplification).[46] H3.3 G34 mutant glioblastomas harbor mutations of the histone H3.3-encoding gene in codon 34 and occur at a median age of 19.5 years with supratentorial hemispheric localization. Gain of chromosome 7 or 1q as well as loss of chromosome 13, 10q, and 4q represent common chromosomal aberrations in this subgroup. Mesenchymal glioblastomas reside within the cerebral hemispheres, occur at a median age of 59 years and are characterized by loss of chromosome 10 and 9p21 as well as gain of chromosome 7. Glioblastomas of the RTK I type with prominent loss of chromosome 10 and 9p21 and gain of chromosome 7 affect the cerebral hemispheres of people at a median age of 64 years. RTK II glioblastomas affect patients at a median age of 61 years, reside within the cerebral hemispheres, and present with gain of chromosome 7, 19, or 20 and loss of chromosome 10 or 9p21. Glioblastomas of the subclass RTK III comprise childhood tumors occurring at a median age of 9 years. Localization within the cerebral hemispheres, EGFR amplification, and loss of chromosome 10 represent typical features of this subgroup.[46]

### *Symptoms and standard treatment of glioblastoma*

Primary glioblastoma typically occurs in the white matter of the cerebrum (supratentorial), whereby the spectrum of symptoms can vary depending on the exact localization.[2] Glioblastoma patients present with headaches and, in severe cases, nausea or vomiting. Confusion, focal neurological deficit, changes of personality, loss of memory, and seizures are associated with glioblastoma as well.[10,12,14] Despite having high migratory potential, dissemination of glioblastoma cells remains usually CNS-restricted without distant metastases.[10,13]

The so-called Stupp protocol includes surgery and adjuvant radiochemotherapy as standard-of-care for newly diagnosed WHO grade IV glioma.[13] The extent of surgical resection correlates with patient outcomes, but it is impossible to achieve complete surgical resection while fully preserving surrounding benign brain tissue[10,13,58] Fractionated radiotherapy applied post-surgery delivers a total of 60 Gy split into 30-33 individual doses.[10,13,59] The excellent bioavailability in the CNS distinguishes TMZ from other chemotherapeutics rendering it the first choice for glioblastoma patients. Concomitant administration during radiotherapy amounts to 75 mg/m$^2$ daily, whereas adjuvant chemotherapy lasting additional six months comprises five monthly doses of 150-200 mg/m$^2$ each. In elderly patients (> 70), maintenance TMZ is tailored to the individual performance status and health situation.[10,12,13,60,61] Median survival of patients with newly diagnosed glioblastoma treated as recommended by the Stupp protocol accounts to 14.6 months, whereas radiotherapy alone achieves 12.1 months.[61]

Seizure management is based on valproate and second-generation antiepileptic drugs such as levetiracetam, lamotrigine, or pregabalin. Carbamazepine, phenobarbital, and phenytoin are avoided as anticonvulsants in glioblastoma, since they accelerate the metabolization of other medication.[10,13,62,63] Dexamethasone (8-16 mg daily) and other corticosteroids symptomatically treat neurological deficits and elevated intracranial pressure caused by glioblastoma-associated brain edema.[10,13]

### *Propensity for disease recurrence*

Glioblastoma cells deeply infiltrate into surrounding benign brain tissue rendering complete surgical resection impossible.[12,49] In addition, glioma stem cells acquire resistance to radiotherapy and TMZ by upregulation of MGMT and activation of other DNA repair pathways.[10,12,64] Moreover, a pronounced high degree of intratumoral heterogeneity is a typical feature of glioblastomas. Under evolutionary pressure exerted by the applied therapies, tumor subclones constantly acquire new mutations leading to an intensified therapeutic situation.[10,49,65] As a consequence, glioblastoma inevitably recurs in most patients accompanied by a decline of median overall survival to 6.2 months.[13,49,66] Of note, a fraction of patients (37% in a study of The Cancer Genome Atlas Research Network) develops a so-called hypermutation phenotype, which is induced by alkylating chemotherapy.[10,34,67] MutS homolog 2 (MSH2) / mutS homolog 6 (MSH6) heterodimers recognize mismatch base pairs formed by thymine and $O^6$-alkylated guanine. MSH6-dependent apoptosis is induced when the number of these mismatches exceeds a certain threshold.[10,67-69] In case loss-of-function mutations affect mismatch repair (MMR) genes such as MSH6 and TMZ is continued, glioblastoma cells acquire therapy resistance. Alkylating agents then facilitate the accumulation of mutations, clonal evolution, and glioblastoma progression instead of inducing apoptosis. Loss of either mutL homolog 1 (MLH1), MSH2, MSH6, or the endonuclease postmeiotic segregation increased 2 (PMS2) is designated as MMR deficiency and occurs predominantly in patients where the primary tumor had MGMT promoter methylation. Hypermutated recurrent grade IV glioma harbor > 30 mutations instead of less than five in primary tumors.[10,34,67]

So far, no standard-of-care has been established to manage disease recurrence in glioblastoma. Up to one third of patients are eligible for surgical resection. However, clinical benefit of re-surgery and re-irradiation is usually very limited. Upon progression on first-line chemotherapy, TMZ dosage can be increased or nitrosoureas such as carmustine can serve as second-line medication. In case inclusion criteria are met, patients may receive experimental therapies evaluated in clinical trials.[10,12,13,49,66,70,71] Constant application of electric fields alternating at a frequency of 200 kHz is capable of interrupting mitosis and inducing apoptosis. Applied subsequently to radiochemotherapy and concomitantly to maintenance TMZ, tumor treating fields (TTF) prolonged overall survival of patients with newly diagnosed glioblastoma by 4.9 months as compared with maintenance chemotherapy alone.[72] In recurrent glioblastoma, TMZ-free TTF therapy *versus* chemotherapy resulted in a median survival of 6.6 or 6.0 months, respectively.[73] The device NovoTTF/Optune® was approved by the U.S. Food and Drug Administration (FDA) for the treatment of recurrent glioblastoma in 2011.[74] The monoclonal antibody bevacizumab is directed against vascular endothelial growth factor (VEGF) to target angiogenesis. It received FDA approval for recurrent glioblastoma in

2009, although an effect on overall survival has so far not been observed.[70,75-77] Molecular therapies are further trying to exploit the regulators of PI3K, mitogen-activated protein kinase (MAPK), and DNA repair pathways as well as EGFR / EGFRvIII, VEGF receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and transforming growth factor β (TGF-β) as targets.[10,12,78] Targeted monotherapies, however, elicit a response in only 0-15% of patients and these rare responses are only transient.[12,79] The reason for this lies in the glioblastoma-driving signaling network harboring a high degree of functional redundancy. Targeting one molecule of this network results in upregulation or activation of another to circumvent effects on proliferation or survival of the tumor cell.[10,12,80] Hence, ongoing and future studies aim at overcoming the mechanisms of therapy resistance in (recurrent) glioblastoma by combining various targeted therapies.[10,12,79]

## 1.3 Medulloblastoma

***Etiology and subgroup-associated mutational and pathway signatures***

Behind pilocytic astrocytoma and malignant glioma, embryonal tumors constitute the third most common brain and CNS tumors (13.1%) in children younger than 14 years. Embryonal tumors comprise medulloblastomas (MB; 64.1%), atypical teratoid rhabdoid tumors (ATRT; 15.9%), and primitive neuroectodermal tumors (PNET; 10.4%), whereby medulloblastomas represent the most common pediatric intracranial malignancies. The peak incidence of medulloblastomas accounting for 66.0% (1,475 cases in the US from 2012-1016) is observed in children aged between 0 and 14 years, whereas only 7.3% of total cases occur in patients older than 40 years (Table 1). The total incidence in children younger than 14 years in the US from 2012-2016 distributed to 36% at age group 0-4 (535 cases), to 41% at age group 5-9 (603 cases), and to 23% at age group 9-14 (337 cases).[1]

The underlying causes of medulloblastoma are in general unclear, with most cases being designated as sporadic.[81,82] High birth weight, maternal diet – especially the uptake of *N*-nitroso compounds – during pregnancy, exposure to pesticides during childhood or the prenatal period as well as HCMV infection have been suggested as risk factors.[8,83] As for glioblastoma, hereditary cancer syndromes are associated with a small proportion (5-6%) of medulloblastoma diagnoses, whereas this fraction is highest in the SHH-activated subgroup (14-20%). Germline mutations of deleted in polyposis 2.5 / adenomatous polyposis coli protein (DP2.5 / APC; Turcot's syndrome) or of suppressor of fused homolog (SUFU) and protein patched homolog 1 (PTCH1) (both Gorlin syndrome) are linked to WNT-activated or SHH-activated medulloblastomas, respectively. The latter distinguish into TP53[WT] and TP53[mut] tumors, whereby germline TP53[mut] (Li-Fraumeni syndrome) medulloblastomas are always pediatric cases. Deficiency of homologous recombination expressed by germline breast cancer type 2 susceptibility protein (BRCA2; Fanconi anemia) or partner and localizer of BRCA2 (PALB2) mutations occurs across SHH-activated, Group 3, and Group 4 subtypes.[81,82,84]
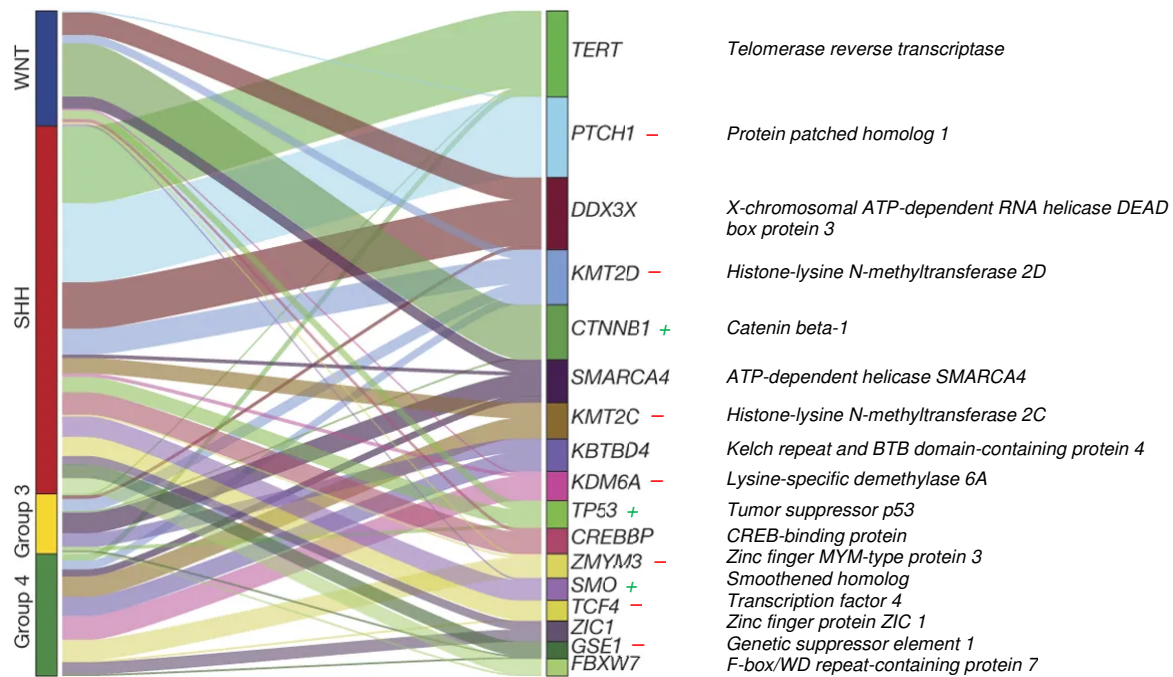
**Figure 3. Recurrently mutated genes in medulloblastoma subgroups.** Genes exceeding a mutation frequency of 5% in the respective study cohort (n=491 medulloblastomas; median age of 8 years [1 month – 50 years]) are depicted in a subgroup-specific manner. Driver genes affected by gain-of function mutations are marked with a **+**, whereas **–** indicates loss-of function driver mutations. Figure taken from Northcott *et al.*[85] and supplemented with information from Northcott *et al.*[85] and UniProt[86].

Recurrent mutations in medulloblastoma are shown on a subgroup-specific level in Figure 3. These participate in the regulation of neuronal development, cell-cell contacts, chromatin modification, cell signaling, and the cell cycle. WNT-activated medulloblastomas show a clear enrichment of mutations affecting histone lysine methylation, the β-catenin nuclear pathway, and the differentiation of CNS neurons. Catenin beta-1 (CTNNB1) mutations and monosomy 6 are present in >80% of WNT-activated tumors, whereas molecules of the SWI/SNF nucleosome-remodeling complex (AT-rich interactive domain-containing protein 1A/2 (ARID1A, ARID2), ATP-dependent helicase SMARCA4) together reach a mutation frequency of around 30%. SHH-activated medulloblastomas recurrently harbor mutations altering CNS neuron differentiation and the SHH-Gli pathway. Mutations of the histone acetyltransferases KAT6B, p300, and CREB-binding protein (CREBBP) as well as of peregrin (BRPF1) and KAT8 regulatory NSL complex subunit 1 (KANSL1), which regulate histone acetyltransferase complexes, are observed in 19% of SHH-activated medulloblastomas. In turn, recurrent mutations in Group 3 and Group 4 medulloblastomas show a mixed pattern of pathway annotations. Group 3 tumors are rather enriched for mutations involved in the Notch and TGF-β pathway, whereas recurrent Group 4 mutations are rather assigned to mechanisms of chromatin modification. Amplifications of the myc proto-oncogene are specific for subgroup 3 (17%), whereas the cases of amplified N-myc proto-oncogene distribute equally to Group 3 and Group 4 medulloblastomas (5-6% each).[85] Further, medulloblastomas have recently been described to exhibit subgroup-characteristic DNA methylation patterns.[46]

### *Pathology and standard treatment*

Medulloblastomas are infratentorial tumors arising in the posterior fossa, whereby a cerebellar localization is most common.[5] WNT-activated tumors typically grow in the cerebellar peduncles or the cerebellopontine angle connecting cerebellum and pons (brain stem). They can infiltrate into the brain stem and the fourth ventricle. SHH-activated medulloblastomas preferentially reside within the cerebellar hemisphere, whereby a small proportion is localized in the midline vermis. Group 3 and Group 4 occupy the midline of the fourth ventricle (median sulcus).[7,8,87,88] Medulloblastoma is *per se* graded as malignant WHO grade IV tumor.[2] CNS-internal metastatic spread is frequently observed in cases of Group 3 and Group 4, whereas SHH- or WNT-activated medulloblastomas rarely disseminate. Overall, two out of five patients present with metastases affecting the thoracic and lumbar spine or the sacrum. Ventricular or subependymal dissemination, not uncommonly already at diagnosis, occurs as well.[5,8] Depending on the assignment to a tumor subgroup, children suffering from medulloblastoma have a 5-year overall survival between <50% (very high-risk: SHH-activated/TP53$^{mut}$ and metastatic Group 3) and >90% (low-risk: WNT-activated and non-metastatic Group 4). High-risk medulloblastomas (metastatic SHH or Group 4 tumors and N-myc-amplified SHH) are characterized by a 5-year overall survival rate of 50-75%, whereas patients with average-risk tumors (non-metastatic / TP53$^{WT}$ / non-N-myc-amplified SHH, non-metastatic / non-myc-amplified Group 3, and non-metastatic Group 4 without loss of chromosome 11) have a 5-year overall survival rate of 75-90%.[87]

Symptoms of medulloblastoma comprise headache, visual and motor coordination disorders, clumsiness, cranial nerve palsies, reduced food intake, general indisposition, nausea, and vomiting.[89,90] Standard-of-care following near or gross total resection depends on subgroup annotation and age. Children older than three to five years suffering from average-risk tumors undergo radiochemotherapy comprising craniospinal irradiation (CSI) with a total dose of 54-55.8 Gy (1.8 Gy per day) from one month post-surgery. 23.4 Gy are delivered to the whole brain and spinal cord, whereby the residual dose is directed to the posterior fossa or tumor bed. For WNT-activated tumors, a reduced dosing regimen is conceivable. Adjuvant chemotherapy lasts around seven weeks and can be followed by maintenance chemotherapy administered every four to six weeks. Chemotherapeutics of choice include vincristine (weekly 1.5 mg/m$^2$), cisplatin (75 mg/m$^2$ for six cycles), cyclophosphamide (1,000 mg/m$^2$/dose for three cycles of two doses each), and lomustine (75 mg/m$^2$ for six cycles). When diagnosed with high- or very high-risk medulloblastoma, protocols comprising radiotherapy (standard, higher, or altered dose) with adjuvant standard or high-dose chemotherapy are applied. Infants younger than three to five years suffering from low- or average-risk tumors receive post-surgery standard chemotherapy only, eventually supplemented with intratumoral chemotherapy. High- or very high-risk disease is treated with standard chemotherapy, optional high-dose chemotherapy, and, if needed, focal or CSI radiotherapy. Treatment of adult medulloblastomas is similar to high-risk tumors in children, whereby the dose delivered to the whole brain and spine is increased to 36 Gy.[8,90]

### Risk of recurrence and long-term sequelae

Although response rates to extensive multimodal therapies are high and immediate, metastatic and high-risk medulloblastomas tend to acquire therapy resistance *via* apoptosis evasion.[5,8] WNT-activated tumors rarely recur, whereas recurrence in SHH-activated medulloblastomas occurs, but remains local. In turn, recurrent Group 3 and Group 4 tumors are mostly even metastatic.[8] Three-quarter of pediatric recurrences are observed within two years. As for primary medulloblastoma, the age of the patient decides on the application of radiotherapy. However, a treatment protocol to manage recurrent medulloblastoma has so far not been established.[8]

Those children belonging to the 70-75% designated as cured, will experience long-term treatment-associated sequelae comprising neurological, developmental, psychosocial, and neuroendocrine deficits or even secondary malignancies such as glioblastoma and meningioma. These are primarily attributed to irradiation of the developing brain. The development of novel, especially targeted or immunotherapies, is aimed at replacing radiotherapy.[5,8,90-92] SMO (SHH pathway) inhibitors such as vismodegib, recombinant soluble tumor necrosis factor-related apoptosis inducing ligand (TRAIL), TRAIL receptor (TRAILR)-agonistic antibodies, N-myc inhibition, targeting of anti-apoptotic Bcl-2 proteins, and pro-apoptotic Bcl-2 homology protein mimetics belong to the most promising approaches. To circumvent mechanisms of early therapy resistance, the preferential use of combination therapies is suggested, whereas targeted monotherapies should be avoided.[5,8,93]

## 1.4 Meningioma

### Risk factors and genetic basis

Meningiomas constituted 37.6% of all primary intracranial neoplasms diagnosed in the US from 2012-2016. They occur at a median age of 66 years and are subdivided into benign (WHO grade I; 80.5%), atypical (WHO grade II; 17.7%), and anaplastic or malignant meningiomas (WHO grade III; 1.7%). Malignant meningiomas make up only 0.4% with an annual average of 355 cases in the US, whereas non-malignant tumors account to a yearly incidence of 30,196 (Table 1).[1,2] Irradiation, e.g. for childhood medulloblastoma, represents an established risk factor for the development of meningeal neoplasia, whereas the role of head trauma, neurosurgery, and sex hormones, especially progesterone, has so far not been clarified.[90,94-97] Hereditary predisposition applies to a minority of meningiomas and occurs in the context of neurofibromatosis type 2 characterized by germline mutations of the tumor suppressor gene neurofibromin-2 (NF2).[95,98,99] Moreover, meningiomas have been associated with Turcot's, BRCA-associated protein-1 (BAP1) tumor predisposition, Cowden, Werner, Li-Fraumeni, Gorlin, multiple endocrine neoplasia type 1, von Hippel-Lindau, and Gardener's syndrome.[16,84,95,99-106] Familial meningiomatosis is caused by germline mutations affecting two components of the SWI/SNF nucleosome-remodeling complex: SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1 and subfamily B member 1 (SMARCE1; SMARCB1).[99,107,108]

NF2 is not only affected by germline, but also by somatic loss-of function mutations present in 40-60% of meningiomas.[95,99,109,110] Related thereto, loss of chromosome 22q harboring both

the NF2 region and SMARCB1 was identified to be causative of sporadic meningiomas.[95,99,108,111] Moreover, BAP1, TERT, SMARCB1, SMO, SUFU, TNF receptor-associated factor 7 (TRAF7), RNA polymerase II subunit A (POLR2A), Krueppel-like factor 4 (KLF4) as well as the protein kinases AKT1, AKT3, and PI3K have been identified to be recurrently mutated in meningiomas, especially such harboring WT NF2. These mutated gene products mainly participate in the mammalian target of rapamycin (mTOR), SHH, and p53 pathway.[110,112-117] High-grade meningiomas harbor a significantly increased mutational load as compared with low-grade meningiomas, apparent on the level of both non-synonymous mutations (on average 23 *versus* ~10 per tumor) and chromosomal copy number alterations (on average 19% *versus* 3% of the tumor genome disrupted). Despite that, no recurrent mutations (defined by a frequency of ≥ 5% in the respective study cohort) apart from NF2 have so far been identified for high-grade meningiomas. Thus, mutated NF2 is designated a driver of meningioma across all WHO grades.[110]

### *Symptoms, structures affected by meningioma, and standard-of-care*

Meningiomas arise from both cerebral and spinal meninges and show a characteristic tail of thickened dura on radiological images.[1,2,118,119] Meningeal neoplasias invade both intra- and extracranial structures comprising the skull, dura mater, and dural sinuses or soft tissue, scalp, and the orbits, respectively.[95,96,119,120] From WHO grade II, an invasion of the brain is observed.[2] Meningiomas manifest with symptoms of increasing intracranial pressure which include – depending on localization and size – headaches, nausea and vomiting, seizures, and visual impairment.[95-97] A small proportion of meningiomas (estimated at 0.1%) forms extracranial and even extraneural metastases affecting predominantly the lungs as well as the pleura, bones, the liver, the mediastinum, and lymph nodes.[120-122]

Benign meningiomas are usually treated with surgical resection to the maximum possible extent alone. However, the specific localization of some tumors, particularly at the skull base, which is affected by 38% of grade I tumors and is rich in neurovascular structures, impedes surgery. This causes considerable morbidity and a 5-year recurrence rate of 22%.[123,124] Atypical meningiomas receive optional adjuvant radiotherapy, whereas irradiation is a fixed pillar in the therapeutic regimen of anaplastic meningiomas. Radiotherapy typically comprises a total dose of 50 to 60 Gy applied in fractions of 1.5 to 2 Gy to the tumor bed and a margin of 0.5 to 1.5 cm.[124,125] Stereotactic radiosurgery represents a sophisticated technique to direct irradiation emitted from a gamma knife to tumor tissue during the course of surgical resection, especially of skull base or posterior fossa meningiomas.[124,126,127] So far, there is no FDA-approved chemotherapeutic agent – neither for primary nor for recurrent or refractory meningeal neoplasms.[120,124] 5-year overall survival is stratified by WHO grade and comes up to 92% for grade I tumors, 78% for atypical meningiomas, and 44% for patients diagnosed with grade III.[123,128]

### *Recurrent meningiomas and emerging targeted treatment strategies*

Local recurrence of grade I meningiomas is rare (5-10%), whereas 5-year recurrence rates mount up to 40% for atypical meningiomas and to 50-80% when diagnosed with a malignant meningeal tumor. Recurrence of grade III meningiomas goes along with a decline in median survival to less than 24 months. So far, no effective therapies have been established to

manage recurrent meningioma.[95,121,124,129,130] Of note, primary tumors treated with adjuvant radiotherapy show an increased amount of non-synonymous mutations and copy number alterations at recurrence.[110]

Recurrent meningiomas can be treated – if possible – with salvage resection and adjuvant radiotherapy or radiosurgery. In addition, these patients may receive hydroxyurea, anti-hormonal therapy (e.g. anti-progesterone with mifepristone), or somatostatin analogs (e.g. octreotide), whereby all these agents can mainly mediate stabilizing effects.[124,131-133] Moreover, mTOR inhibitors such as everolimus and vistusertib are under investigation for use in recurrent or progressive meningioma.[124,134] Anti-angiogenic therapy is increasingly applied and can be based on bevacizumab or small molecules (e.g. sunitinib and vatalanib) targeting VEGFR or PDGFR.[124,135,136] As for medulloblastoma, the SMO inhibitor vismodegib is evaluated for use in meningeal neoplasias.[93,124] In case eligibility criteria are fulfilled, participation in clinical trials represents a further option for patients suffering from recurrent meningioma.[124]

# 2 Cancer immunotherapy

## 2.1 Key actors for effective anti-cancer immune responses

To protect the host from pathogens, infected or transformed cells, the immune system discriminates between self and non-self. It is made up of two major pillars: the innate and the adaptive system, which both have cellular as well as humoral components. The cellular part of the innate immune system encompasses macrophages, dendritic cells (DCs), granulocytes, mast cells, and natural killer cells, whereas the complement system and antimicrobial peptides predominantly build the humoral part.[137,138] Professional antigen-presenting cells (APCs), especially DCs, connect the two subsystems by presenting fragments of incorporated antigens to naïve T lymphocytes of the adaptive immune system. These peptide fragments are bound to cell surface molecules of the major histocompatibility complex (MHC) and are specifically recognized by a T-cell receptor (TCR).[138] When naïve antigen-specific T cells bind to peptide-MHC complexes and receive co-stimulatory signals as well as cytokines from an APC, they get activated, proliferate, and differentiate into CD4$^+$ helper T cells (Th cells; Th1 and Th2 as subtype), CD8$^+$ cytotoxic T lymphocytes (CTLs), or CD4$^+$CD25$^+$ regulatory T cells (Treg cells). CTLs are responsible for the elimination of affected host cells, whereas Tregs suppress immune effector functions.[138-140] In secondary lymphoid tissues, Th2 cells can induce the differentiation of antigen-experienced B lymphocytes into plasma cells. The latter produce antibodies, the humoral effectors of the adaptive immune system.[138,141] Besides antigen specificity and clonality, the formation of long-lived memory B and T lymphocytes is a hallmark of adaptive immunity.[138,142] Immune responses are orchestrated and regulated by numerous cytokines and chemokines produced by both innate and adaptive cells.[137-139,142]

## 2.2 Human leukocyte antigens present peptides to T lymphocytes

### 2.2.1 Expression and structure of HLA molecules

For adaptive immunity, MHC presentation of peptides derived from intracellular or incorporated proteinaceous antigens to T cells is an essential feature. In humans, the MHC is called human leukocyte antigen complex (HLA).[10,143] The short arm of chromosome 6 (6p21.3) harbors the HLA gene cluster comprising the regions HLA class I-III. Molecules belonging to the tumor necrosis factor (TNF) family and the complement system are encoded by HLA class III, whereas HLA class I and II transcripts are translated into proteins involved in the processing and presentation of antigens.[10,144] The α chains of both the classical (HLA-A, -B, -C) and the non-classical (HLA-E, -F, -G) HLA class I molecules are gene products of the HLA class I region. In addition, the stress-induced MHC class I polypeptide-related sequence proteins (MIC-A, -B) and the human hemochromatosis protein (HFE) are transcribed from this region as well.[10,145] The genes for HLA class II α and β chains, the peptide transporter associated with antigen processing (TAP), the chaperone tapasin, and the low molecular weight proteins (LMP) 2 and 7, two proteasome subunits induced by interferon (IFN), are included in the HLA class II region. Further, HLA-DM and HLA-DO, two non-classical HLA class II molecules involved in antigen processing, are encoded here (Figure 4 A).[10,146]

Chromosome 15 harbors the genetic information for $\beta_2$ microglobulin ($\beta_2$m). $\beta_2$m is neither polymorphic nor participates in building the peptide-binding cleft, but forms heterodimers with the α chains of classical HLA class I molecules (Figure 4 B). All nucleated cells express HLA-A, -B, and -C on their surface to provide CTLs with antigenic peptides. To fulfill their function as professional APCs, DCs, macrophages, B lymphocytes, Kupffer, and Langerhans cells express HLA class II.[10,143,145,147] However, in recent years it has become evident that HLA class II expression is not absolutely restricted to APCs, but may also occur on epithelial (e.g. thyroidal or intestinal cells), endothelial, further lymphoid cells (e.g. granulocytes and mast cells), and even on solid malignancies (e.g. glioblastoma and renal cell carcinoma)[10,145,147-149] – either constitutively or IFN-γ-induced.[145,150] In heterodimeric HLA class II molecules, both the α and β chain participate in formation of the peptide-binding cleft (Figure 4 B) exposing antigenic peptides to TCRs.[143,145]

Polygenicity and polymorphism characterize the HLA gene cluster. Per chromosome, one HLA-A, -B, and -C allele each is encoded, which together constitute the HLA class I haplotype (Figure 4 A). Thus, every individual can have cell-surface expression of up to six HLA class I allotypes.[10,145] The IPD-IMGT/HLA Database (release 3.40.0, 2020-04-20) listed a total of 6,082 HLA-A alleles (3,794 proteins), 7,255 HLA-B alleles (4,648 proteins), and 5,842 HLA-C alleles (3,503 proteins), in June 2020. Among the 7,301 HLA class II alleles, were 3,357 isoforms of HLA-DR β chain alleles (2,378 proteins), 1,556 HLA-DP β allele (1,016 proteins), and 1,826 HLA-DQ β allele (1,213 proteins) variants.[151] In some haplotypes, the expression of two functional HLA-DR β chains is observed (HLA-DRB1, -DRB3, -DRB4, and -DRB5 loci).[145] In contrast to HLA class II β chains, α polypeptides are hardly subject to polymorphism (2 HLA-DR α, 68 HLA-DP α, and 106 HLA-DQ α protein isoforms).[151]
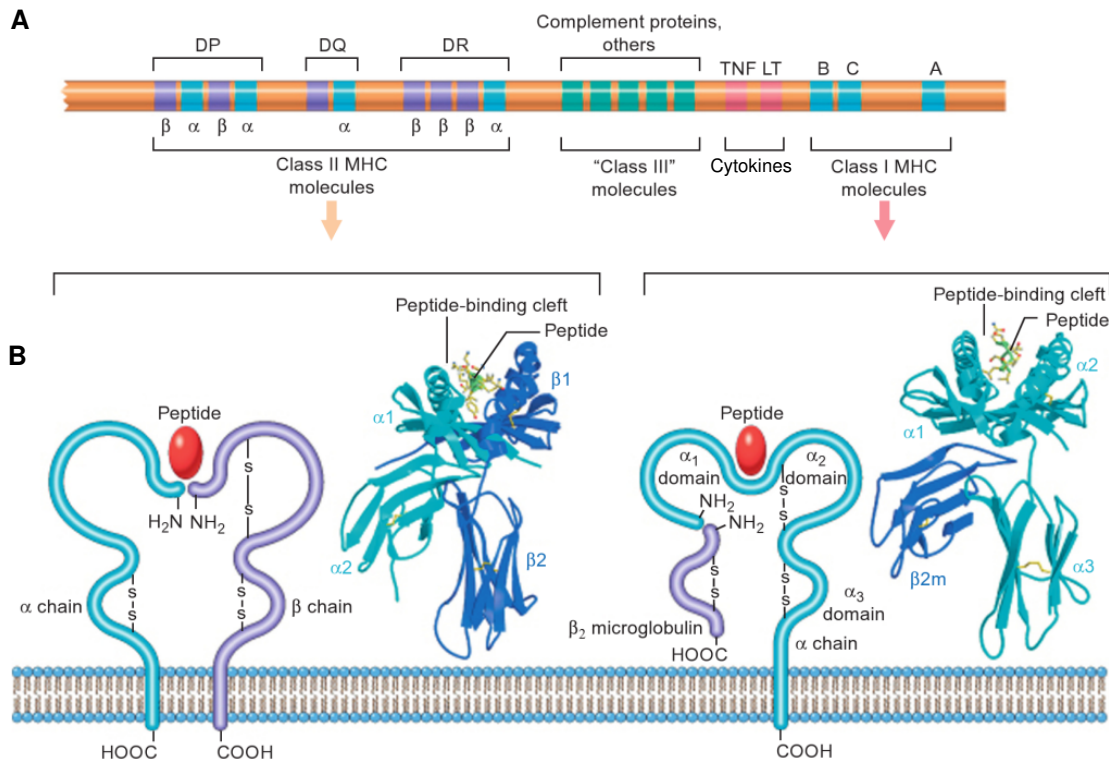
**Figure 4. Structure of the HLA on genomic and protein level. (A) Organization of the HLA gene cluster on the sixth chromosome (6p21.3).** The three gene regions (I-III) harbor the protein-coding information for HLA molecules themselves, other proteins of the antigen processing and presentation machinery as well as for cytokines and complement factors. **(B) Schematic illustration of HLA class I- and II-peptide complexes.** HLA class I-presented epitopes have only contact to the α polypeptide of the heterodimer (right), whereas the peptide-binding cleft of HLA class II molecules is formed by both the α and β chain (left). Figure taken from Bhagavan *et al.*[152]; slightly modified.

## 2.2.2  Antigen processing and HLA-peptide complex formation

HLA class I ligands primarily originate from intracellular antigens deriving from both the host cell itself and from pathogens such as viruses residing inside the cell.[153] Multicatalytic complexes with proteinase activity, so-called proteasomes, show nuclear and cytoplasmic localization. The catalytic activity is harbored by a core barrel of 20 Svedberg units (S) that is capped by two 19S subunits. These caps recognize proteins marked with ubiquitin for degradation. Upon unfolding of the substrate, it is introduced into the barrel, where peptide bonds are cleaved hydrolytically.[10,154] Cleavage products are released into the cytoplasm,[154] from where TAP selectively translocates them into the endoplasmic reticulum (ER).[155] There, peptides are further trimmed by aminopeptidases, especially the ER aminopeptidase associated with antigen processing (ERAAP), to be of appropriate length for association with HLA class I molecules. HLA class I α polypeptides are synthesized at ER-bound ribosomes, are simultaneously imported into the rough ER lumen, and are bound by calnexin, an integral membrane chaperone. Upon binding of $\beta_2$m to the α chain, the complex associates with the chaperone calreticulin and the thiol reductase ERp57 assisting HLA molecules in proper folding.[10,155,156] TAP, calreticulin, ERp57, and the TAP-associated tapasin form the peptide-loading complex (PLC). The PLC recognizes processed peptides and α chain-$\beta_2$m heterodimers and allows them to form a stable complex. HLA class I-peptide complexes dissociate from the PLC and undergo vesicular transport from the ER to the Golgi apparatus.

In secretory Golgi vesicles, the complexes traffic to the plasma membrane in which they are integrated through membrane fusion (Figure 5 A).[10,155,156] HLA class I ligands have a typical length of eight to ten amino acids (AA) and an extended conformation.[157,158] Slightly longer peptides may be accommodated by bulging out in the center and slightly protruding from the groove.[158,159] HLA class I molecules have five exceptionally polymorphic peptide-binding pockets (A-F), whereby the second and last pocket (B and F) authoritatively define biochemical properties of binding peptides for most allotypes. The interacting AAs of the ligand are designated as anchor residues and represent the peptide motif of the corresponding HLA class I allotype. Usually, these anchors are the C-terminal residue and the second AA counted from the N-terminus.[10,160,161]
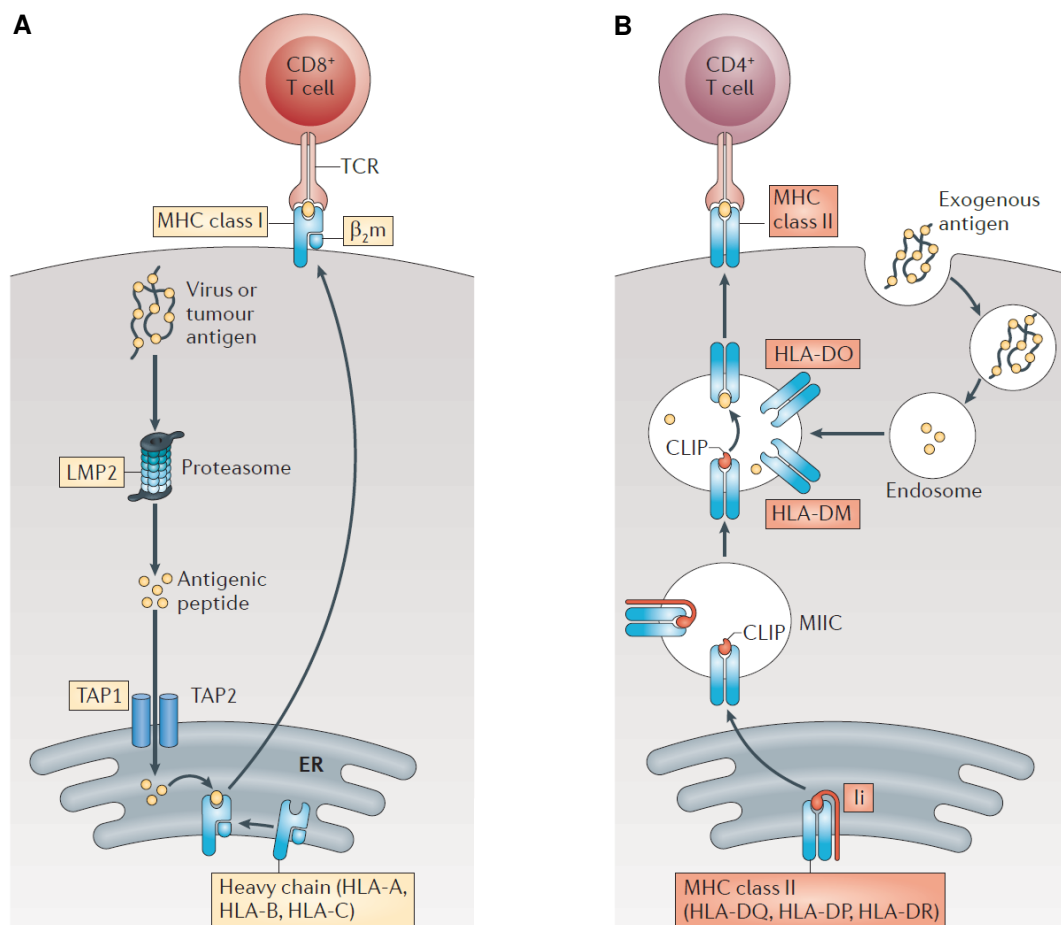


**Figure 5. Loading of HLA molecules with antigenic peptides. (A) Processing of HLA class I antigens.** Antigenic peptides are generated by the proteasome, bind to newly synthesized HLA class I molecules in the ER, and HLA-peptide complexes are integrated into the plasma membrane. **(B) Generation of HLA class II-peptide complexes.** Membrane proteins or extracellular antigens are broken down in vesicles and resulting peptides are loaded in MIIC on HLA class II molecules. This schematic leaves out the step of initial translocation of the HLA class II-Ii complexes to the plasma membrane and subsequent endocytosis. Figure adapted from Kobayashi *et al.*[162].

Extracellular antigens as well as membrane proteins can be incorporated by phagocytosis, (clathrin-mediated) endocytosis, or by macropinocytosis and do subsequently undergo proteolytic degradation in phagolysosomes, early endosomes, macropinosomes, and the antigen-processing compartment. Through autophagy, parts of the cytosol can be engulfed by membranes and degraded in autophagosomes. This allows intracellular antigens to be

presented on HLA class II, which is of utmost importance in DCs and thymic epithelial cells to establish central and peripheral tolerance to self-antigens.[10,163-165] HLA class II α and β chains newly synthesized at ER-resident ribosomes heterodimerize and the peptide-binding cleft is occupied by the non-polymorphic invariant chain (Ii). The complex passes the trans-Golgi network and traffics to the plasma membrane. Clathrin-mediated endocytosis of the complex and translocation to multivesicular late endosomal-lysosomal MHC class II antigen-processing compartments (MIIC) is facilitated by Ii.[10,163,164,166] Ii is cleaved in intraluminal vesicles within the MIIC, whereby the class II-associated invariant chain peptide (CLIP), a remnant of Ii, is still bound to the peptide-binding cleft. HLA-DO regulates the enzyme HLA-DM which removes CLIP from the HLA class II heterodimer to render it peptide-receptive.[10,163,164,166] Association of ligands and HLA class II molecules takes place in (auto-)phagolysosomes and in the MIIC. HLA class II-peptide complexes become plasma membrane-bound *via* vesicles or tubules originating from the MIIC that fuse with the cell membrane (Figure 5 B).[10,163,164,166] HLA class II ligands have a typical length of 15 to 17 AA.[167] However, longer peptides of up to 25 AA can be accommodated by protrusion of the C- and N-terminus from the groove as well.[168] The anchor residues of HLA class II-presented peptides which mediate binding specificity reside within the approximately central core region spanning nine AA.[10,158,169] HLA class II molecules are renowned for promiscuous binding of peptides (with distinct core regions) due to decreased length preferences and degenerate peptide motifs.[169-171]

The phenomenon of cross-presentation describes HLA class I presentation of extracellular antigens on DCs for the activation of naïve CTLs. One of the mechanisms accomplishing this is the phagosome-to-cytosol pathway. Macropinosomes and phagosomes are believed to derive from ER membranes and do hence comprise tapasin, TAP, and the protein transporter complex Sec61. The latter allows antigens to escape from endocytic vesicles into the cytoplasm.[10,172] Proteins are proteasomally degraded, cleavage products are imported into the ER and occupy the peptide-binding cleft of HLA class I molecules. Alternatively, peptides leaving the proteasome may undergo phagosomal import *via* TAP, where they encounter ER-derived HLA class I heterodimers. A third mechanism depends on cathepsin S, a cysteine protease that is contained in endosomes. Besides taken up antigens that are broken down by cathepsin S, endosomes do also encompass HLA class I molecules either deriving from the ER or from the plasma membrane in the course of recycling processes. Upon association of peptides and HLA class I molecules, complexes are integrated into the cell membrane.[10,172]

## 2.3 Cancer immunotherapy for intracranial neoplasms

### 2.3.1 Immunotherapy – a contemporary concept to treat malignancies

The idea that recognition and elimination of tumor cells through cells of the innate and adaptive immune system is as simple as it is ingenious.[173,174] The beginnings of cancer immunotherapies reach back to 1893, when William Bradley Coley observed concomitant tumor regression in cancer patients suffering from *Streptococcus*-induced erysipelas after surgery. Based on a first hypothesis, he systematically infected patients with *Streptococcus* inducing remarkable anti-tumor effects.[175] Immune responses directed against cancer cells have two classes of antigens as targets: tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs). Processing of mutation-bearing proteins (TSAs) can produce so-called neo-epitopes. These can represent deletions or insertions, single nucleotide variants (SNVs), gene fusions, and frameshift mutations. Further, neo-antigenic HLA ligands can originate from mutations leading to splice site alterations or from defective products of usually untranslated RNA sequences.[174,176,177] Cryptic peptides arising from cancer-specific antisense transcripts, novel unannotated open reading frames, non-coding regions (5' and 3' untranslated regions (UTR), introns, intergenic regions), non-canonical reading frames of protein-coding regions, or unconventional (proteasomal) splicing events as well as peptides harboring tumor-exclusive post-translational modifications (PTMs) can also be (non-mutated) neo-epitopes.[177-186] WT genes can encode for tissue-specific differentiation antigens (TAAs) and cancer-testis (CTAs) or germline antigens (TSAs).[174,187-190] Viral antigens, particularly when derived from oncogenic viruses, are another source of TSAs,[10,174,191-194] whereas protein overexpression in malignant cells gives rise to TAAs.[10,174,191,195] The major advantage of TSAs over TAAs is real tumor specificity rendering 'on-target off-tumor' toxicities virtually impossible. Additionally, TSA-specific T cells do not underlie central tolerance to self-antigens that has to be overcome for anti-tumor responses and are thus expected to be strongly immunogenic. However, this might not be applicable for CTAs with ectopic expression in the thymus resulting in the deletion of highly affine TCRs.[196,197] Targeting early oncogenic driver mutations by immunotherapy further has the advantage of homogenous expression (and probably HLA presentation) across subclones, which is highly preferable in heterogenous tumors such as glioblastoma.[49,65,198]

Despite this large variety of potential targets, immune responses against tumors may be inefficient or even absent. In 2011, the famous hallmarks of cancer as defined by Hanahan and Weinberg were supplemented with 'immune evasion'.[199] Robert Schreiber summarized the steps to immune evasion as the three phases of immunoediting which are governed by interaction and co-evolution of immune and tumor cells. In the elimination phase, the immune system successfully defeats outgrowth of tumor cells, as it eliminates transformed cells during immunosurveillance. Few surviving cells enter the second phase. Tumor formation is not yet possible, since tumor cell division and eradication are at equilibrium. Selection pressure caused by the immune system in combination with genetic instability foster the development of new variants and subclones. Once cancer cells succeed in immune evasion, this results in clinical manifestation of tumor formation.[200,201] The reasons for insufficient or even lacking tumor antigen-specific immune responses encompass inadequate HLA presentation of the tumor antigen (on APCs), scarceness of cytokines (e.g. interleukin 2 (IL-2), IL-12) and/or co-stimulatory molecules (e.g. CD80, CD86) required for T-cell activation, a lack of immune

effector cells at the site of action, and immunosuppression originating from the tumor microenvironment (TME).[202,203] The latter can be mediated by immunosuppressive cells (e.g. Tregs, myeloid-derived suppressor cells (MDSCs), inhibitory B lymphocytes), high levels of inhibitory cytokines (TGF-β, IL-10) and signals (e.g. cytotoxic T-lymphocyte-associated antigen 4 (CTLA4, CD152), inhibitory Ig-like transcripts (ILTs), programmed cell death protein 1 (PD-1, CD279), programmed cell death ligand 1 / 2 (PD-L1, CD274, B7-H1 / PD-L2, CD273, B7-DC), Fas cell surface death receptor (Fas), Fas ligand (FasL), TRAIL, TRAILR, impaired antibody-mediated opsonization, a metabolic milieu which is adverse for immune cells, or elevated levels of enzymes degrading factors essential for T cells or producing inhibitory metabolites (e.g. arginase-1 (ARG1), heme oxygenase-1 (HO-1), indoleamine 2,3-dioxygenase-1 (IDO1)).[203-206] Cancer immunotherapy aims at breaking immune evasion by reconstituting or modulating anti-cancer immune responses.[10,173,176,203,207] In general, this is pursued by three strategies: provision of the (appropriate) antigen (1), supply of T-cell co-stimulation or blockade of T-cell co-inhibition (2), and transfer of effector cells (3).[202] The so far most common approaches include vaccination with antigenic substances (1) or antigen-presenting DCs (1, 2), monoclonal antibodies intervening in T-cell activation (2), recombinant cytokines (2), adoptive T-cell transfer (3), and oncolytic viruses (multifaceted mode of action).[10,174,203]

### *Cancer vaccines*

For vaccination-based immunotherapies, whole tumor cell lysates, antigen- or epitope-encoding expression vectors, processable long peptides or recombinant antigens as well as short peptides in the form of exact CD8+ or CD4+ T-cell epitopes directly binding to HLA molecules, are employed. *In vitro*, the antigen presentation of DCs can be modified by pulsation with antigens or peptides, cancer cell lysate, or nucleic acids (RNA / DNA) encoding the respective TAA or TSA. Upon administration, these antigen-presenting DCs are capable of priming adaptive immunity. Vaccines can increase both the repertoire and the number of (targetable) antigens presented on HLA paving the way to induce anti-cancer immune responses.[10,196,203,208,209] However, sipuleucel-T (Provenge), a vaccine for metastatic castration-resistant prostate carcinoma composed of autologous APCs,[210] has so far remained the only therapeutic cancer vaccine receiving FDA approval.[203]

Short peptide vaccines have several clear advantages as compared to other vaccination approaches. On the one hand, they have a clearly defined composition which is also beneficial to evaluate immunogenicity prior to administration as well as to monitor CD8+ and CD4+ T-cell responses.[211] On the other hand, peptide synthesis is well feasible in good manufacturing practice (GMP) grade without being disproportionately expensive allowing for *de novo* synthesis for single patients. Despite the high degree of individuality of peptide vaccines inherent to the HLA system, peptides for vaccine composition may also be chosen 'off-the-shelf' from a previously built up warehouse.[196,212] Peptide vaccines using a mix of targetable epitopes have demonstrated to both have an exceptional safety profile and to elicit clinically significant anti-tumor responses. The latter is most likely not possible by targeting a single antigen.[196,203] However, overall clinical response rates come up to a maximum of 5% indicating that the effectiveness of cancer vaccines does not solely depend on the selection of the right target. Great potential is seen for the combination with other immunotherapies such as

checkpoint inhibition or adoptive T-cell transfer (discussed in the next sections) and the use of an appropriate adjuvant has a decisive impact.[185,196,213]

Adjuvants can protect the delivered peptide and mediate a depot effect, promote antigen uptake by APCs, and, most importantly, provide a more general stimulus to APCs inducing antigen-specific responses of the desired Th1/CTL type.[196] Montanide™ ISA 51, also designated as analogue of incomplete Freund's adjuvant, is a delivery adjuvant used for oil-in-water formulations of vaccines and has both protective and depot effects.[196,214] In turn, the popular adjuvant granulocyte-macrophage colony-stimulating factor (GM-CSF) is capable of recruiting and activating APCs at the site of injection. However, this effect is far weaker than expected and doses above 100 μg can even lead to T-cell inhibition and proliferation of MDSCs.[196,215] Other adjuvants bind to so-called toll-like receptors (TLR) resulting in strong stimulation of APCs accompanied by an increase in HLA expression, cytokine and chemokine secretion, as well as the number of costimulatory and adhesion molecules.[196] Deoxycytidyl-deoxyguanosin oligodeoxynucleotides (CpG ODNs), a TLR9 ligand, was the first TLR agonist tested in a clinical trial of a therapeutic cancer vaccine (in combination with Montanide™ ISA 51) eliciting outstanding T-cell responses.[196,216] Hiltonol® is composed of polyinosinic-polycytidylic acid stabilized by polylysine and carboxymethylcellulose (Poly-ICLC) which activates TLR3, but has not been tested in large cohorts yet. Single-stranded RNA or Imiquimod (Aldara® 5% cream) represent TLR7/8 activators. The results whether patients benefit from topical Aldara® application at the site of injection are controversial, whereas RNA adjuvants are still under development.[196] Recently, Pam$_3$Cys-GDPKHPKSF (XS15), a novel adjuvant mimicking the bacterial lipopeptide Pam$_3$Cys-Ser-Ser, has been described. First applications of this TLR1/2 ligand as adjuvant for peptide vaccines appear very promising (internal data created at the Department of Immunology, University of Tübingen).[196,217] The optimal composition of therapeutic cancer vaccines remains an open question and is under intense investigation. Combinations of adjuvants acting in different manner, chimeric TLR agonists, as well as concomitant administration of other immunotherapies such as monoclonal antibodies or recombinant cytokines are possibilities to enhance the efficacy of cancer vaccines.[196]

### *Recombinant cytokines*

Recombinant cytokines are supposed to supplement the immune system with missing signals to elicit anti-tumoral T-cell responses. Their use was the first proof of the effectiveness of cancer immunotherapy.[218] IL-2 is a stimulator of T-cell proliferation and acts *via* the highly affine α chain (CD25) of the IL-2 receptor complex. Natural killer (NK) and B cells have IL-2 receptors lacking CD25. In these cells, IL-2 induces proliferation as well as stimulation of NK-cell cytotoxicity and B-cell differentiation, respectively.[10,203,219] Despite having been approved by the FDA, recombinant IL-2 (Proleukin®, former aldesleukin) is rarely used in clinical routine owing to severe toxicities and limited efficacy.[203] The latter is in part reasoned by the coincident expansion of CD4$^+$CD25$^+$ Tregs.[203,220] Novel approaches to reduce IL-2 toxicity by altering pharmacokinetic properties include coupling to polyethylene glycol (PEG) or to Fc domains of antibodies thus increasing half-life of the molecule. Further, the pharmacodynamic profile is optimized by inhibiting binding to highly affine IL-2 receptors expressed by Tregs. Targeting IL-2 to the site of action can be achieved by chimeric molecules equipped with TME-specific

antibodies.[218] Besides IL-2, FDA approval was only given to one further recombinant cytokine: type I interferon (IFN-α), as native molecule and as half-life-optimized PEGylated version.[218,221] IFN-α acts both on the TME by being anti-angiogenic and on tumor cells by promoting apoptosis and inhibiting proliferation.[218,222] DCs and T cells mature under the influence of IFN-α, which is also an immunostimulant.[218,223] However, recombinant IFN-α is rarely used in the clinics. This might be overcome by engineering novel variants with reduced toxicity and optimized binding properties. Fusion of IFN-α and apolipoprotein A-I improves the pharmacokinetic profile, whereas so-called AcTakines (activity-on-Target cytokines) deliver the cytokine to DCs *via* a Clec9A-specific antibody domain. Moreover, mutation of the cytokine itself can contribute to improved binding affinity.[218,224,225]

CTLs and NK cells exert their cytotoxic activity, proliferate, and release cytokines upon stimulation with IL-15.[218,226,227] In contrast to IL-2, it does not promote the concomitant expansion of Tregs.[218,228] First in-human applications failed due to severe side effects and later studies did not show a clear clinical benefit of IL-15 treatment.[218,229] Current approaches try to increase stability of this small molecule and to generate fusion proteins with better binding properties. RLI is a chimeric molecule consisting of IL-15 and the sushi domain bearing the binding domain of the IL-15 receptor α.[218,230] Inclusion of apolipoprotein A-I might be a way to optimize delivery of the construct.[218,231] IL-12, IL-21, and even IL-10, which has so far been designated as immunosuppressive cytokine but was shown to prevent apoptosis of CTLs following antigen recognition, are currently under intense investigation for application as cancer immunotherapies. In addition, there are ongoing studies evaluating the effects of targeting pro-tumor cytokines such as TNF-α, TGF-β, or the colony stimulating factor 1 (CSF-1) by antibodies, small interfering RNA (siRNA), cytokine traps, and inhibitors of cytokine receptor signaling.[218]

### *Agonistic and antagonistic monoclonal antibodies*

The category of monoclonal antibodies comprises agonistic ones acting on co-stimulatory molecules and antagonistic ones blocking co-inhibitory receptors, so-called checkpoint inhibitors. In 2018, Tasuku Honjo and James P. Allison were honored with the Nobel Prize in Physiology and Medicine for their work on immune checkpoints.[232] CTLA4 exceeds the affinity of CD28 to CD80 (B7-1) and CD86 (B7-2) expressed on APCs thus preventing CD28-CD80 or CD28-CD86 interactions that enable T-cell activation.[233,234] CTLA-4-blocking antibodies (Ipilimumab) both facilitate T-cell effector function and deplete Tregs that constitutively express CTLA4 by antibody-dependent cellular cytotoxicity (ADCC).[10,235-237] When PD-1+ T cells bind to PD-L1 or PD-L2, this induces an inhibitory signaling cascade rendering the T cell anergic. While hematopoietic and parenchymal cells express PD-L1 to prevent autoimmunity, PD-L2 is preferentially expressed by APCs and Th2 cells. Various types of cancer cells hijack the PD-1–PD-L1/PD-L2 axis as mechanism of immune evasion.[10,203,206,238-240] Monoclonal antibodies blocking PD-1 (Pembrolizumab, Nivolumab, Cemiplimab) or PD-L1 (Atezolizumab, Durvalumab, Avelumab) have the capability to elicit anti-tumor responses and have been approved by the FDA for several cancer entities.[241-248] Antibodies blocking V-domain immunoglobulin suppressor of T-cell activation (VISTA), lymphocyte activation gene-3 (LAG-3, CD223), B- and T-cell lymphocyte attenuator (BTLA, CD272), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), B7 homolog 3 protein (B7-H3, CD276), T-cell

immunoglobulin and ITIM domain (TIGIT), or sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15) are currently under development and designated as 'next-generation checkpoint inhibitors'. Furthermore, the combination of several checkpoint antibodies is considered to have high therapeutic potential.[203,241,243,249] The response to checkpoint inhibition correlates with the mutational burden and, for antibodies targeting the PD-1–PD-L1/PD-L2 axis, with PD-1, PD-L1, and PD-L2 expression on tumor and T cells. Further predictive biomarkers include the pre-therapy tumor burden and site of metastases, the diversity and (relative) composition of the peripheral immune cell repertoire, cytokine profiles, levels of immunosuppressive cells (e.g. MDSCs, Tregs), the amount and type of tumor-infiltrating lymphocytes (TILs), the patient's performance status, or the occurrence of immune-mediated side effects. The exploitation of predictive biomarkers is of importance to weigh up the side effects of checkpoint blockade and the expected clinical benefit.[241]

Engagement of co-stimulatory molecules can also contribute to the enhancement of anti-tumor T-cell responses. So far, none of these agonistic antibodies have received FDA approval for cancer immunotherapy. OX40 (CD134) belongs to the TNF receptor family and is expressed by CD8$^+$ and CD4$^+$ T lymphocytes upon antigen recognition. OX40-activating antibodies promote T-cell proliferation and activation of effector functions.[203,250] Agonistic antibodies acting on 4-1BB (CD137) can enhance NK-mediated ADCC and even restore cytotoxicity of T cells.[203,251] Engagement of the glucocorticoid-induced tumor necrosis factor receptor (GITR), which is expressed on activated DCs, NK cells, regulatory and effector T cells as well as B cells, enhances the activity of the targeted cell. In the T-cell compartment, the immunosuppressive effect of Tregs is reduced, whereby TCR signaling and proliferation of CTLs and Th cells are enhanced.[203,252] CD40-targeted immunotherapy stimulates APCs, T cells, and anti-tumoral responses mediated by cytotoxic cells of the myeloid lineage[203,253]

A more sophisticated approach to target co-stimulatory or -inhibitory molecules is based on aptamers. The specificity and affinity of these RNA or DNA oligonucleotides is comparable to monoclonal antibodies. Thus, they represent an additional tool to unleash anti-cancer T-cell responses.[254] Another technique for future cancer immunotherapies are bi-specific antibody formats, so-called bi-specific antibodies for T-cell redirection and activation (BiTes). Antibodies with dual specificity are aimed at redirecting immune cells to the tumor. Blinatumomab, an anti-CD19/CD3 BiTe, brings CD3$^+$ T lymphocytes into spatial proximity of CD19$^+$ leukemia cells.[255] Similar effects can be achieved with fusion proteins consisting of a soluble TCR and an antigen-binding domain, named immune-mobilizing monoclonal T-cell receptors against cancer (ImmTACs). Upon TCR binding to the tumor cell, polyclonal T lymphocytes are recruited *via* the CD3-specific part of the ImmTAC.[256]

### *Adoptive T-cell transfer*

It is suggested that the affinity, frequency, and functionality of tumor-specific T cells correlate with the efficacy in eliminating cancer cells. Autologous T cells that recognize tumor antigens can be enriched and expanded *in vitro* to be subsequently used as passive immunotherapy. Enrichment of TAA- or TSA-specific T cells can be achieved by selecting peripheral or tumor-infiltrating T-cell clones.[10,203,257-259] Upon lympho-depleting chemotherapy which eliminates immunosuppressive factors such as Tregs or so-called 'homeostatic cytokine sinks', expanded

T cells are re-infused.[203,260] Lymphodepletion goes along with the risk of severe infections and T-cell reconstitution is not only deferred, but often also incomplete which may ultimately also contribute to cancer recurrence.[261,262] Therefore, alternative treatment regimens try to circumvent lymphodepletion by concomitant administration of CTL-promoting cytokines such as IL-15.[262] Despite eliciting clinically relevant responses e.g. in melanoma,[259] adoptive T-cell therapy faces several obstacles: collection and expansion of T cells, especially TILs, is laborious and tumor-specific T cells are often rare and/or of low affinity.[203] Genetic modification represents an option to equip T lymphocytes with highly affine TCRs specific for a pre-defined target antigen.[203,263] Nevertheless, this approach does still require the target antigen to be processed and presented on HLA. Chimeric antigen receptors (CARs) link TCR signaling and antigen recognition of an antibody in one molecule thus rendering T-cell binding HLA-independent. CAR-expressing T cells can not only recognize unprocessed surface antigens, but also gangliosides, carbohydrates, proteoglycans, and glycosylated proteins.[10,203,264] Moreover, co-stimulatory molecules and T cell-activating factors can be introduced into CAR-T cells by transfection.[203,265,266]

CAR-T cells have enormous potential for immunotherapies and two CD-19-specific constructs, tisagenlecleucel and axicabtagene ciloleucel, have already been approved by the FDA for use in hematological malignancies.[267] However, a major drawback of CARs is an unfavorable profile of adverse events including cytokine release syndrome and neurotoxicity as well as limited ability to target solid tumors.[203,267] So-called suicide genes (e.g. Cetuximab-targetable truncated EGFR (EGFRt), ganciclovir-sensitive hygromycin phosphotransferase-herpes simplex virus 1 thymidine kinase (HyTK), truncated CD19 (CD19t), or drug-inducible caspase-9) increase the safety of CAR-T-cell therapies.[268-273] To overcome immunosuppression in the TME, CAR-T cells redirected for universal cytokine killing (TRUCKs) are a sophisticated approach. TRUCK-T cells express stimulatory cytokines such as IL-18 upon CAR engagement at the site of disease. This might be an option to locally augment CAR-T-cell activity.[203,274,275] Homing of CARs to the TME may be facilitated by using constructs with an antigen-binding domain similar to BiTes.[203,276]

### *Oncolytic viruses*

Employing so-called oncolytic viruses for immunotherapy is in concept similar to the experiments of Coley.[277,278] Virotherapy is based on the idea that lytic replication happens almost exclusively in tumor cells instead of affecting all replicating cells. It is expected that the immunosuppressive mechanisms exhibited by the tumor itself as well as the TME facilitate the infection of cancer cells.[277] This results in immunogenic cell death characterized by the release of danger signals and tumor antigens. Additionally, oncolytic viruses have been shown to enhance the number of TAAs cross-presented on HLA class I.[10,203,279,280] This way, they are capable of both enhancing pre-existing and inducing *de novo* anti-tumor immunity. In contrast to targeted therapies, the big advantage of oncolytic viruses is being not solely dependent on one specific receptor.[277] So far, Talimogene laherparepvec (T-Vec, Imlygic), a herpes virus modified to express GM-CSF, has remained the only oncolytic virus with FDA approval for use in metastatic melanoma.[203,277,281] Besides herpes viruses, virus families tried to be exploited for virotherapy encompass *Paramyxo-* (e.g. *measles morbillivirus*, *mumps rubulavirus*), *Retro-* (e.g. *Moloney Leukemia Virus*), *Adeno-*, *Pox-* (e.g. *Vaccinia virus*, *Fowlpox*), *Parvo-*,

*Rhabdo-* (e.g. *Vesicular Stomatitis Virus*), *Reo-*, and *Picornaviridae* (e.g. *Seneca Valley Virus*, *Coxsackie-*, *Rhino-*, *Poliovirus*) as well as the *Newcastle disease virus*.[10,277,282] Reduced off-target tropism and increased virulence for cancer cells is achieved by genetic modification.[277,283] Intratumorally injected, the safety profile of oncolytic viruses – measured by the rate of (serious) adverse events – is superior to that of checkpoint inhibitors. Despite local injection, oncolytic viruses such as T-Vec are capable of inducing distant responses, for instance in metastases.[277,283,284]

## 2.3.2  Current status of immunotherapy for intracranial neoplasias

For decades, the brain has been denoted an immune privileged organ shielded by the blood-brain barrier of the cerebrovascular endothelium. This begs the question, whether immunotherapy is a realistic therapeutic concept for brain tumors. In 1948, Peter Brian Medawar provided the first evidence for intracranial immune effector cells. Following allogeneic skin transplantation, heterotopic skin grafts of the same donor were rejected in the brain.[10,285,286] Likewise, transplants into the brain parenchyma were shown to be responsible for accelerated rejection of orthotopic skin grafts.[286,287] Upon injection of T cells and DCs into the brain, these migrated to cervical lymph nodes indicating that the brain is not segregated from peripheral immunity and immunosurveillance.[288,289] In 2014, the existence of meningeal lymphatic vessels located along dural sinuses was proven by two independent groups. This system accomplishes the drainage of immune cells as well as cerebrospinal and brain interstitial fluid from the CNS to deep cervical lymph nodes.[290,291]

Microglia express MHC class II as well as co-stimulatory molecules and are responsible for the phagocytosis of tumor cells (e.g. gliosarcoma) damaged by CD8$^+$ T cells. Consequently, they constitute an interface for interaction with peripheral immune cells.[10,292-300] Activated T cells were also evidenced to penetrate into the CNS where final maturation and proliferation of tumor-specific CTLs takes place.[10,301-307] In melanoma patients, i.v. administered T cells have reached brain metastases eliciting clinical responses.[308] Three routes allow leukocytes to penetrate from the periphery into the CNS: leptomeningeal vessels (blood-to-subarachnoid space), the blood-brain barrier (blood-to-perivascular space), and the choroid plexus (blood-to-CSF) (Figure 6). Under pathological conditions, traffic rates increase dramatically.[309-311] Following antigenic challenge, both the CSF and the brain parenchyma home memory T cells.[309,311,312] As explained in 1.4, meningiomas originate from the meninges lying beyond the blood-brain barrier. Thus, these should be more easily accessible to drugs and immune cells as compared with glioblastomas or medulloblastomas.[313]

Human glioblastomas, medulloblastomas, and meningiomas are infiltrated by both CD4$^+$ and CD8$^+$ T cells indicated by the detection or isolation of TILs from surgical specimens.[129,314-317] In glioblastoma and high-grade meningioma, low amounts of CD3$^+$CD8$^+$FOXP3$^-$ TILs (relative to CD4$^+$ TILs) are described as negative prognostic factor for disease progression[129,318] and survival of glioma-bearing mice was shown to be CTL-mediated.[289] For medulloblastomas, no correlation between T-cell infiltration and clinical outcome has been observed.[317] Spontaneous intratumoral T-cell responses seem to be rare in glioblastoma. However, peptide vaccination is capable of promoting T-cell infiltration and eliciting TIL responses to both mutated neo-

epitopes and WT antigens, which is another indicator of the invalidity of an absolute immune privilege concept.[314,315]
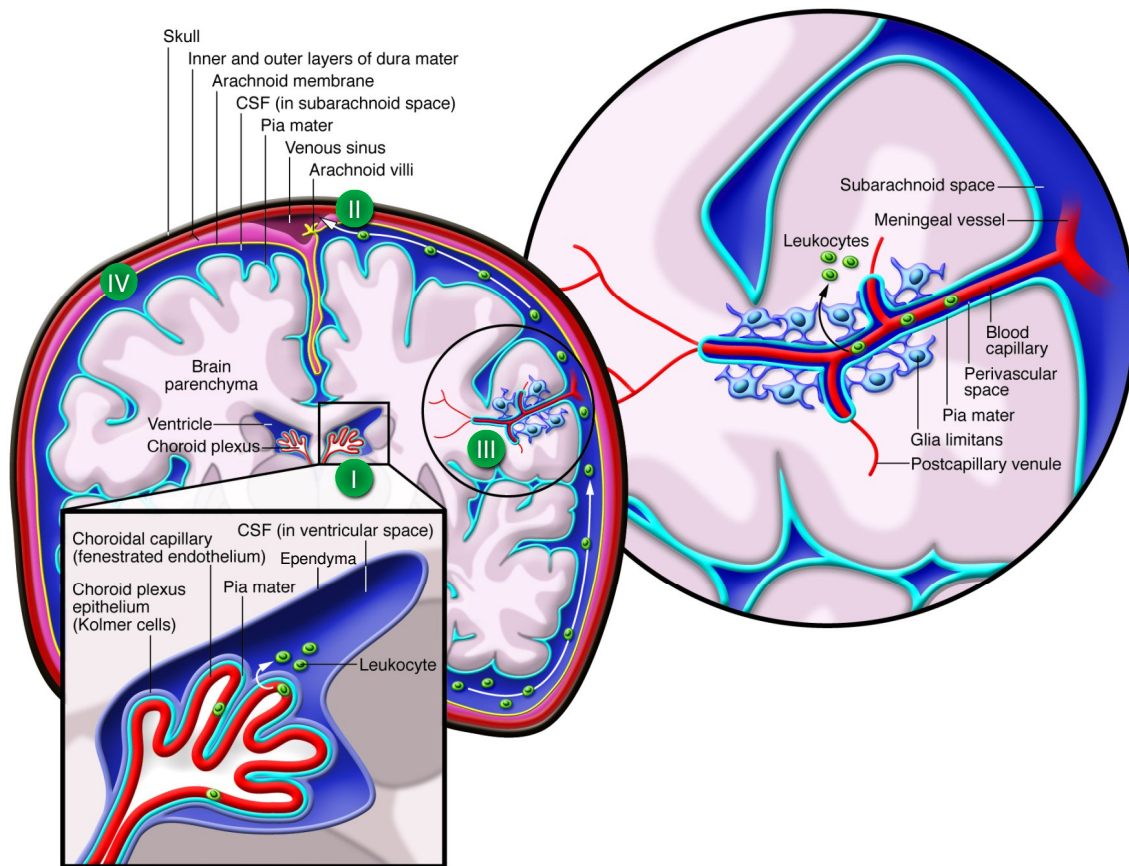


**Figure 6. Leukocyte routes into and within the CNS.** The brain parenchyma is separated from the skull by three meningeal layers, namely the pia mater, the arachnoid membrane, and the dura mater (from inside to outside). The subarachnoid space lies between pia mater and arachnoid membrane and is filled with CSF. The latter is produced in the choroid plexus, where leukocyte transmigration from the blood into CSF takes place (I). CSF-resident leukocytes penetrate the pia mater and the glia limitans, which is formed by astrocytes, to access the brain parenchyma. Alternatively, leukocytes can enter the parenchyma *via* the ependyma which lines the ventricular system. At the venous sinus, the uptake of leukocytes from CSF into the blood is mediated by arachnoid villi (II). The brain is supplied with blood by branches from meningeal vessels. Blood capillaries are located in the so-called perivascular space and reach deeply into the brain parenchyma. Immune cells can pass the blood-brain barrier by trafficking across vascular endothelium, the subarachnoid space, the pia mater, and the glia limitans (III). Leukocytes may also leave the blood by diapedesis to enter the CNS *via* the leptomeninges encompassing the arachnoid membrane and the pia mater (IV). Image taken from Wilson *et al.*[311]; slightly modified.

In order to reach the designated site of action, acellular immunotherapeutic agents have to permeate into the brain. The proportion of drugs which can do so was estimated at less than 5%.[319] Recombinant cytokines such as IFN-α can be engineered to be blood-brain barrier-permeable by coupling to Apolipoprotein A-I.[224] The ability to cross the human blood-brain barrier was also evidenced for oncolytic viruses such as ParvOryx01 applied in an early clinical trial for the therapy of glioblastoma.[320] So-called nanoscale immunoconjugates (NICs), chimeric molecules of antibodies and biopolymer scaffolds, can pass the blood-brain barrier, which is not applicable e.g. for unmodified anti-CTLA4 or anti-PD-L1 antibodies.[319,321,322] Likewise, integration of a 'Brain Shuttle module' into therapeutic antibodies enables transferrin

receptor-dependent transcytosis into and optimized lysosome sorting in the CNS.[323] Primary intracranial neoplasms – excepting hypermutated recurrent glioblastomas – are in general characterized by low mutational loads.[324] Checkpoint blockade has significant clinical benefit e.g. in metastatic melanoma or non-small cell lung cancer, whereby response rates correlate with the mutational burden. Thus, checkpoint inhibitors as monotherapy are not expected to have the same therapeutic efficacy in brain tumors as observed for other cancer entities.[325-327] Glioblastomas largely express PD-L1,[328] whereas medulloblastomas are mostly PD-L1 negative[317] and PD-L1 expression is rarely observed in meningiomas.[329] This further diminishes the expected clinical benefit of anti-PD-L1 therapy for the latter two cancer entities. Checkpoint antibodies represent passive immunotherapies that are not capable of priming *de novo* immune responses rendering pre-existing anti-cancer T cells a prerequisite for their effectiveness. This suggests that patients suffering from brain tumors and lacking endogenous anti-tumor immunity do not benefit from checkpoint inhibition as monotherapy. However, combinations of checkpoint blockade with other (active) immunotherapies such as cancer vaccines might be promising for intracranial neoplasias.[10,203,330]

In conclusion, it can be stated that the anatomically reasoned dogma of the brain being devoid of immunity is no longer sustainable. Especially under pathological conditions, immune cells and cytokines enter the CNS and there is growing evidence for effective intracranial immune responses. This suggests to pursue cancer immunotherapy as means to counteract immunosuppression unleashing intracranial anti-cancer immune responses. As recently shown for peptide vaccination in glioblastoma, the timing and dosage of concomitant medication such as dexamethasone has a major impact on the effectiveness of immunotherapy and consequently requires in-depth evaluation.[315] Immunotherapy has shown to be feasible for immunologically cold brain tumors with low mutational burden, whereby the clinical benefit of standard therapy has still not been outcompeted by immunotherapeutic approaches. In the following, the current knowledge on tumor antigens in glioblastoma, medulloblastoma, and meningioma as well as a summary of recent or ongoing clinical trials will be presented. Searching the PubMed database for publications dealing with immunotherapy yielded 1,582 hits for glioblastoma and only 140 or 68 entries for medulloblastoma or meningioma, respectively.[331] The imbalance between these three tumor entities with a profound lack of immunotherapeutic approaches in medulloblastoma and meningioma is also reflected by the number of clinical trials registered at ClinicalTrials.gov to investigate immunotherapeutic intervention in glioblastoma (n=92), medulloblastoma (n=8), or meningioma (n=6).[332]

### *Tumor antigens in glioblastoma*

For immunotherapeutic approaches targeting glioblastoma, a large variety of TAAs has been suggested. The target selection of WT antigens in a recent phase I clinical trial of multi-peptide vaccination conducted by the Glioma Actively Personalized VAccine Consortium (GAPVAC) was based on natural HLA presentation as well as RNA expression data. The source proteins of vaccine peptides comprised ankyrin repeat domain-containing protein 40 (ANR40), chondroitin sulfate proteoglycan 7 / brevican core protein (CSPG7), CDK4, ceramide synthase 1 (CerS1), clusterin (CLUS), cysteine and glycine-rich protein 2 (CSRP2), serine/threonine-protein kinase DCLK2 (DCLK2), dihydropyrimidinase-related protein 4

(DRP-4), eukaryotic translation initiation factor 4E (eIF4E), elongation of very long chain fatty acids protein 2 (ELOV2), glutamate receptor 4 (GluR4), ionotropic glutamate receptor kainate 3 (GluK3), ATP-sensitive inward rectifier potassium channel 10 (KCJ10), melanoma-associated antigen F1 (MAGE-F1), membrane-associated guanylate kinase inverted 2 (MAGI2), protein MTSS 2, X-linked neuroligin-4 (NLGNX), Y-linked neuroligin-4 (NLGNY), neuronal cell adhesion molecule (NRCAM), ORM1-like protein 1 (ORML1), protocadherin gamma-C5 (PCDGM), pecanex-like protein 3 (PCX3), pleckstrin homology domain-containing family A member 4 (PKHA4), receptor-type tyrosine-protein phosphatase zeta (PTPRZ), DNA repair and recombination protein RAD54B (RAD54B), seizure 6-like protein (SE6L1), transforming acidic coiled-coil-containing protein 3 (TACC3), ubiquitin carboxyl-terminal hydrolase 11 (UBP11), vacuolar protein sorting-associated protein 13B (VP13B), abnormal spindle-like microcephaly-associated protein (ASPM), protein crumbs homolog 1 (CRUM1), dedicator of cytokinesis protein 7 (DOCK7), brain-type fatty acid-binding protein (B-FABP), acyl-CoA 6-desaturase (FADS2), constitutive coactivator of PPAR-gamma-like protein 2 (F120C), neuronal membrane glycoprotein M6-b (GPM6B), HEAT repeat-containing protein 1 (HEAT1), heparan sulfate 2-O-sulfotransferase 1 (HS2ST), integrin alpha-7 (ITA7), the uncharacterized protein LOC728392, leucine zipper putative tumor suppressor 1 (LZTS1), oligodendrocyte transcription factor 2 (OLIG2), E3 ubiquitin-protein ligase Praja-2 (PJA2), 60S ribosomal protein L7a (RL7A), excitatory amino acid transporter 4 (EAA4), structural maintenance of chromosomes protein 4 (SMC4), alpha-2,8-sialyltransferase 8E (SIA8E), transmembrane protein 231 (TM231), transmembrane protein 255A (T255A), zinc finger protein 3 (ZNF3), and baculoviral IAP repeat-containing protein 5 / survivin (BIRC5).[314] The development of GAPVAC-101 included a phase I trial of the invariant peptide cocktail IMA950, which contained additional peptides derived from chondroitin sulfate proteoglycan 4 (CSPG4), insulin-like growth factor 2 mRNA binding protein 3 (IF2BP3), met proto-oncogene / hepatocyte growth factor receptor (c-Met), and tenascin C (TN-C).[330]

The glioblastoma-associated antigens IL-13 receptor alpha 2 (IL13Rα2), receptor tyrosine-protein kinase erbB-2 (HER2), tyrosinase-related protein 2 (TRP2), Wilms' tumor gene 1 (WT1), absent in melanoma 2 (AIM2), melanocytes lineage-specific antigen gp100 (gp100), and melanoma-associated antigen 1 (MAGE-A1 / CT1.1) have also been evaluated in in-human trials employing e.g. DCs pulsed with peptides, CAR-T cells, or peptide vaccination.[333-336] Besides MAGE-A1, expression of the CTAs X antigen family member 3 (XAGE3 / CT12.3), Opa-interacting protein 5 / protein Mis18-beta (OIP5 / CT86), transcriptional repressor CTCFL (CTCFL / CT27), and actin-like protein 8 (ACTL8 / CT57) was evidenced in WHO grade IV glioma.[10,209,337,338] By comparing the repertoire of HLA ligands naturally presented on both primary glioblastoma and glioblastoma stem-like cells but not on healthy tissues, Neidert *et al.* described Ras and Rab interactor 1 (RIN1), hepatocyte cell adhesion molecule (HEPACAM), glypican-1 (GPC1), DnaJ homolog subfamily C member 25 (DJC25), alpha-tubulin N-acetyltransferase 1 (ATAT), paired box protein Pax-6 (PAX6), raftlin-2 (RFTN2), transforming growth factor beta-2 proprotein (TGFB2), protein AF1q, EF-hand domain-containing protein 1 (EFHC1), MICOS complex subunit MIC25 (MIC25), NmrA-like family domain-containing protein 1 (NMRL1), transmembrane prolyl 4-hydroxylase (P4HTM), plasminogen activator inhibitor 1 (PAI1), and prostaglandin F2 receptor negative regulator (FPRP) as further candidate targets for cancer immunotherapy.[149] Overexpression renders ephrin type-A

receptor 2 (EphA2), B7-H3, telomerase, and EGFR candidates for glioblastoma immunotherapy as well.[339-343]

Neo-epitopes are mainly eligible for fully individualized immunotherapeutic approaches. However, the EGFRvIII and IDH1R132H mutations may be exploited as targetable neo-antigens in larger cohorts, as they occur in 25-30% or 5-15% of adults suffering from primary glioblastoma, respectively.[27,37,342,344,345] Targeting the histone H3 K27M variant is currently under investigation for pediatric high-grade as well as diffuse midline (non-hemispheric) glioma characterized by a subgroup-specific mutation frequency of up to 30%.[198,346] HCMV-directed immunotherapy, especially against the tegument protein pp65, has been subject of multiple clinical trials in glioblastoma and was even associated with clinical benefit. However, this therapeutic option is still under controversial discussion.[10,209,341,347-350]

### *Targets for the immunotherapy of medulloblastoma*

The number of potential targets for the immunotherapy of medulloblastoma is substantially lower as compared with glioblastoma. However, several CT antigens have been proposed based on expression data and correlation with poor patient outcome. These comprise melanoma antigen preferentially expressed in tumors (PRAME), sperm autoangiogenic protein 17 (SPA17 / CT22), MAGE-A1, New York esophageal squamous cell carcinoma-1 (NY-ESO-1 / CT6.1), G antigen 1 (GAGE-1 / CT4.1), synaptonemal complex protein 1 (SYCP1 / CT8), solute carrier organic anion transporter family member 6A1 (SO6A1 / CT48) as well as the melanoma-associated antigens 3, 4, 6, C1, and C2 (MAGE-A3 / CT1.3; MAGE-A4 / CT1.4; MAGE-A6 / CT1.6; MAGE-C1 / CT7.1; MAGE-C2 / CT10).[268,351,352] Non-CTAs suggested for medulloblastoma immunotherapy include disialoganglioside GD2, Her2, EGFR, glypican-2 (GPC2), and B7-H3, whereby all antigens excepting the latter two have been subject of recent or ongoing clinical trials. [353-357]

Despite low mutational burden, somatic mutations in medulloblastoma were found to yield immunogenic predicted neo-epitopes. However, shared mutations translating into targetable neo-antigens for a larger number of patients have so far not been identified.[324,358] As for glioblastoma, targeting HCMV-derived antigens has been suggested for medulloblastoma, but remains under controversial debate.[83,347]

### *Antigens in meningioma immunotherapies*

Immunotherapeutic approaches in meningioma face a profound lack of suitable target antigens. Most of the candidates including B7-H3,[359] gp100,[360] NY-ESO-1,[361,362] IL13Rα2,[363] survivin[364], TRP-2,[360] and WT1[365] have also been proposed as targets for glioblastoma or medulloblastoma. In addition, members of the CT45 gene family (CT45A1-3, CT45A5-10) having ≥ 90% sequence identity[86,362,366] and synaptonemal complex protein 1 (SYCP1 / CT8) have been suggested as immunotherapeutic targets.[230,232,233] Besides having in general low mutational load, little is known about the presence of neo-antigens in meningioma. In high-grade meningiomas characterized by a higher number of somatic mutations as compared with low-grade meningiomas, no common mutational signature (apart from NF2 alterations) has been identified so far. Thus, one can expect that targeting neo-epitopes in meningioma remains suitable for fully individualized approaches only.[110]

### *Evaluation of immunotherapy for intracranial neoplasms*

Recent and ongoing clinical trials evaluating different immunotherapeutic approaches in glioblastoma, medulloblastoma, or meningioma are summarized in Table 2.

**Table 2. Exemplary clinical trials evaluating immunotherapies for the treatment of intracranial neoplasms (pp. 34-39).** Design and results of immunotherapeutic studies in glioblastoma (red), medulloblastoma (orange), and meningioma (blue). Listed adverse events do only refer to applied immunotherapies and not to concomitantly administered standard therapy. Abbreviations not introduced in the text above: anaplastic astrocytoma (AAST), Autologous Lymphoid Effector Cells Specific Against Tumour Cells (ALECSAT), brain stem glioma (BSG), complete response (CR), cobalt gray equivalent (CGE), delayed-type hypersensitivity (DTH), Epstein-Barr Virus (EBV), glioblastoma (GBM), intradermal (i.d.), intranodal (i.n.), intratumoral (i.t.), intravenous (i.v.), keyhole limpet hemocyanin (KLH), meningioma (MNG), months (mo), overall survival (OS), peripheral blood mononuclear cells (PBMCs), progressive disease (PD), progression-free survival (PFS), partial response (PR), PEP-CMV in Recurrent Medulloblastoma / Malignant Glioma (PRiME), radiological response (RR), subcutaneous (s.c.), stable disease (SD), time to progression (TTP), total tumor RNA (TTRNA), *ex vivo* expanded Autologous Lymphocyte Transfer (xALT), year (y), plaque-forming units (pfu).

| Treatment Reference | Therapeutic schedule | Phase # Patients | Immuno-genicity | Outcome | Adverse events |
|---|---|---|---|---|---|
| **Monoclonal antibodies** | | | | | |
| **Monoclonal antibodies: checkpoint inhibitors** | | | | | |
| Atezolizumab[367] | Post-radiochemotherapy ± bevacizumab 1,200 mg atezolizumab i.v. every 3rd week | Ia n=16 recurrent GBM | | n=1 PR n=3 SD 4.2 mo median OS 1.2 mo median PFS | n=3 grade 3 toxicities (brain edema, asthenia, aspartate aminotransferase elevation) |
| CheckMate 143[368]: nivolumab ± ipilimumab | Group 1: 3 mg/kg nivolumab every 2nd week Group 2: 4 doses 1 mg/kg nivolumab + ipilimumab 3 mg/kg every 3rd week, then 3 mg/kg nivolumab Group 3: 4 doses 3 mg/kg nivolumab + ipilimumab 1 mg/kg every 3rd week, then 3 mg/kg nivolumab | I recurrent GBM Group 1: n=10 Group 2: n=10 Group 3: n=20 | | n=1/0/1 PR n=2/2/4 SD 1.9/1.5/2.1 mo median PFS 10.4/9.2/7.3 mo OS | n=0/9/6 grade 3-4 toxicities Nivolumab monotherapy tolerated best, side effects determined by ipilimumab dose |
| Pembrolizumab[369] | Group 1: 200 mg pembrolizumab i.v. 19-9 days before surgery (neoadjuvant), adjuvant 200 mg i.v. every 3rd week Group 2: 200 mg i.v. every 3rd week (adjuvant) | II, randomized recurrent GBM Group 1: n=16 Group 2: n=16 | | 13.7 / 7.5 mo median OS 3.3 / 2.4 mo median PFS | n=10/7 grade 3-4 adverse events; n=1 grade 3 pneumonitis and n=1 grade 4 elevation of alanine aminotransferase (both in group 1) |
| Nivolumab[370] | Nivolumab i.v., 4 doses of 240 mg every 2nd week, 12 monthly doses of 480 mg | II, ongoing n=180 adults with recurrent / refractory rare CNS tumors | | Planned: OS, 6mo-PFS, RR | |
| Avelumab[371] | Post-surgery Avelumab i.v., 10 mg/kg every 2nd week for 3 mo, concomitant proton therapy 20 CGE / week | Ib, ongoing n=12 recurrent / progressive grade I-III MNG | Planned: changes in TILs | Planned: RR, PFS, OS | |

| Treatment Reference | Therapeutic schedule | Phase # Patients | Immuno-genicity | Outcome | Adverse events |
|---|---|---|---|---|---|
| **Monoclonal antibodies: others** | | | | | |
| **Bevacizumab[372]** | 60 Gy (2 Gy/day, 6 weeks) + 75 mg/m² TMZ daily (max. 49 days), subsequent maintenance TMZ 150-200 mg/m² 5 successive days/mo (6-12 mo) Experimental treatment from week 4 of radiotherapy Group 1: bevacizumab 10 mg/kg i.v. every 2nd week Group 2: placebo | III newly diagnosed GBM Group 1: n=320 Group 2: n=317 | | 15.7 / 16.1 mo median OS 10.7 / 7.3 mo median OS | Hypertension, thromboembolism, visceral perforation, thrombocytopenia, neutropenia, fatigue in both groups, but more common in group 1 |
| **Bevacizumab[373]** | Bevacizumab + irinotecan ± TMZ Group 1: n=5 10 mg/kg bevacizumab + 125-150 mg/m² irionotecan i.v. every 2nd week + 150 mg/m² oral TMZ 5 days/mo Group 2: n=2 bevacizumab + irinotecan (as group 1) Group 3: n=2 15 mg/kg bevacizumab i.v. + 90 mg/m² oral irinotecan for 5 successive days + 1.5 mg/m² vincristine i.v. every 3 weeks | Retrospective n=9 children with recurrent MB | | 11 mo median TTP 13 mo median OS n=6 PR, n=3 CR, n=3 PD, n=1 SD | n=2 grade III neutropenia, n=2 grade III thrombocytopenia, n=1 grade III elevated liver function test, n=1 grade III diarrhea |
| **[131]I-3F8 anti-GD2[355]**: radio-immunotherapy | Oral potassium iodide and liothyronine (5-7 days before and until 2 weeks after therapy) Max. 4 intraventricular injections (2 mg with 10 mCi/dose) + oral prior acetaminophen and diphenhydramine + oral / i.v. 0.5-1 mg dexamethasone 2×/day for 3 days + ceftriaxone 1 h post-infusion | II n=43 high-risk / recurrent MB / PNET | | 24.9 mo median OS, 11 mo median PFS, 5y-OS 44.9%, 6mo-PFS 57.1% | Self-limiting fever, headache, nausea, vomiting n=2 bradycardia, headache, fatigue, n=1 CSF pleocytosis |
| **Bevacizumab[374]** | Monthly cycles (8 in median) of 10 mg/kg bevacizumab i.v. (day 1, 15) + oral 10 mg everolimus | II n=17 refractory progressive grade I-III MNG | | 22 mo median PFS n=15 SD (in median for 10 mo), n=1 PD | Grade 1-2 adverse events: n=9 thrombocytopenia, n=1 proteinuria, Grade 3 adverse events: n=2 proteinuria, n=1 colitis, n=1 thrombocytopenia |
| **Cancer vaccines** | | | | | |
| **Cancer vaccines: peptide vaccination** | | | | | |
| **GAPVAC-101[314]**: warehouse WT peptides (APVAC1; 33 HLA-A*02:01 / 26 HLA-A*24:02 ligands of 9-10 AA; 3 HLA-DR peptides of 14-18 AA) and individualized neo-epitopes (APVAC2; 19 AA) | Following surgery and radiochemotherapy; 400 µg/peptide i.d. + 75 µg GM-CSF i.d. + 1.5 mg poly-ICLC s.c.; maintenance TMZ APVAC1: 7 warehouse peptides according to transcriptome and immunopeptidome data + 2 promiscuous HLA-DR peptides + 1 viral marker peptide; 12 applications in median (n=15 patients) APVAC2: predicted neo-epitopes, naturally presented WT peptides of 9-10 AA not part of APVAC1 as 2nd choice; 10 applications in median (n=11 patients) | I, single-arm n=15 newly diagnosed GBM (HLA-A*02:01+ / -A*24:02+) | APVAC1: 12/13 patients sustained CD8+ central memory responses; functional T cells (killing assay) APVAC2: 8/10 neo-epitope-specific multifunctional Th1 responses; 1/6 WT antigens immunogenic | 29 mo median OS, 14.2 mo median PFS | Mild-to-moderate injection site reactions; n=2 anaphylactic reaction; n=1 brain edema |

| Treatment Reference | Therapeutic schedule | Phase # Patients | Immuno-genicity | Outcome | Adverse events |
|---|---|---|---|---|---|
| **NOA-16**[344]: IDH1R132H (20 AA) | Peptide in Montanide s.c. + topical Imiquimod Group 1: 4-6 weeks post radiotherapy Group 2: at day 10 of 4th TMZ cycle Group 3: post radiochemotherapy at day 10 of 1st maintenance TMZ cycle 8 vaccinations (completed by n=29) | I, completed n=32 IDH1R132H+ newly diagnosed grade III-IV astrocytoma | 24/30 patients IDH1R132H-specific T cells 26/30 IDH1R132H-specific antibodies | n=4 PD; n=28 SD Planned: PFS | Grade 1 reactions, n=1 serious adverse event |
| **ACT IV**[37]: Rindopepimut (13 AA EGFRvIII peptide-KLH conjugate) | Following surgery and radio-chemotherapy Group 1: 500 µg rindopepi-mut + 150 µg GM-CSF i.d. Group 2: 100 µg KLH i.d. 2 priming doses (day 1 + 15), then monthly injections + maintenance TMZ until progression / intolerance | III EGFRvIII-expressing newly diagnosed GBM, group 1 n=369; group 2 n=372 | Robust anti-EGFRvIII response (humoral) | Group 1: 20.1 mo OS, 2-y survival 30% Group 2: 20.0 mo OS, 2-y survival 19% | Mild-to-moderate injection site reactions and single cases of rash (both groups); n=2 allergic reactions; n=1 fatal pulmonary thromboembolism |
| **PRiME**[347,375]: PEP-CMV (26 AA human pp65 peptide + CMV glycoprotein B conjugated to KLH) | PEP-CMV + Montanide (1:1) i.d. 3 doses every 2nd week, followed by monthly administration for a maximum of 10 y | I, ongoing n=30 recurrent MB or grade III-IV glioma | Planned: vaccine-specific T-cell and antibody responses | | |
| **Cancer vaccines: tumor cells and lysates** | | | | | |
| **ERC1671**[376]: autologous and allogeneic (3 GBM patients) whole inactivated tumor cells (1×10⁵-1×10⁶ cells) and lysates (1×10⁵-1×10⁶ cells) | Group 1: from 1 mo post-surgery monthly ERC1671 + 500 µg GM-CSF i.d., 50 mg / day oral cyclophosphamide for 4 days (beginning of every cycle) + 10 mg/kg bevacizumab every 2nd week Group 2: placebo vaccination + oral placebo, bevacizumab as group 1 | II, randomized recurrent GBM Group 1: n=5 Group 2: n=4 | Group 1: CD4⁺ T-cell numbers correlate with OS | 12.1/7.6 mo median OS 7.3/5.4 mo median PFS | n=4/8 grade 3 adverse events |
| **Autologous formalin-fixed tumor**[377] | 1 mo after surgery and radio-chemotherapy, 1 vaccination/week (5 i.d. injections each) + 500 ng tuberculin (1:1 soluble and microparticles), maintenance TMZ | I/IIa n=24 primary GBM | n=23 tested patients: DTH response to autologous GBM tissue induced by vaccination | 8.2 mo median PFS 22.2 mo median OS n=9 with strong DTH response: 29.5 mo median PFS | n=19/1 grade 1/2 injection site reactions n=3 fever, n=2 headache, n=2 seizures, n=1 appetite loss |
| **Autologous tumor cell vaccine, stem cell transplantation and xALT**[378] | Post-surgery induction chemotherapy i.v., tumor cell vaccine + GM-CSF every 2nd week (up to 5 doses) s.c., high-dose chemotherapy i.v., autologous stem cell transplantation, ~3 mo later autologous lymphocytes i.v. + 5 doses IL-2 every 2nd day | II, completed n=30 recurrent / refractory primary high-grade brain tumors | Planned: deconvolute cellular antitumor response | | |
| **Cancer vaccines: DCs** | | | | | |
| **DCVax®-L**[379]: autologous DCs pulsed with autologous tumor lysate | Post-surgery + radiochemotherapy, experimental treatment (day 0/10/20, then mo 2/4/8, from mo 12 half-yearly) + maintenance TMZ 150-200 mg/m² for 5 days / mo Group 1: DCVax®-L 2.5×10⁶ DCs i.d. Group 2: placebo PBMCs Cross-over: upon progression, DCVax®-L for patients from group 2 | III, cross-over design newly diagnosed GBM Group 1: n=232 Group 2: n=99 Cross-over: n=54 | | 23.1 mo median OS (both groups incl. cross-over patients) | Grade 3-4 reactions: n=3 cerebral edema, n=2 seizures, n=1 lymph gland infection |

| Treatment Reference | Therapeutic schedule | Phase # Patients | Immuno-genicity | Outcome | Adverse events |
|---|---|---|---|---|---|
| **Audencel**[380,381]: autologous DCs loaded with autologous tumor lysate | Group 1: 60 Gy radiation (2 Gy/fraction) + 75 mg/m$^2$ TMZ, adjuvant TMZ 150-200 mg/m$^2$ for 5 days / mo Group 2: radiochemotherapy as group 1, from week 3 of adjuvant TMZ n=3 weekly, then monthly i.n. vaccinations of 1-5×10$^6$ DCs (for 7 mo in median), 3-mo intervals until vaccines were used up | II, randomized newly diagnosed GBM Group 1: n=42 Group 2: n=34 | Enhanced Th1 anti-tumor immune responses upon vaccination | 28.4%/24.5% 12 mo-PFS 18.9/18.8 mo median OS | n=7 flu-like symptoms, n=6 injection site reactions |
| **Autologous DCs** pulsed with **HLA-A*01-/A*02-restricted peptides** derived from Her2, TRP-2, gp100, MAGE-1, AIM-2, IL13Rα2[334] | Post-surgery + radiochemotherapy I.d. injection every 2$^{nd}$ week, n=3 vaccinations | I HLA-A*01$^+$/-A*02$^+$ patients with confirmed expression of ≥ 3 TAAs n=17 primary GBM, n=3 recurrent GBM, n=1 BSG | 33% of primary GBM respond (≥ 1.5-fold increased IFN-γ secretion by peptide-stimulated PBMCs after vaccination) | Primary GBM: 16.9 mo median PFS, 38.4 mo median OS | n=9 grade 1, n=2 grade 2 toxicities |
| **Autologous DCs** loaded with an **allogeneic brain tumor stem cell line**[382] | DCs i.d. + topical Imiquimod Group 1: 5×10$^6$ DCs Group 2: 10×10$^6$ DCs Group 3: 15×10$^6$ DCs 4 vaccinations every 2$^{nd}$ week + 10 monthly vaccinations | I, completed n=8 recurrent MB / GBM / ependymoma / AAST | | Planned: TTP | |
| **Autologous DCs** loaded with **autologous tumor lysate**[383] | Post-surgery, 0.25-11.9×10$^6$ DCs i.d. for 2 lymph node regions each + topical Imiquimod Group 1: 2 vaccinations every 2$^{nd}$ week, then monthly Group 2: 5 vaccinations every 2$^{nd}$ week, then monthly Group 3: 4 weekly vaccinations, monthly i.d. injection of tumor lysate (220-3,125 µg protein) Group 4: treatment as group 3, altered DC maturation | Feasibility study in children n=33 glioma, n=5 MB / PNET, n=4 ependymoma, n=3 ATRT | | MB / PNET: 5.7 mo median OS; 3.7 mo median PFS | Mild injection site reactions; n=8 fatigue, n=5 headache, n=3 fever, n=1 flu-like symptoms |
| **Adoptive T-cell transfer** | | | | | |
| **IL13Rα2-specific CAR-T cells**[384,385]: autologous T cells transduced with IL13Rα2-specific, 4-1BB-costimulatory CAR and CD19t | 3 weekly intracavitary / intratumoral / intraventricular infusions, continued until product is used up Case report: 6 intracavitary + 10 intraventricular infusions of 2-10×10$^6$ CAR-T cells | I n=92 recurrent / refractory IL13Rα2$^+$ GBM Case report: n=1 recurrent multifocal GBM | Planned: cytokine, CAR-T-cell, TIL levels Case report: Increased cytokine and immune cell levels in CSF | Planned: PFS, OS, RR Case report: regression of all foci for 7.5 mo (upon intraventricular administration), possible IL13Rα2-low recurrence | Case report: grade 1-2 headache, fatigue, myalgia, olfactory auras |
| **Her2-specific CAR-T cells**[333]: autologous EBV-, adenovirus-, and CMV-specific CD4$^+$ and CD8$^+$ T cells (optimized persistence) transduced with Her2-specific CAR | 1×10$^6$ – 1×10$^8$ CAR-T cells/m$^2$ i.v. n=11 1 infusion, n=6 multiple infusions | I n=17 progressive / recurrent Her2$^+$ GBM | CAR-T-cell persistence for ≥ 6 weeks and ≤ 18 mo in 7/15 patients | n=1 PR; n=7 SD; n=8 PD 11.1 mo median OS from 1$^{st}$ infusion 24.5 mo median OS from diagnosis | n=2 grade 2 seizures, n=1 grade 2 headache |
| **ALECSAT**[386]: autologous CTLs and NK cells | 3 i.v. infusions of 10×10$^6$-1×10$^9$ activated and expanded cells | I, completed n=23 recurrent GBM | | Planned: RR | |

| Treatment Reference | Therapeutic schedule | Phase # Patients | Immuno-genicity | Outcome | Adverse events |
|---|---|---|---|---|---|
| **Re-MATCH[387]**: TTRNA-loaded xALT and TTRNA-loaded autologous DCs | Following myeloablative chemotherapy 1 dose TTRNA-xALT i.v. ($3\times10^7$/kg) 3 doses TTRNA-DCs i.d. ($1\times10^7$; every 2nd week) | I/II, ongoing I: n=9 II: n=35 Recurrent MB, PNET | Planned: T-cell and anti-body response, cytokine profile, TLR activation, lymphocyte subset phenotypes | Planned: PFS, OS, RR | |
| **Her2-specific CAR-T cells[353]**: autologous CD4+ and CD8+ T cells transduced with Her2-specific CAR and EGFRt | 2 cycles of 1 weekly infusion for 3 weeks each, 1 week off between cycles Group 1: tumor cavity infusion Group 2: ventricular infusion | I, ongoing n=36 children / adolescents with Her2+ recurrent / refractory CNS tumors | | Planned: RR | |
| **EGFR806-specific CAR-T cells[356]**: autologous CD4+ and CD8+ T cells transduced with EGFR-specific CAR and EGFRt | Intra-patient dose escalation supratentorial tumors: tumor cavity infusion infratentorial / leptomeningeal tumors: ventricular infusion | I, ongoing n=36 children / adolescents with EGFR+ recurrent / refractory CNS tumors | | Planned: RR | |
| **NY-ESO-1-specific T cells[361]**: autologous T cells transduced with TCR recognizing HLA-A*02:01-presented NY-ESO-1 | Conditioning non-myeloablative lymphodepleting chemotherapy, 7 days later T cells + IL-2 720,000 IU/kg i.v. every 8 h for max. 5 days | II, ongoing NY-ESO-1+ HLA-A*02:01+ recurrent / refractory MNG, melanoma, breast / non-small cell lung / hepatocellular cancer | | Planned: clinical response | |
| **GRm13Z40-2 CAR-T cells[271,363]**: allogeneic glucocorticoid-resistant ganciclovir-sensitive CTL line expressing IL-13-Zetakine (IL13Rα2-specific CAR) and HyTK | Bi-weekly i.t. infusion (day 1, 3) + IL-2 i.t. (day 1/2-5) until progression / toxicity ± dexamethasone | I, completed Recurrent / refractory brain tumors | Planned: anti-GRm13Z40-2 responses (allograft rejection) | | |
| **Oncolytic viruses** | | | | | |
| **ParvOryx01[320,388]**: replication-competent parvovirus H-1 | Group 1: 1 i.t. injection (50% of total dose), 10 days later tumor resection + virus application (remaining 50%) in resection cavity Group 2: 5 i.v. infusions (10% of total dose each), 2nd dose as group 1 Dose escalation: $1\times10^6$-$1\times10^9$ pfu total dose in both groups | I/IIa Recurrent / refractory GBM Group 1: n=12 Group 2: n=6 | n=18 dose-dependent anti-viral antibodies, n=9 (of 12 tested patients) anti-viral CD8+ T-cell responses n=3 (of 6 tested patients) anti-tumor T-cell response | n=12 PD 27% 6-mo PFS, 3.7 mo median PFS 15.5 mo median OS | n=1 consciousness upon administration |
| **G207[389]**: oncolytic herpes simplex 1 virus | $1.15\times10^9$ pfu split into 1 pre- (2-5 days) and 1 post-surgery dose | Ib n=6 recurrent GBM | n=5 virus-infected tumor cells n=4 increased CD3+ TIL numbers | 3 mo median PFS 23 mo median OS from primary diagnosis 6.6 mo median OS from administration | n=2 seizure, n=2 hemiparesis, n=2 fever, n=1 somnolence, n=1 motor neuropathy, n=1 neglect, n=1 decreased mental status |

| Treatment Reference | Therapeutic schedule | Phase # Patients | Immuno-genicity | Outcome | Adverse events |
|---|---|---|---|---|---|
| **Delta-24-RGD**[390]: replication-competent adenovirus | Intracerebral infusion of $10^7$ / $10^8$ / $10^9$ / $10^{10}$ / $3\times10^{10}$ / $10^{11}$ virus particles | I/II n=30 recurrent GBM n=3 per dose level + n=6-9 after dose escalation treated with $10^{11}$ virus particles | | Planned: PFS, 6mo-PFS, OS, 6mo-OS, 12mo-OS | |
| **G207**[391]: oncolytic herpes simplex 1 virus | 1st cohort: 1 intracranial infusion (close to tumor site) 2nd cohort: 1 intracranial infusion + 5 Gy after 24 h | I, ongoing n=15 children with recurrent / refractory cerebellar brain tumors | Planned: HSV-1 antibody titers | Planned: PFS, OS, performance status | |
| **PVSRIPO**[392]: oncolytic polio-rhinovirus recombinant | 1 i.t. infusion | Ib, ongoing n=12 children / adolescents with recurrent supratentorial grade III glioma / GBM / MB / ATRT | | Planned: 24mo-OS | |

# 3   Immunopeptidomics

## 3.1   The immunopeptidome

The entirety of peptides presented by the HLA molecules of a cell or tissue is designated as the immunopeptidome, HLA ligandome, or HLA peptidome. Isolating these HLA ligands and employing subsequent liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS) is a way to comprehensively view the antigenic signature of human neoplasms. Here, mass spectrometry (MS) represents the central component warranting unbiased peptide identification. In contrast to epitope prediction-based studies, the major strength of immunopeptidomics as approach to define candidate targets for cancer immunotherapies is focusing on those peptides that are naturally presented and thus of pathophysiological relevance.[177] Target discovery approaches based on genome or transcriptome sequencing with subsequent *in silico* prediction of HLA ligands yield a considerable number of false positives while missing others. This is reasoned by the poor stoichiometric relationship between RNA transcripts and HLA-presented peptides. The immunopeptidome represents an autonomous layer strongly influenced by the antigen processing machinery and can thus not be expected to mirror the proteome or even the transcriptome.[177,393-396] Moreover, it has become evident that not the entire antigen translates into HLA ligands, but mainly so-called hotspot regions give rise to HLA-presented peptides thus shaping the immunopeptidome.[177,397-400]

As shown e.g. for renal cell and epithelial ovarian carcinoma, glioblastoma, acute myeloid and chronic lymphocytic leukemia, or multiple myeloma, downregulation of surface HLA expression by tumors is far less common than originally expected and should thus not be designated a hallmark of cancer immune evasion anymore.[149,177,315,401-405] For immunopeptidomics, primary human samples clearly outcompete artificial systems, especially permanent monoclonal cell lines, as they substantially more accurately reflect the biology of human malignancies, the genetical diversity among patients, and provide a high degree of translationability into individualized treatment attempts.[177] Analyses of the HLA peptidome can be performed with bulk or dissociated tumor tissue,[149,401] primary cell lines established from solid tumors,[406] leukemia cells,[403-405] cancer cell-derived soluble HLA molecules,[407] or extracellular vesicles as starting material.[177,408] Immunopeptidomics can in principle cover all sources of HLA ligands and associated PTMs, whereby the majority of HLA ligands originates from WT canonical proteins. Cryptic peptides (2.3.1) were estimated to contribute with 6.5-13% to the entirety of HLA ligands.[177,179] Proving neo-antigenic peptides to be naturally presented on cancer tissue by LC-MS/MS, has remained a major obstacle.[177,409] The proportion of neo-antigenic HLA ligands is expected to be even smaller than that of cryptic ones, with only one mutated peptide being on average identified among $1.1 \times 10^4$ HLA class I peptides and from 1,800 non-synonymous somatic mutations.[177]

In recent years, the concomitant development of orbitrap mass analyzers, chromatographic systems with enhanced performance, and algorithms for automated spectra interpretation have vastly improved speed, sensitivity, resolution, and mass accuracy of data acquisition and/or data analysis. Altogether, this enabled the fast ascent of immunopeptidomics as method of choice for state-of-the-art antigen discovery studies.[177] Nowadays, it is possible to extract and identify several thousands of unique HLA ligands from a single primary tissue sample.[397,401,410] Four immunopeptidomic studies in glioblastoma have been performed, whereby not the entire datasets have been made publicly available.[149,313,402,403] The published content of one study was even restricted to those HLA-A*02:01, -A*24:02, and -DR-presented peptides applied in a clinical trial of peptide vaccination (GAPVAC-101).[314] Two of the remaining datasets were acquired on a time of flight mass spectrometer (Q-TOF) or an LTQ Orbitrap XL, allowing the presumption that peptide identifications will increase when using another device such as the Orbitrap Fusion Lumos with higher resolution and scan rate. Moreover the number of primary samples came up to only four and nine, respectively.[149,411] The fourth publication focuses on comparing the immunopeptidome of ten glioblastoma tissues to that of 142 samples of soluble HLA molecules obtained from 52 glioblastoma patients as well as 30 ankylosing spondylitis patients and 6 healthy donors as control. These data were also searched for known CTAs, however, tissue samples were part of the same cohort as used for the GAPVAC-101 project.[314,396] So far, no study investigating the immunopeptidome of medulloblastoma has been published. HLA class I ligands have been isolated from twelve primary meningioma samples. These data were part of a training dataset (n=2) and used as benchmark dataset (n=10) for a model predicting length distribution and binding specificity across HLA class I allotypes and not reviewed for potential targets for cancer immunotherapy.[412]

## 3.2 Materials and methods for immunopeptidome analyses

### 3.2.1 Materials

Substantial parts of the material lists presented on the following pages are congruent with listings in 'Freudenmann LK. Mapping the HLA Ligandome of Primary *versus* Recurrent Disease in Glioblastoma Multiforme by Mass Spectrometry'[10].

### *Chemicals*

| | |
|---|---|
| Acetonitrile $CH_3CN$ (AcN; MS grade) | J.T.Baker®, Center Valley (USA) |
| Baker water $H_2O$ (MS grade) | J.T.Baker®, Center Valley (USA) |
| CHAPS 3-[(3-cholamidopropyl) dimethyl-ammonio]-1-propanesulfonate 1.2% (w/v) | PanReac AppliChem, Darmstadt (Germany) |
| Cyanogen bromide (CNBr)-activated sepharose 4B | GE Healthcare, Little Chalfont (UK) |
| Dimethyl sulfoxide (DMSO) $(CH_3)_2SO$ | Merck Millipore, Billerica (USA) |
| Dulbecco's phosphate-buffered saline (DPBS) without $CaCl_2$ and $MgCl_2$ | Gibco™ / Thermo Fisher Scientific, Waltham (USA) |
| Formic acid (FA; MS grade) | Merck, Darmstadt (Germany) |
| Glycine | Merck, Darmstadt (Germany) |
| Hydrogen chloride HCl | Carl Roth, Karlsruhe (Germany) |
| Phosphate-buffered saline (PBS) without $CaCl_2$ and $MgCl_2$ | Produced in-house by Claudia Falkenburger |
| Protease inhibitor cocktail tablets | cOmplete™ / Roche, Basel (Switzerland) |
| Sodium bicarbonate $NaHCO_3$ | Merck Millipore, Billerica (USA) |
| Sodium chloride NaCl | VWR Chemicals / VWR International, Leuven (Belgium) |
| Sodium hydroxide NaOH | Carl Roth, Karlsruhe (Germany) |
| Trifluoroacetic acid $C_2HF_3O_2$ (TFA; MS grade) | Sigma-Aldrich, St. Louis (USA) |

### *Solutions and buffers*

| | |
|---|---|
| A* | 0.1% TFA in Baker $H_2O$ |
| $AB_E$ | 32.5% AcN / 0.2% TFA in Baker $H_2O$ |
| $A_{Load}$ | 1% AcN / 0.05% TFA in Baker $H_2O$ |
| Coupling buffer | 0.5 M NaCl / 0.1 M $NaHCO_3$ in $ddH_2O$ adjusted to pH 8.3 with HCl (at room temperature) |
| Lysis buffer (1×) | 2× lysis buffer / PBS mixed 1:1 |
| Lysis buffer (2×) | 33 ml PBS / 0.4 g CHAPS / 1 protease inhibitor cocktail tablet |

### *Antibodies*

**Table 3. HLA class I- or II-specific murine monoclonal antibodies employed for HLA-immunoprecipitation.** All antibodies were kindly produced in-house by Claudia Falkenburger using murine B lymphocyte hybridoma cells.

| Clone | Isotype | Specificity | Reference |
|---|---|---|---|
| W6/32 (ATCC® HB-95™) | $IgG_{2\alpha}$ | HLA-A, -B, -C | [413] |
| L243 (ATCC® HB-55™) | $IgG_{2\alpha}$ | HLA-DR | [414] |
| Tü39 | $IgG_{2\alpha}$ | HLA-DP, -DQ, -DR | [415] |

## *Consumable supplies*

| | |
|---|---|
| Acclaim® C18 PepMap RSLC column 50 µm × 25 cm | Thermo Fisher Scientific, Waltham (USA) |
| Acclaim® C18 PepMap RSLC column 75 µm × 2 cm | Thermo Fisher Scientific, Waltham (USA) |
| Amicon-Ultra 0.5 ml centrifugal filter unit 10 kDa | Merck Millipore, Billerica (USA) |
| Amicon-Ultra 0.5 ml centrifugal filter unit 3 kDa | Merck Millipore, Billerica (USA) |
| DeckWorks® low binding pipet tips 1-200 µl | Corning, Corning (USA) |
| Eppendorf adapter for rotors with 15 ml bore holes (allows using 5 ml conical tubes) | Eppendorf, Hamburg (Germany) |
| Eppendorf Safe-Lock tubes 1.5 ml | Eppendorf, Hamburg (Germany) |
| Eppendorf Safe-Lock tubes 2 ml | Eppendorf, Hamburg (Germany) |
| Eppendorf screw cap tubes 5 ml | Eppendorf, Hamburg (Germany) |
| Falcon tubes 15 ml | Greiner Bio-One, Frickenhausen (Germany) |
| Falcon tubes 50 ml | Greiner Bio-One, Frickenhausen (Germany) |
| Maxymum Recovery® universal filter tips 1000 µl | Axygen, Union City (USA) |
| Maxymum Recovery® universal tips 0.5-10 µl | Axygen, Union City (USA) |
| Millex®-SV low protein binding PVDF Durapore® syringe filter unit 5.0 µm | Merck Millipore, Billerica (USA) |
| Petri dishes ø 30 mm | Greiner Bio-One, Frickenhausen (Germany) |
| Petri dishes ø 60 mm | Greiner Bio-One, Frickenhausen (Germany) |
| Polypropylene autosampler vials 250 µl | Thermo Fisher Scientific, Waltham (USA) |
| Polypropylene caps for 250 µl vials | Thermo Fisher Scientific, Waltham (USA) |
| Protein LoBind tubes 1.5 ml | Eppendorf, Hamburg (Germany) |
| Protein LoBind tubes 2 ml | Eppendorf, Hamburg (Germany) |
| SafeSeal® SurPhob filter tips 1250 µl | Biozym Scientific, Hessisch Oldendorf (Germany) |
| Surgical disposable scalpels | Aesculap AG, Tuttlingen (Germany) / B. Braun Melsungen AG, Melsungen (Germany) |
| Syringe Luer-Lok™ Tip 20 ml | Becton Dickinson, Drogheda (Ireland) |
| Syringe Luer-Lok™ Tip 30 ml | Becton Dickinson, Drogheda (Ireland) |
| Syringe Luer-Lok™ Tip 50 ml | Becton Dickinson, Drogheda (Ireland) |
| ZipTip$_{C18}$® Pipette Tips with 0.6 µl resin bed volume | Merck Millipore, Billerica (USA) |

## *Basic equipment*

| | |
|---|---|
| Double socket, 13 mm span | neoLab Migge, Heidelberg (Germany) |
| Econo-Column® low pressure glass chromatography columns, 0.5 cm × 5 cm | Bio-Rad, München (Germany) |
| Econo-Column® low pressure glass chromatography columns, 1 cm × 5 cm | Bio-Rad, München (Germany) |
| Hamilton syringe 50 µl | Sigma-Aldrich, St. Louis (USA) |
| Hamilton syringe 500 µl | Sigma-Aldrich, St. Louis (USA) |
| Lyophilization glass 600 ml, 105 mm outer diameter | Martin Christ Gefriertrocknungsanlagen, Osterode am Harz (Germany) |
| Micro pestle for 1.5 ml Eppendorf tubes | Carl Roth, Karlsruhe (Germany) |
| Polypropylene Luer adapters with 1/8″ lock rings and 1/8″ teflon tubing | Cole-Parmer, Vernon Hills (USA) |
| Potter glass | Wheaton, Milville (USA) |

| | |
|---|---|
| Rotilabo® tripod mount, 250 mm x 142 mm, M10 threaded hole | Carl Roth, Karlsruhe (Germany) |
| Rotilabo® tripod rod, 600 mm, M10 thread | Carl Roth, Karlsruhe (Germany) |
| Two-way stopcocks with female to male Luer fitting | Bio-Rad, München (Germany) |
| Tygon® 3350 2 stopper 104 mm distance, 2.06 mm inner diameter, 381 mm silicone tube | Hirschmann, Neckartenzlingen (Germany) |
| Tygon® 3350, 2.4 mm inner diameter, 0.8 mm wall thickness, 15 m silicone tube | neoLab Migge, Heidelberg (Germany) |
| Universal stand clamp Remanit 4301, 240 mm length, 90 mm span | Carl Roth, Karlsruhe (Germany) |

## *Devices*

| | |
|---|---|
| Autosampler WPS-3000PL (RS) UltiMate™ 3000 Series | Dionex, Sunnyvale (USA) |
| Biofuge Pico centrifuge | Heraeus, Pforzheim (Germany) |
| Branson sonifier 250 | Emerson Industrial Automation, Danbury (USA) |
| Chiller ThermoFlex 900 | Thermo Fisher Scientific, Waltham (USA) |
| DURAN® beaker glass 1000 ml | Schott, Wertheim (Germany) |
| E2M28 rotary vacuum pump | Edwards, Feldkirchen (Germany) |
| Freeze dryer VaCo 2 | ZIRBUS technology, Bad Grund (Germany) |
| IKA® KS 250 basic shaker | Sigma-Aldrich, St. Louis (USA) |
| IKA® MS 3 basic vortex mixer | Sigma-Aldrich, St. Louis (USA) |
| KF-2-110 cold trap | H. Saur Laborbedarf, Reutlingen (Germany) |
| LTQ Orbitrap XL | Thermo Fisher Scientific, Waltham (USA) |
| Megafuge 1.0 R centrifuge | Heraeus, Pforzheim (Germany) |
| MIKRO 200 R centrifuge | Andreas Hettich, Tuttlingen (Germany) |
| Nano/Cap System NCS-2500RS UltiMate™ 3000 Series | Dionex, Sunnyvale (USA) |
| Nano/Pump System NCP-3200RS UltiMate™ 3000 Series | Dionex, Sunnyvale (USA) |
| Nanospray Flex™ ion source | Thermo Fisher Scientific, Waltham (USA) |
| Nanospray ion source | Thermo Fisher Scientific, Waltham (USA) |
| Orbitrap Fusion Lumos | Thermo Fisher Scientific, Waltham (USA) |
| Potter-Elvehjem tissue homogenizer | Omni International, Kennesaw (USA) |
| RC6 chemistry-HYBRID-vacuum-pump | vacuubrand, Wertheim (Germany) |
| rotarus® smart 30 peristaltic pump | Hirschmann, Neckartenzlingen (Germany) |
| RV8 oil-sealed rotary high vacuum pump | Edwards, Feldkirchen (Germany) |
| Sogevac® single-stage, oil-sealed rotary vane pump SV 65 BIFC | oerlikon leybold vacuum, Köln (Germany) |
| Solvent Rack SRD-3x00 UltiMate™ 3000 Series | Dionex, Sunnyvale (USA) |
| SpeedVac vacuum concentrator | BACHOFER, Reutlingen (Germany) |
| Tube rotator L28 | LABINCO, Breda (Netherlands) |
| Ultra-low freezer DF8517GL | Skadi, Wilmington (USA) |
| Ultrasonic cleaner | JSP, Los Angeles (USA) |
| VM-300 vortex mixer | neoLab Migge, Heidelberg (Germany) |

## *Software and databases*

| | |
|---|---|
| Allele Frequency Net Database | [416] |
| area_picker.R (2015) | In-house R script, written by Linus Backert |
| BioVenn | [417] |
| Catalogue of Somatic Mutations in Cancer (COSMIC) | [418] |
| Chromeleon™ 6.80 Chromatography Data System | Thermo Fisher Scientific, Waltham (USA) |
| DAVID Bioinformatics Resources 6.8 | [419,420] |
| DCMS Link 2.14 for Xcalibur | Thermo Fisher Scientific, Waltham (USA) |
| DB Browser for SQLite 3.11.2 | Open-source software |
| dbSNP | [421] |
| Discoverer Daemon 1.4 | Thermo Fisher Scientific, Waltham (USA) |
| GraphPad Prism 6.0 | GraphPad Software, La Jolla (USA) |
| Genotype-Tissue Expression (GTEx) database | [422] |
| HLA-C motifs | [423] |
| Hotspots.R (2018) | In-house R script, written by Leon Bichmann |
| Inkscape 0.92.4 | Open-source software |
| Immune Epitope Database (IEDB): population coverage | [424] |
| In-house HLA class I SQL database (version 2019/04/25) | Benign primary samples (n=418):<br>n=10 adrenal glands, 10 aortae, 98 peripheral blood samples, 27 bone marrows, 12 brains, 11 cerebella, 8 colons, 10 esophagi, 4 gall bladders, 11 hearts, 12 kidneys, 14 livers, 14 lungs, 17 lymph nodes, 5 mammae, 10 skeletal muscles, 31 ovaries, 9 pancreases, 18 prostates, 11 skins, 12 small intestines, 1 spinal cord, 12 spleens, 10 stomachs, 13 thyroid glands, 9 tongues, 7 tracheae, 10 urinary bladders, 2 uteri<br><br>Malignant primary samples (n=874):<br>n=4 acute lymphocytic leukemias, 97 acute myeloid leukemias, 1 anaplastic astrocytoma, 10 atypical teratoid rhabdoid tumors, 3 bladder cancers, 59 breast cancers, 98 chronic lymphocytic leukemias, 21 chronic myeloid leukemias, 38 colorectal cancers, 23 ependymomas, 6 esophageal cancers, 4 fibroses, 30 gastric cancers, 1 gastrointestinal stromal tumor, 2 germ cell tumors, 50 glioblastomas, 28 hepatocellular carcinomas, 1 leiomyosarcoma, 1 melanoma, 33 meningiomas, 1 merkel-cell carcinoma, 13 multiple myelomas, 24 neuroblastomas, 18 non-small cell lung carcinomas, 1 oligodendroglioma, 28 oropharyngeal squamous cell carcinomas, 3 osteosarcomas, 139 ovarian carcinomas, 1 pancreatic cancer, 1 paraganglioma, 1 parotis carcinoma, 13 polycythemia veras, 8 primary myelofibroses, 28 prostate carcinomas, 79 renal cell carcinomas, 1 sarcoma, 5 subependymomas<br>The number of leukemia samples includes few genetic duplicates due to multiple HLA ligand isolations from sorted cell populations or treated cells |

| | |
|---|---|
| In-house HLA class II SQL database (version 2019/04/25) | Benign primary samples (n=364): n=10 adrenal glands, 10 aortae, 85 peripheral blood samples, 23 bone marrows, 12 brains, 11 cerebella, 8 colons, 10 esophagi, 4 gall bladders, 11 hearts, 13 kidneys, 13 livers, 14 lungs, 17 lymph nodes, 5 mammae, 10 skeletal muscles, 2 ovaries, 9 pancreases, 6 prostates, 12 skins, 12 small intestines, 1 spinal cord, 12 spleens, 9 stomachs, 4 thymi, 13 thyroid glands, 9 tongues, 7 tracheae, 10 urinary bladders, 2 uteri |
| | Malignant primary samples (n=626): n=2 acute lymphocytic leukemias, 125 acute myeloid leukemias, 1 anaplastic astrocytoma, 3 bladder cancers, 59 breast cancers, 69 chronic lymphocytic leukemias, 20 chronic myeloid leukemias, 2 colorectal cancers, 23 ependymomas, 4 fibroses, 41 glioblastomas, 4 hepatocellular carcinomas, 1 melanoma, 33 meningiomas, 13 multiple myelomas, 16 neuroblastomas, 18 non-small cell lung carcinomas, 1 oligodendroglioma, 28 oropharyngeal squamous cell carcinomas, 3 osteosarcomas, 78 ovarian carcinomas, 1 pancreatic cancer, 1 paraganglioma, 1 parotis carcinoma, 11 polycythemia veras, 8 primary myelofibroses, 54 renal cell carcinomas, 1 sarcoma, 5 subependymomas The number of leukemia samples includes few genetic duplicates due to multiple HLA ligand isolations from sorted cell populations or treated cells |
| jVenn | [425] |
| Ligandosphere | In-house software |
| List of 366 established TAAs and CTAs collected and translated into UniProt accessions by Lena Mühlenbruch and Michael Ghosh from the following resources: Cancer-related proteins deposited in UniProt (search term: disease:cancer AND reviewed:yes AND organism:"Homo sapiens (Human) [9606]"; 2017/09/01) | [86] |
| Cheever *et al.* (2009) | [426] |
| CTDatabase (2019/08/16) | [427] |
| Cancer Immunity Peptide Database (version: 2016) | [191] |
| LTQ Tune Plus 2.5.5 SP2 | Thermo Fisher Scientific, Waltham (USA) |
| MHC Motif Viewer | [428] |
| Microsoft Office 2010 and Professional Plus 2016 | Microsoft Corporation, Redmond (USA) |
| NetMHC 4.0 Motif Viewer | [429] |
| NetMHCpan-4.0 | [430-432] |
| Notepad++ v7.8.1 | Open-source software |
| Orbitrap Fusion Lumos Tune Application 2.1.1565.23 | Thermo Fisher Scientific, Waltham (USA) |
| Percolator 2.04 (2012) | Department of Genome Science at the University of Washington, Seattle (USA) |
| PhosphoMotif Finder of the Human Protein Reference Database | [433] |
| Proteome Discoverer 1.4.1.14 | Thermo Fisher Scientific, Waltham (USA) |
| replicate_merger.R (2016) | In-house R script, written by Linus Backert |
| RStudio 3.4.2 | Open-source software |

| | |
|---|---|
| saturation_analysis.R (2016) | In-house R script, written by Linus Backert |
| Scintilla and SciTE | Open-source software |
| SEQUEST search engine | [434] |
| Scripts for complex SQL queries:<br>sql_protein_benign_counter_classI_groupedtissue.R (2017)<br>sql_protein_benign_counter_classII_groupedtissue.R (2017)<br>sql_protein_counter_malignant_classI.R (2017)<br>sql_protein_counter_malignant_classII.R (2017)<br>sql_sequence_counter_benign_classI_exactMatch_case-insensitive.R (2018)<br>sql_sequence_counter_benign_classII_exactMatch_case-insensitive.R (2018)<br>sql_sequence_counter_malignant_classI_exactMatch_case-insensitive.R (2018)<br>sql_sequence_counter_malignant_classII_exactMatch_case-insensitive.R (2018) | In-house R scripts, written by Leon Bichmann |
| Skyline | [435] |
| SYFPEITHI | [436] |
| UniProt | [86] |
| Venn Diagram Plotter 1.5.5228.29250 | Open-source software |
| volcano_plotter_from_area_all_conds_hardcoded_lim2_new_style.R (2015) | In-house R script, written by Linus Backert |
| Xcalibur 2.1.0.1160 with Foundation 1.0.2.65 | Thermo Fisher Scientific, Waltham (USA) |
| Xcalibur 2.2 SP1 with Foundation 2.0 SP1 | Thermo Fisher Scientific, Waltham (USA) |
| Xcalibur 4.0.27.10 with Foundation 3.1 SP1 | Thermo Fisher Scientific, Waltham (USA) |

## 3.2.2 Methods

Following the pioneering work of Kirsten Falk and Olaf Rötzschke in the laboratory of Hans-Georg Rammensee, the protocol underwent several optimization processes, but core components have been retained. In brief, the workflow is composed of seven steps: preparation of tissue or cell lysate, immunoaffinity chromatography to pull down HLA-peptide complexes, acidic elution of HLA molecules and their ligands, purification of HLA ligands, peptide identification by LC-MS/MS, and subsequent data analysis (Figure 7).[437,438] The major part of the protocol presented on the following pages resembles the methods section in 'Freudenmann LK. Mapping the HLA Ligandome of Primary *versus* Recurrent Disease in Glioblastoma Multiforme by Mass Spectrometry'[10].
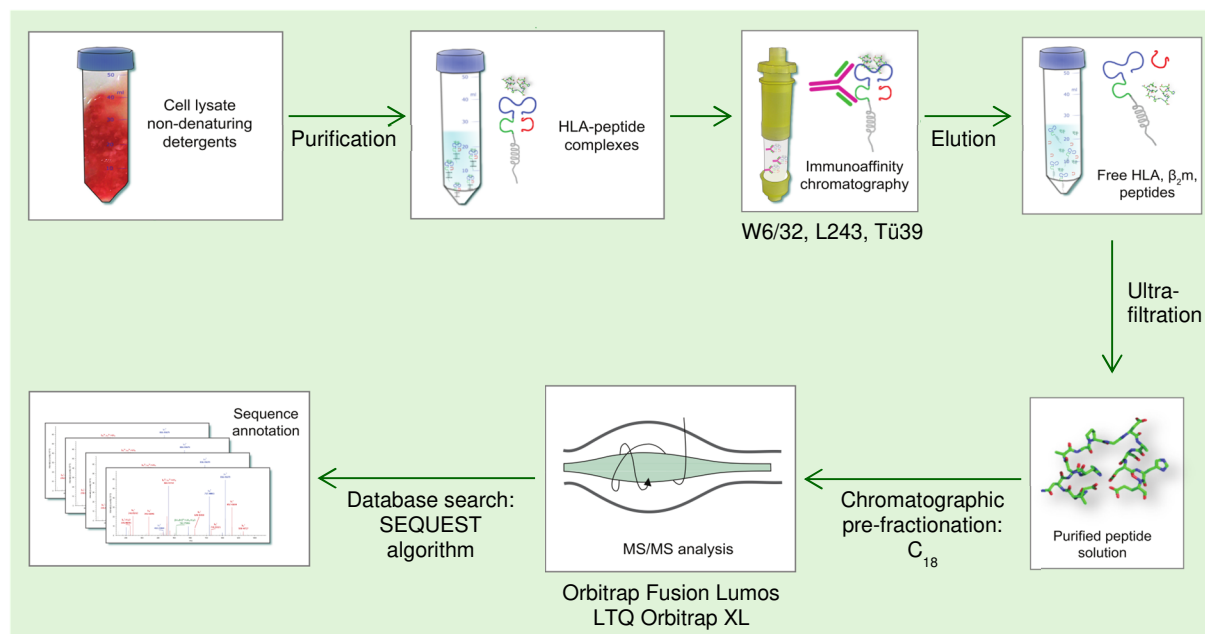
**Figure 7. Isolation and analysis of HLA ligands from intracranial neoplasias.** Here, icons depict schematics of HLA class I-peptide complexes. Modified from Freudenmann *et al.*[177]

### *Preparation of lysate from tumor tissue*

All steps for preparation of tissue lysate from fresh frozen surgical specimens were performed at 4°C in the cold room. The entire amount of tissue was never used up for immunopeptidomics alone in order to perform parallel analyses including whole exome (glioblastoma) and RNA sequencing (glioblastoma and medulloblastoma), DNA methylation profiling (planned for glioblastoma and medulloblastoma), and HLA typing (all samples) from the identical piece of tissue. As mentioned in 1.2, the degree of intratumoral heterogeneity is especially high in glioblastoma. In order to subject cells of every subclone – as far as possible – to every analytical method, tissue was chopped first and tiny pieces were then collected to either isolate DNA and RNA (≥ 20 mg) or HLA ligands. Since RNA degradation sets in as soon as tissue thaws, samples were kept on dry ice throughout almost the whole time and immediately returned to -80°C after tissue collection for sequencing until analysis at CeGaT GmbH (Tübingen).

Tissue for the preparation of cell lysate was weighed in appropriately sized petri dishes (30 or 60 mm in diameter). Having added one volume of 2× lysis buffer, tissue was chopped finely using forceps and scalpel. Tissue was transferred into a potter glass cleaned with 70% ethanol, $dH_2O$, and flushed out with PBS before. Two volumes of 1× lysis buffer each were used to rinse the petri dish twice and added to the potter glass as well. With a Potter-Elvehjem tissue homogenizer, samples were mashed for approximately 2 min, whereby the potter glass was kept on ice to avoid warming of the lysate. The latter was transferred into appropriately sized tubes (5 / 15 / 50 ml) and the potter glass was rinsed twice with two volumes of 1× lysis buffer each, that were subsequently added to the lysate. In case a substantial amount of sample was still left in the potter glass, another washing step – if necessary, with a slightly increased volume of lysis buffer – was performed. Samples were then incubated on the shaker at 200 revolutions per minute (rpm) for 1 h. To further disrupt cell membranes, five cycles of pulsed sonication (duty cycle output control = 5; 60% duty cycle) each lasting 20 s were applied to

the samples. To prevent lysates from warming, tubes were kept on ice and cool-down breaks of 20 s were performed after every cycle. After another incubation on the shaker for 1 h, samples were centrifuged at 4,000 rpm and 4°C for 1 h. For lysates prepared from ≥ 1 g of tissue, centrifugation of the supernatant was repeated once. Employing 5.0 µm low protein binding filter units, samples were subsequently sterile filtrated into appropriately sized tubes (5 / 15 / 50 ml).

For tissue samples weighing less than 150 mg, tissue was manually minced with a micro pestle in 1.5 ml Eppendorf tubes instead of using the Potter-Elvehjem tissue homogenizer. Sonification was performed in an ultrasonic bath for five times 20 s with cool-down breaks on ice of 20 s each in between. Moreover, these samples were spun down only once at 13,000 rpm and 4°C for 1 h. Lysates with a volume of less than 2 ml (corresponds to ~222 mg tissue) were not filtered before being directly injected into either only the upper column equipped with W6/32 antibodies or into both columns. The outflow of the lower column with HLA class II-specific antibodies was straightly transferred to the upper column instead of being collected in and aspirated from a Falcon tube in between.

### *Isolation of HLA ligands by immunoaffinity chromatography*

Immunoaffinity chromatography requires a solid phase, CNBr sepharose, to which HLA-specific antibodies are bound. Per 1 mg of antibody, 40 mg of CNBr-activated sepharose were weighed in and resuspended in 45 ml 1 mM HCl (in ddH$_2$O) by inverting for five times. At room temperature, the suspension was rotated for 1 h to be then spun down at 300 rpm and without break for 4 min. Having carefully decanted the supernatant, 20 ml coupling buffer and the respective antibody were added. Falcon tubes in which the antibodies had been stored were rinsed with ~10 ml of coupling buffer, added to the sepharose as well, and the total volume was adjusted to 45 ml with coupling buffer. After inverting for five times, tubes were rotated for 2 h and centrifuged as previously. The sepharose-antibody matrix was resuspended in 45 ml 0.2 M glycine (in ddH$_2$O) and rotated for 1 h to reduce unspecific binding activity. Sepharose was pelleted, washed twice with DPBS, adjusted with DPBS to a concentration of 1 mg antibody/40 mg sepharose per ml, and stored at 4°C. Newly coupled antibodies were only approved for patient samples upon passing quality control comprising isolation of HLA-peptide complexes from a standardized lysate (equivalent to $4.5 \times 10^7$ cells) prepared from the B-lymphoblastoid cell line JY.

Glass chromatography columns were cleaned thoroughly with 70% ethanol and afterwards with dH$_2$O by means of a 50 ml syringe. Before assembly of the chromatographic system in the cold room, possible sample residues were removed from peristaltic pumps, cassettes as well as tubes and the latter were examined for tightness. All cassettes mounted in the same peristaltic pump were manually adjusted to an equal flow rate at 10 rpm pump speed. For each sample, two columns were set up one above the other, so that the flowthrough of the upper column equipped with HLA class I-specific antibodies directly dropped into the lower column equipped with HLA class II-specific antibodies. By pipetting, sepharose-antibody matrices were resuspended and a minimum volume of 1 ml was added to each column. To precipitate HLA class II-peptide complexes, the antibodies L243 and Tü39 were mixed 1:1. For samples weighing more than 1 g, the amount of antibody was proportionally increased to an equivalent

of 1 mg per 1 g of tissue. Econo-Columns® with a diameter of 0.5 cm have a maximum capacity of 3 mg antibody-sepharose matrix so that columns with a diameter of 1 cm were used for samples above 3 g. The peristaltic pump was connected to the upper column equipped with W6/32 so that PBS flowed through both columns into a waste beaker glass. The liquid phase was changed to 1× lysis buffer and the system was subsequently cycled until preparation of tissue lysates had been finished.

Having linearized the system again, filtrated samples were aspirated from tubes and the system was cycled as soon as the lysate reached the lower column or before the tube ran dry, respectively. To manually load small samples, liquid was drained from either the upper or both chromatography columns and the lysate was directly pipetted onto the sepharose-antibody matrix. In order to prevent technical artefacts, samples subjected to relative quantitation of HLA ligands later on, were always run with the same peristaltic pump and under use of the identical antibody batches. Immunoaffinity chromatography was then performed overnight. To wash columns the next morning, PBS was pumped linearly through the system for 30 min followed by ddH$_2$O for 1 h. Having let columns run dry and washed the lids with ddH$_2$O in a beaker glass, 1 µl 10% TFA per 1 mg antibody and 100 µl 0.2% TFA were added with a 50 µl or a 500 µl Hamilton syringe, respectively. In case of large tissue samples, the amount of 0.2% TFA was increased until the entire antibody-sepharose matrix was covered. In a rack on the shaker, columns were incubated at 300 rpm and 4°C for 30 min. Using an air-filled 50 ml syringe, HLA molecules and peptides were eluted into 1.5 or 2 ml Protein LoBind Eppendorf tubes. This procedure was repeated for a total of four times, whereby incubation time on the shaker was reduced to 15 min and 0.2% TFA was used solely from the second to the fourth elution. Having completed the elution of HLA-peptide complexes, chromatography columns were cleaned thoroughly with dH$_2$O, 70% ethanol, and again with dH$_2$O using a 50 ml syringe.

### *Purification of peptides and preparation for mass spectrometry*

In parallel to washing the chromatography columns and later shaking them for 30 min, Amicon filter units to separate peptides from HLA molecules were prepared. HLA class I peptides were ultrafiltrated at a pore size of 3 kDa, whereas longer HLA class II peptides were purified at a pore size of 10 kDa. As leak test, filters were filled with 500 µl Baker H$_2$O and spun down at 13,000 rpm and room temperature for 7 min. Remaining volume inside all filters of the same pore size was then compared and filter units with significantly divergent fill level were replaced. After discarding the flowthrough and banging the filters upside down onto paper towels to empty them, four more washing steps were performed. Centrifugation time was increased to 15 min for all of them. Washing was performed by sequentially adding 500 µl 0.1 N NaOH, Baker H$_2$O, and 0.2% TFA. 500 µl 0.2% TFA were again pipetted into the filter unit and run through only upon the elution of HLA-peptide complexes had been completed.

Eluates were centrifuged at 13,000 rpm and 4°C for 5 min, before not more than 500 µl supernatant were filled into the respective filter inlay which had been placed into a new collection tube. Amicon filters were spun down, until the volume had completely run through and the flowthrough was collected in appropriately sized Protein LoBind Eppendorf tubes (1.5 / 2 ml). This was repeated until the entire amount of eluate had been ultrafiltrated. Predominantly to elute hydrophobic peptides from the membrane, filters were then washed

with 450 µl $AB_E$ which was subsequently added to the sample. An additional loose lid was perforated and placed on each Protein LoBind tube. These were frozen to the core at -80°C for at least 1 h. Peptide extracts were subsequently lyophilized to a target volume of ~30 µl.

Desalting of peptide solutions was achieved by reversed-phase liquid chromatography using $ZipTip_{C18}$® Pipette Tips.[439] During the entire procedure, a volume of 10 µl was repetitively aspirated and dispensed in different solvents as well as the sample, whereby it was strictly avoided to introduce any air into the $C_{18}$ matrix. First, the resin was cleansed by quickly pipetting up and down in ~500 µl $AB_E$ for ten times followed by equilibration in ~500 µl A* for ten pipetting cycles as well. Peptides contained in the respective sample, which had been centrifuged at 13,000 rpm and 4°C for 5 min, were bound to the $C_{18}$ matrix by slowly aspirating and dispensing for ten times. Desalting was achieved during three very slow pipetting cycles in another tube containing ~500 µl A*. Bound peptides were subsequently eluted from the resin by quickly pipetting up and down in an autosampler vial filled with 35 µl $AB_E$ for ten times. The steps from A*, *via* sample and A*, to the autosampler vial ($AB_E$) were repeated for another four times. Upon completion of five cycles, $ZipTip_{C18}$® Pipette Tips were cleansed in ~500 µl $AB_E$ as before and stored until next use. Samples planned to be compared by relative quantitation of HLA ligands (e.g. primary and recurrent tumor pairs or meningioma and autologous dura), were desalting using the identical $ZipTip_{C18}$® Pipette Tip in order to reduce technical variability. The hydrophobic solvent content of $AB_E$ was removed by reducing the volume to 1 to 5 µl in the SpeedVac and $A_{Load}$ was subsequently added to the desired target volume of 25 to 35 µl.

After establishment of direct injection, a method to subject peptide solutions to LC-MS/MS without prior purification *via* $ZipTip_{C18}$® Pipette Tips, this was done for every possible sample. Besides less cumbersome sample handling, the big advantage of direct injection is a reduction in possible technical artefacts and thus better reproducibility and comparability of samples. This is of particular importance when relative quantitation of peptides in two autologous samples (e.g. primary *versus* recurrence or tumor *versus* benign) is performed. Direct injection worked well for glioblastoma and medulloblastoma, but not for meningioma samples, since these tended to be sticky throughout the whole experiment and blocked – despite sterile filtration – not only the sepharose-antibody matrices during immunoaffinity chromatography, but also the trapping and separation columns of the liquid chromatography system. However, it should be noted that remnants of CHAPS might cause ion suppression during electrospray ionization and this method should therefore not be applied for big samples lysed with large amounts of this zwitterionic detergent.[440] For direct injections, samples lyophilized to ~30 µl were spun down at 13,000 rpm and 4°C for 10 min, supernatants were transferred into autosampler vials and reduced to 1 to 5 µl by vacuum centrifugation. $A_{Load}$ was added to the desired target volume of 25 to 35 µl.

Autosampler vials were stored at -80°C, thawed in an ultrasonic bath for 2.5 min, sublimated $A_{Load}$ was replenished, and air bubbles were removed thoroughly prior to LC-MS/MS.

## Peptide identification by LC-MS/MS and subsequent database search

### LC-MS/MS

Analysis of peptide extracts was performed by nanoflow high-performance liquid chromatography (Dionex UltiMate™ 3000 Series liquid chromatography system) and tandem mass spectrometry. For each tissue and HLA class, a minimum of three technical replicates consuming 5 µl sample and lasting 130 min each were acquired. During the first 10 min, sample was loaded onto the trapping column at 50°C and a flow rate of 4 µl/min, whereas it was gradually eluted from the separation column from min 10 to 100 at 50°C and 175 nl/min (LTQ Orbitrap XL) or 300 nl/min (Orbitrap Fusion Lumos), respectively. During this 90 min gradient, the proportion of hydrophobic solvent (NC pump solvent B) increased from to 3 to 40% (equivalent to 2.4 to 32.0% AcN). Between min 101 and 106, it mounted up to 95% to remove remaining sample from the column. Pre-fractionated peptides eluting from the separation column were introduced into an online-coupled tandem mass spectrometer, either the LTQ (linear trap quadrupole) Orbitrap XL (meningioma samples) or the Orbitrap Fusion Lumos (glioblastoma, medulloblastoma, and meningioma samples). Both devices were equipped with a nanospray ion source and were operated in data-dependent acquisition (DDA) mode. In a survey scan, masses of protonated precursor ions of a defined mass-to-charge ratio (m/z) were determined (MS$^1$ spectra) and the 5 (LTQ Orbitrap XL) or $n$ (Orbitrap Fusion Lumos: top $n$) most intense / abundant peptides were isolated for fragmentation, respectively. Peptides were fragmented by collision with nitrogen and fragment (MS$^2$) spectra were subsequently recorded. Detailed settings of both LC-MS/MS systems are listed in Table 4.

**Table 4. LC-MS/MS settings for the identification of peptides eluted from HLA class I or II molecules.** Abbreviations not introduced in the text above: collision-induced dissociation (CID), higher-energy induced dissociation (HCD). Table layout and listed parameters for the Orbitrap Fusion Lumos are largely congruent with Table 4 in [10].

| Parameter | LTQ Orbitrap XL | | Orbitrap Fusion Lumos | |
|---|---|---|---|---|
| | **HLA class I peptides** | **HLA class II peptides** | **HLA class I peptides** | **HLA class II peptides** |
| **Internal name of the method used** | top5CID_400-650mz_90min Gradient_3sDynExcl | top5CID_300-1500mz_90min Gradient_3sDynExcl | DDA_400-650mz_ qIS_CIDOT(_6Port) | DDA_400-1000mz_ qIS_HCDOT(_6Port) |
| *LC flow rate* | | | | |
| **Loading pump [µl/min]** | 4 | | 4 | |
| **Nano pump [nl/min]** | 0-100 min: 175; 101-130 min: 275 | | 300 | |
| *LC solvents* | | | | |
| **Loading pump solvent A** | 0.1% FA / 1% AcN | | 0.05% TFA / 1% AcN | |
| **Loading pump solvent B** | 0.1% FA / 80% AcN | | 0.15% FA / 80% AcN | |
| **Nano pump solvent A** | 0.15% FA | | 0.15% FA | |
| **Nano pump solvent B** | 0.15% FA / 80% AcN | | 0.15% FA / 80% AcN | |
| *MS$^1$* | | | | |
| **Permitted positive charge states** | 2-3 | ≥ 2 | 2-3 | 2-5 |
| **Permitted m/z** | 400-650 | 300-1,500 | 400-650 | 400-1,000 |
| **Detector type** | Orbitrap | | Orbitrap | |
| **Resolution** | 60,000 | | 120,000 | |
| **MS$^1$ precursor selection** | Top 5; quadrupole | | Top $n$; quadrupole | |

| *MS²* | | | |
|---|---|---|---|
| **Fragmentation type** | CID | CID | HCD |
| **Normalized collision energy [%]** | 35 | 35 | 30 |
| **Localization** | Ion trap | Ion trap | Ion-routing multipole |
| **Detector type** | Ion trap detector | Orbitrap | |
| **Resolution** | not specified | 30,000 | |
| **Data type** | Centroid | Centroid | |
| *Dynamic exclusion* | | | |
| **Exclusion after *n* times** | 1 | 1 | 1 |
| **Duration [s]** | 3 | 7 | 10 |
| **Mass tolerance [ppm]** | ± 10 | ± 2.5 | |

*Sequence annotation to uninterpreted MS/MS spectra and data export*

So far, MS/MS spectra had only been recorded, but not yet interpreted. Annotation to a peptide sequence was accomplished by database search using the SEQUEST search algorithm[434] which Proteome Discoverer 1.4.1.14 embeds as processing node. To identify peptides derived from canonical WT antigens, the Swiss-Prot release from September 27th 2013 (20,279 reviewed protein sequences) served as reference database. Subsequently, the Percolator algorithm 2.04 estimated the false discovery rate (FDR) by decoy database search. Target FDR was set to ≤ 5%. Parameters for processing of raw files compiled by either the LTQ Orbitrap XL or the Orbitrap Fusion Lumos are given in Table 5.

To guarantee that every single measurement was technically accurate, not only the total ion chromatogram, loading and nano pump pressures were reviewed, but also a database search was performed for every technical replicate. For all analyses excepting those aimed at relative quantitation of HLA ligand abundances, the maximum number of available technical replicates was co-processed and exported peptide and protein lists served as base for downstream analyses. Due to the permission of methionine oxidation as dynamic modification, sequence duplicates with either oxidized (m) or reduced (M) methionine residues were included in the peptide lists. These two peptide variants were treated equally, whereby only the oxidized version was reported, in case the reduced one was present as well.

**Table 5. Parameters for database search and data export in Proteome Discoverer.** Abbreviations not introduced in the text above: signal-to-noise ratio (S/N), Fourier Transform Mass Spectrometry (refers to Orbitrap detection; FTMS). Table layout and listed parameters for the analysis of data compiled by the Orbitrap Fusion Lumos are congruent with Table 5 in [10].

| Parameter | LTQ Orbitrap XL | | Orbitrap Fusion Lumos | |
|---|---|---|---|---|
| | HLA class I peptides | HLA class II peptides | HLA class I peptides | HLA class II peptides |
| *Raw file processing* | | | | |
| **Internal name of workflow** | SequestIT | SequestIT_ ClassII | SequestOT | SequestOT_ ClassII |
| *Spectrum properties filter* | | | | |
| **Precursor mass [Da]** | 800-5,000 | | 800-5,000 | |
| **Minimum peak count** | 1 | | 1 | |
| **S/N threshold (FTMS)** | 1.5 | | 1.5 | |

| Input data | | | | |
|---|---|---|---|---|
| **Enzyme name** | No-enzyme (unspecific) | | No-enzyme (unspecific) | |
| **Maximum missed cleavage sites** | 0 | | 0 | |
| **Peptide length [# of AA]** | 8-12 | 8-25 | 8-12 | 8-25 |
| *Scoring options* | | | | |
| **Maximal delta Cn** | 0.05 | | 0.05 | |
| *Tolerances* | | | | |
| **Precursor mass tolerance [ppm]** | ± 5 | | ± 5 | |
| **Fragment mass tolerance [Da]** | ± 0.5 | | ± 0.02 | |
| *Spectrum matching* | | | | |
| **Use of neutral loss ions** | a, b, y ions | | a, b, y ions | |
| **Weight of a, c, x, z ions** | 0 | | 0 | |
| **Weight of b or y ions** | 1 | | 1 | |
| *Modifications* | | | | |
| **Permitted dynamic modification** | Oxidation / +15.995 Da (M) | | Oxidation / +15.995 Da (M) | |
| **Maximum modifications per peptide** | 3 | | 3 | |
| **Permitted static modification** | None | | None | |
| *Data export* | | | | |
| **Internal name of filter set** | Class I.filters | Class II.filters | Class I.filters | Class II.filters |
| **Minimum peptide confidence** | Medium (≤ 5% FDR) | | Medium (≤ 5% FDR) | |
| **Peptide length [# of AA]** | 8-12 | 8-25 | 8-12 | 8-25 |
| **Search engine rank** | 1 | | 1 | |
| **Peptide grouping** | Enabled | | Enabled | |
| **Protein grouping** | Disabled | | Disabled | |

### Deconvolution of HLA class I datasets

As elucidated in 2.2.2, HLA class I allotypes have relatively strict binding preferences to particular peptide motifs. Peptides obtained from HLA-immunoprecipitation (HLA-IP) with W6/32 originate from up to six different HLA-A, -B, and -C molecules. To deconvolute this multi-allelic dataset, binding prediction algorithms are employed, which calculate the probability for each peptide to bind to one of the patient's HLA class I allotypes. Ligandosphere is an in-house software which offers, among others, a stand-alone version of NetMHCpan-4.0[431,432] and an enhanced version of SYFPEITHI.[436] Peptides were designated as HLA class I ligands, when predicted as such by at least one of the two algorithms. The binding threshold for SYFPEITHI predictions was set to ≥ 60% of the maximum score of the respective position-specific scoring matrix. Binding peptides according to NetMHCpan-4.0, which is based on artificial neural networks and trained with both binding affinity and immunopeptidomic data,[432] had a percentile rank score ≤ 2%. Proteins not represented by HLA class I ligands were subsequently removed from the protein lists. HLA annotation of class II peptides is still difficult owing to degenerate peptide motifs and promiscuous binding.[177]

### Label-free quantitation of relative HLA ligand abundances

For label-free quantitation (LFQ) of relative HLA ligand abundances in two autologous samples (primary and recurrent glioblastoma, meningioma and tumor-free dura), the total amount of substance analyzed per technical replicate, represented by the summed area under the curve (AUC) of all peptides, was adjusted for the two conditions. For this purpose, a so-called dose-

finding measurement was performed for every sample, separately for HLA class I and II. LFQ was only performed when both samples yielded at least 500 peptides and a total AUC of $1 \times 10^9$ or 400 peptides and a total AUC of $9 \times 10^8$ in the dose-finding run for the Orbitrap Fusion Lumos or the LTQ Orbitrap XL, respectively. For HLA class I-eluted peptides, the total AUC was only calculated from those assigned as HLA class I ligands. The higher concentrated sample was diluted with $A_{Load}$ accordingly to reach the same amount of substance as the lower concentrated one. In case a dilution factor of more than eight was calculated, a new dose-finding run was acquired upon 1:8 dilution. A total number of five (adjusted) technical replicates were measured per condition and HLA class for LFQ experiments. As quality control, the HLA class I ligand or HLA class II peptide yield of each technical replicate was not permitted to deviate by > 30% of the mean peptide yield of all five adjusted measurements. In case a sample had to be heavily diluted for adjustment, two further undiluted replicates were performed in terms of qualitative analyses.

The five adjusted replicates of each condition were co-processed, protein and peptide lists were exported with standard filters, and peptide lists were merged for both conditions to compile the seedlist. For semi-quantitative HLA class I Volcano plots, this seedlist was filtered for ligands, as described previously. In addition, peptides of each replicate were exported without any filter being applied to the data, so that every peptide spectrum match (PSM) including such with a rank ≥ 2 was reported with corresponding scores. Intensities (AUC of the extracted ion chromatogram of precursor ions) of those peptides contained in the seedlist were subsequently picked from the unfiltered lists. Reproducibility of intensities across technical replicates is exemplarily shown in Supplementary Figure 4 and Supplementary Figure 11. For each peptide and each condition, mean AUC across all LFQ-MS runs as well as the ratio of mean AUC in condition A *versus* condition B was calculated. So-called 'one hit wonders' (peptides not detected in at least two technical replicates) were excluded from semi-quantitative Volcano plots. The fold-change of peptides exclusively detected in one condition was calculated by replacing zero values with the median of the five least intensities, which represents the limit of detection specific for the respective sample. Further, a normalization step computing PSM intensities in proportion to the total intensity of precursor ions in technical replicates was included. To test for significance, two-tailed *t*-tests with implemented correction for multiple testing according to the Benjamini-Hochberg (BH) procedure were conducted.

***Comparative profiling against an in-house database and definition of presentation hotspots for HLA class II-presented antigens***

To define glioblastoma-, medulloblastoma-, and meningioma-associated peptides and antigens, protein and peptides were queried against an in-house SQL database (3.2.1) comprising immunopeptidomic datasets from 30 benign primary human organs other than testis (n=418 samples for HLA class I; n=364 samples for HLA class II). The term tumor-exclusive was assigned to peptides and antigens that were never identified on CNS-related tissues (brain, cerebellum, spinal cord, and for meningiomas also dura which was not part of the benign database) and for which a maximum of one non-CNS-related sample was positive. Likewise, the presence of glioblastoma-, medulloblastoma-, or meningioma-associated peptides and proteins was checked in HLA peptidome datasets of up to 37 different primary human malignancies (n=874 samples for HLA class I; n=626 samples for HLA class II).

Comparative profiling is not capable of reflecting length variants and common core sequences of HLA class II-restricted peptides. Vimentin yielded a total of 29 distinct meningioma-exclusive peptides with presentation frequencies up to 67%, however, almost identical peptide sequences differing only in 1 AA in length were identified on benign tissues (Figure 36 A, Supplementary Figure 10 A). To circumvent this problem, all source proteins represented by at least one tumor-exclusive HLA class II-presented peptide were subjected to hotspot analysis. Unique peptides were aligned to human source protein sequences (as deposited in the UniProt[86] database for taxonomy ID 9606) and the proportion of all peptides covering a given position was calculated separately for identifications on malignant and benign tissues. Tumor-associated hotspots were defined to have a minimum length of eight AA and to be covered by peptides identified in at least five (medulloblastoma / meningioma) or eight (glioblastoma) patients, while not having matching sequences in benign samples.

# 4 Aim of the study

Primary brain and CNS tumors account for an average yearly incidence of 81,148 new cases in the US with 15,944 people dieing from malignant CNS cancers every year. They represent the eighth most common tumors in adults older than 40 years and even the most common pediatric tumor entity. Throughout the last decades, the outcome of intracranial neoplasms has remained poor calling for intensive research to establish novel therapies. Especially for recurrent glioblastomas, medulloblastomas, and meningiomas, no treatment protocols or standard-of-care guidelines have been established so far. Cancer immunotherapy has elicited remarkable responses in various cancer entities including glioblastoma. This suggests to employ immune-based therapies for intracranial neoplasias aimed at both preventing (or at least prolonging the time to) relapse, which is a common event especially in glioblastoma, and offering an option to manage disease recurrence. Peptide-specific immunotherapy targeting antigens with natural and tumor-specific presentation on HLA molecules may contribute to significantly improve prognosis and quality of life of patients suffering from glioblastoma, medulloblastoma, or meningioma. Despite extensive research on immunotherapeutic targets in gliomas, this has so far not translated into clinical benefit for patients. Of note, especially the antigenic landscape of recurrent tumors has so far not been investigated at all. Medulloblastoma and meningioma are even characterized by a profound lack of potential targets for cancer immunotherapy. HLA class II-presented antigens have not been adequately adressed for any of these three tumor entities. The aim of the present thesis is to define such tumor-associated peptides by employing an immunopeptidome-centric approach to a large number of primary human glioblastoma, medulloblastoma, and meningioma samples. This will allow adressing both HLA class I- and II-restricted peptides in future immunotherapeutic efforts. In the form of a meta-analysis, the immunopeptidomic data acquired from these three intracranial neoplasias will be compared. This will provide insight into whether the immunopeptidome views the different cellular origin of glioblastoma, medulloblastoma, and meningioma cells. Moreover, it will be analyzed whether shared or even pan-brain tumor antigens exist. These projects are intended to contribute to the development of innovative therapeutic strategies for patients suffering from intracranial neoplasms.

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# CHAPTER 2

## Multi-omics analysis identifies novel antigens for glioblastoma immunotherapy and delineates dynamics during progression from primary to recurrent disease

# 1 Abstract

Glioblastoma is the most aggressive and most frequent primary CNS tumor. Despite extensive research, patients have not sufficiently benefited from recent scientific developments including immune checkpoint inhibitors. However, glioblastoma has been shown to be vulnerable to T cells targeting non-mutated peptides presented on HLA molecules. Immunotherapeutic intervention is typically administered subsequent so standard radiochemotherapy contributing to potential hypermutation and clonal evolution perhaps drastically altering the antigenic repertoire. Thus, we have for the first time investigated the immunopeptidomic landscape of 24 recurrent glioblastomas in comparison with 38 primary tumors, including 22 autologous pairs of samples, to define tumor antigens robustly presented at both recurrent and primary disease, while addressing not only HLA class I- but also HLA class II-restricted peptides. Relative quantitation of HLA ligand abundances, comparative profiling, and functional annotation clustering further delineated antigenic profiles and features associated with primary or recurrent glioblastoma, respectively. Moreover, this multi-omics approach provides the first evidence for natural HLA presentation of two neo-antigenic peptides in human brain tumors. Taken together, we defined a set of glioblastoma-associated antigens representing prime candidates to be pursued in the development of future immunotherapies as being naturally, exclusively, and frequently presented on both primary and recurrent neoplasias. This may contribute to improve the therapeutic situation in recurrent glioblastoma lacking evidence-based protocols.

# 2 Introduction

Glioblastoma represents the most frequent and most aggressive primary CNS neoplasia accounting for a 5-year relative survival rate of only 6.8%.[1] Extensive research has been conducted, however, a sufficient translation of scientific knowledge into clinical benefit has so far stayed out. The standard-of-care still comprises surgery and adjuvant radiochemotherapy according to the Stupp protocol and evidence-based therapeutic regimens to manage disease recurrence do not exist.[2-6] Glioblastoma recurrence is inevitable, as these diffusely growing tumors deeply infiltrate into surrounding benign brain tissue rendering complete surgical resection virtually impossible.[2-4,7] Radio- and especially adjuvant and concomitant chemotherapy are expected to significantly alter the repertoire of potential targets by clonal evolution and, in a fraction of patients, by somatic hypermutation.[8,9] Although few datasets investigating the antigenic landscape of glioblastoma have been published, none has so far addressed recurrent tumors despite this is the condition, in which experimental therapies are initially applied to be evaluated for clinical effectiveness. Likewise, HLA class II-restricted targets have been inadequately examined by previous studies.[10-14]

We employ a multi-omics aproach comprising HLA peptidome analyses, whole exome and transcriptome sequencing, and DNA methylation profiling. Comparison with a large dataset of benign immunopeptidomes enabled the definition of glioblastoma-associated antigens and peptides as candidate targets for cancer immunotherapy. Antigens were reviewed for CTA-like expression profiles by querying the GTEx database, which contains RNA expression data acquired across a large set of benign human tissues.[15] This also allowed excluding such with

CNS-specific expression and to identify antigens not known to be expressed in any tissue. Moreover, exome sequencing of tumors and autologous blood cells may allow the identification of neo-antigenic HLA ligands, especially in hypermutated recurrent tumors, in a fully individualized setting or disprove the efficient translation of non-synonymous mutations into peptides naturally presented on HLA, as already done for several cancer entities including primary glioblastoma.[12,16-19] Besides yielding candidate targets for immunotherapy, this study provides a deep insight into glioblastoma biology during progression from primary to recurrent disease. To adress this, we compared the immunopeptidomic landscape of primary and recurrent tumors in a predominantly autologous setting, whereby mutational, transcriptional, and DNA methylation profiling will follow.
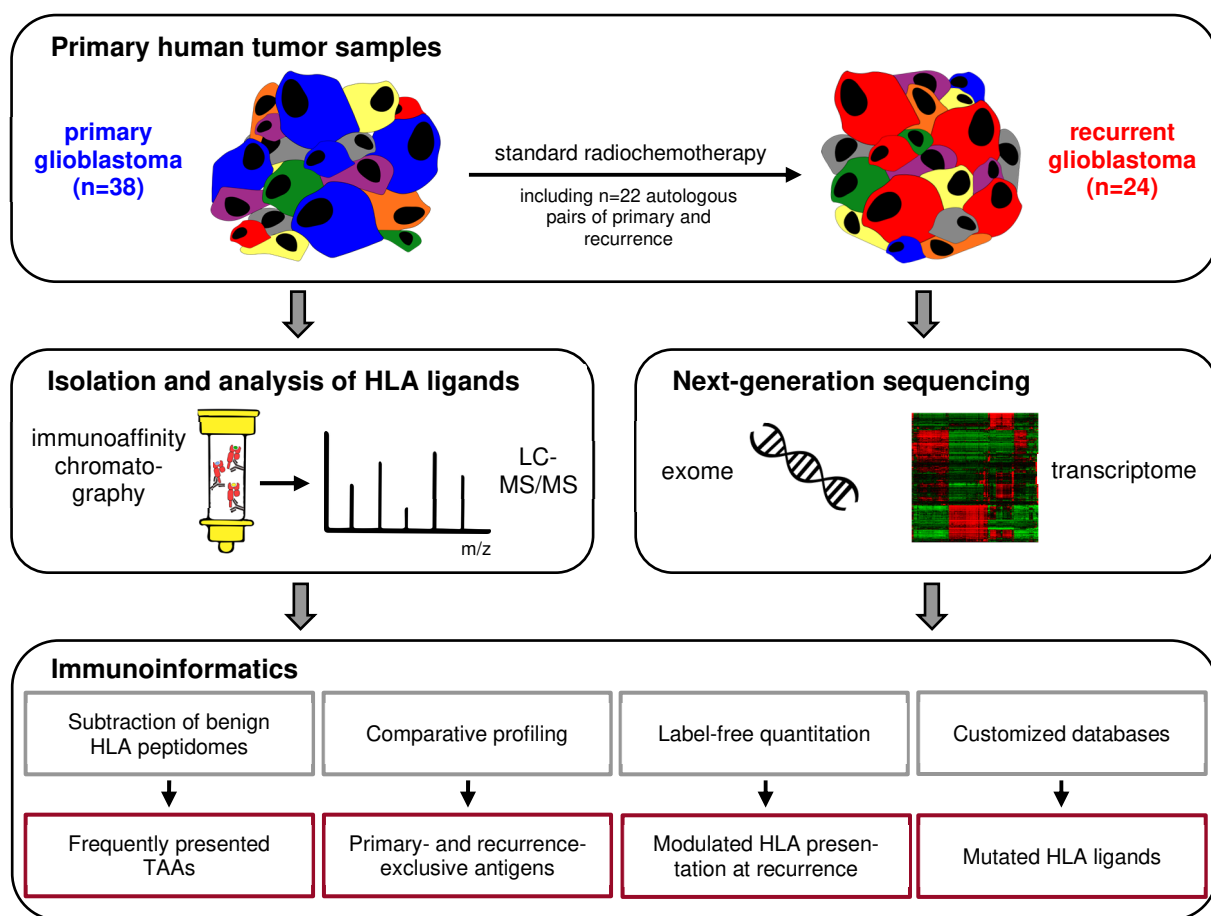


**Figure 8. Multi-omics analysis of glioblastoma with a particular focus on the similarities and differences between primary and recurrent disease.** While the analysis of immunopeptidomic data has been completed, the investigation of whole exome and transcriptome data as well as integration with LC-MS/MS data has been performed for n=13 primary tumors and is currently being extended to all patients with paired primary and recurrent glioblastomas being available. The two middle panels of the figure were kindly provided by PD Dr. med. Marian Christoph Neidert.

# 3   Methods

### *Patient collective*

Written informed consent of the 40 patients included in the present study was obtained in accordance with the Declaration of Helsinki protocol and the local review boards (Kantonale Ethikkommission Zürich / KEK-ZH-Nr. 2015-0163; Ethik-Kommission der Ärztekammer

Hamburg / PV4904) before surgery. Patients underwent surgery at the Department of Neurosurgery of the University Hospital Zürich or of the University Medical Center Hamburg-Eppendorf. Tissue samples were snap frozen in liquid nitrogen and stored at -80°C until use. All patients had histopathologically confirmed glioblastoma and specimens along with clinical metadata were kindly provided by PD Dr. med. Marian Christoph Neidert and Dr. med. Julia Velz (University Hospital Zürich, Department of Neurosurgery), Dr. Konstantina Kapolou (University Hospital and University of Zürich, Department of Neurology, Laboratory for Molecular Neuro-Oncology) as well as by Prof. Dr. med. Manfred Westphal and Dr. med. Malte Mohme (University Medical Center Hamburg-Eppendorf, Department of Neurosurgery).

All patients had histopathologically confirmed glioblastoma (all IDH1$^{WT}$ excepting n=2 lacking IDH1-status and n=1 IDH1$^{mut}$ at recurrence; n=17 MGMT$^+$ / n=20 MGMT$^-$ / n=3 lacking MGMT-status) with 38 and 24 samples obtained during surgery at primary or recurrent disease, respectively. The present study population had a female-to-male ratio of 4:5 and a median age of onset of 67 [33-86] years. Autologous primary and recurrent tumors enabling LFQ of relative HLA class I and II ligand abundances were available from 22 patients. The median amount of tissue subjected to HLA-IP accounted to 893 [119-2759] mg for primary and to 451 [35-5834] mg for recurrent glioblastomas. Individual patient and sample characteristics are given in Table 6 and Figure 9, whereby a closer look on HLA allotype frequencies in the study cohort is provided in CHAPTER 5.

**Table 6. Study cohort comprising 40 glioblastoma patients (n=38 primary and n=24 recurrent tumors).** Age of onset was defined as the age at initial diagnosis. The MGMT-status differentiates tumors into such with (+) and without (–) MGMT promoter methylation, whereas the IDH1-status distinguishes glioblastomas lacking a mutation of the IDH1 gene (WT) from those with mutant IDH1 (mut). HLA class II allotypes were only available for samples sequenced at HistoGenetics. Abbreviations not introduced in the text above: primary glioblastoma (P), recurrent glioblastoma (R), not determined (n.d.). #Sample mass not used for further calculations (part of sample lost during HLA-IP).

| Internal sample ID | Gender | Age of onset [years] | IDH1-status MGMT-status | PFS from primary [months] | Time from P to R surgery [months] | OS from primary [months] | HLA typing | Sample mass HLA-IP [mg] P / R | Direct injection P / R | Relative quantitation HLA class I / II |
|---|---|---|---|---|---|---|---|---|---|---|
| Autologous pairs of primary and recurrent glioblastoma | | | | | | | | | | |
| **GBM2** | ♂ | | WT | n.d. | 10 | n.d. | A*02:01;A*03:01;B*07:02;B*15:01;C*07:02;C*03:04 | | | + / + |
| P N1-06 | 67 | | + | | | | | 978 | – | |
| R N162-06 | | | | | | | | 526 | – | |
| **GBM3** | ♂ | | WT | n.d. | 11 | n.d. | A*02:01;A*01:01;B*27:05;B*18:01;C*02:02;C*07:01 | | | – / + |
| P N82-12 | 68 | | – | | | | | 1862 | – | |
| R N56-13 | | | | | | | | 174 | – | |
| **GBM4** | ♂ | | WT | n.d. | 10 | n.d. | A*03:01;A*03:01;B*07:02;B*07:02;C*07:02;C*07:02 | | | + / – |
| P N119-08 | 65 | | – | | | | | 332 | – | |
| R N73-09 | | | | | | | | 534 | – | |
| **GBM5** | ♀ | | WT | n.d. | 12 | n.d. | A*01:01;A*01:01;B*13:02;B*15:01;C*03:03;C*06:02 | | | + / + |
| P N12-05 | 66 | | + | | | | | 729 | – | |
| R N108-06 | | | | | | | | 375 | – | |
| **GBM6** | ♀ | | WT | n.d. | 12 | n.d. | A*02:05;A*03:01;B*35:01;B*15:03;C*04:01;C*12:03 | | | + / – |
| P N156-08 | 63 | | – | | | | | 1258 | – | |
| R N146-09 | | | | | | | | 253 | – | |
| **GBM7** | ♀ | | WT / | n.d. | 46 | n.d. | A*11:01;A*02:01;B*07:02;B*56:01;C*01:02;C*02:02 | | | + / + |
| P N163-07 | 47 | | mut | | | | | 893 | – | |
| R N182-10 | | | + | | | | | 566 | – | |
| **GBM8** | ♀ | | n.d. | n.d. | 24 | n.d. | A*68:01;A*03:01;B*07:02;B*44:02;C*07:02;C*07:04 | | | – / – |
| P N122/07 | 40 | | n.d. | | | | | 119 | + | |
| R N131/09 | | | | | | | | 82 | + | |
| **GBM9** | ♀ | | WT | n.d. | 7 | n.d. | A*33:05;A*01:01;B*08:01;B*14:02;C*07:01;C*08:02 | | | + / + |
| P N45/14 | 40 | | + | | | | | 1471 | + | |
| R N203/14 | | | | | | | | 917 | + | |
| **GBM10** | ♂ | | n.d. | n.d. | 60 | n.d. | A*02:01;A*03:01;B*07:02;B*07:02;C*07:02;C*07:02 | | | – / – |
| P N207/10 | 52 | | – | | | | | 252 | + | |
| R N476/15 | | | | | | | | 1167 | + | |

| ID | Age/Sex | WT/± | | | | HLA typing | Value | Sign | +/+ |
|---|---|---|---|---|---|---|---|---|---|
| **GBM11** | ♂ | WT | n.d. | 20 | n.d. | A*02:05;A*02:01;B*41:01;B*07:02;C*07:01;C*07:02 | | | + / + |
| P N138/15 | 74 | + | | | | | 663 | + | |
| R N546/15 | | | | | | | 310 | + | |
| **GBM12** | ♂ | WT | 6 | 6 | 9 | A*11:01;A*02:01;B*35:01;B*15:01;C*01:02;C*04:01 | | | + / + |
| P ZH560 | 54 | − | | | | | 206 | + | |
| R ZH588 | | | | | | | 5834 | − | |
| **GBM13** | ♀ | WT | 15 | 15 | n.d. | A*01:01;A*02:01;B*08:01;B*13:02;C*06:02;C*07:01 | | | − / − |
| P ZH794 | 72 | n.d. | | | | DRB1*03:01;DRB1*07:01;DRB3*01:01;DRB4*01:01; | 734 | − | |
| R ZH917 | | | | | | DQB1*02:01;DQB1*02:01;DQA1*02:01;DQA1*05:01 | 546 | + | |
| **GBM14** | ♂ | WT | 6 | 13 | 18 | A*33:01;A*02:01;B*35:03;B*14:02;C*08:02;C*04:01 | | | + / + |
| P ZH301 | 68 | n.d. | | | | | 250 | + | |
| R ZH384 | | | | | | | 2784# | − | |
| **GBM15** | ♂ | WT | 5 | 16 | n.d. | A*24:02;A*02:01;B*44:02;B*44:03;C*05:01;C*02:02 | | | − / + |
| P ZH399 | 54 | − | | | | | 348 | + | |
| R ZH535 | | | | | | | 95 | + | |
| **GBM16** | ♂ | WT | 3 | 36 | 41 | A*03:01;A*01:01;B*07:02;B*08:01;C*07:01;C*07:02 | | | + / + |
| P ZH415 | 59 | + | | | | | 893 | + | |
| R ZH622 | | | | | | | 344 | + | |
| **GBM17** | ♂ | WT | 9 | 10 | 19 | A*01:01;A*03:01;B*44:03;B*57:01;C*04:01;C*06:02 | | | − / − |
| P ZH746 | 72 | − | | | | DRB1*07:01;DRB1*13:05;DRB3*02:02;DRB4*01:01; | 922 | − | |
| R ZH855 | | | | | | DQB1*02:01;DQB1*03:01;DQA1*02:01;DQA1*05:01 | 735 | + | |
| **GBM18** | ♂ | WT | 10 | 11 | n.d. | A*03:01;A*11:01;B*07:02;B*08:01;C*07:01;C*07:02 | | | − / − |
| P ZH813 | 57 | − | | | | DRB1*03:01;DRB1*04:04;DRB3*01:01;DRB4*01:01; | 1655 | − | |
| R ZH895 | | | | | | DQB1*02:01;DQB1*03:02;DQA1*03:01;DQA1*05:01 | 2147 | − | |
| **GBM19** | ♂ | WT | 11 | 11 | n.d. | A*02:01;A*24:02;B*40:02;B*44:02;C*01:02;C*05:01 | | | − / − |
| P ZH818 | 68 | − | | | | DRB1*01:01;DRB1*13:01;DRB3*02:02;DQB1*05:01; | 662 | − | |
| R ZH904 | | | | | | DQB1*06:03;DQA1*01:03;DQA1*01:01 | 40 | + | |
| **GBM20** | ♀ | WT | 19 | 23 | n.d. | A*29:02;A*02:01;B*44:03;B*44:02;C*05:01;C*16:01 | | | + / + |
| P ZH669 | 69 | − | | | | | 140 | + | |
| R ZH909 | | | | | | | 35 | + | |
| **GBM21** | ♀ | WT | 38 | 38 | n.d. | A*02:01;A*02:01;B*35:03;B*44:02;C*12:03;C*07:04 | | | − / + |
| P ZH561 | 33 | + | | | | | 310 | + | |
| R ZH862 | | | | | | | 215 | + | |
| **GBM22** | ♀ | WT | 7 | 20 | 23 | A*03:01;A*24:02;B*35:03;B*40:01;C*03:04;C*12:03 | | | − / − |
| P ZH736 | 76 | + | | | | DRB1*09:01;DRB1*11:01;DRB3*02:02;DRB4*01:01; | 1074 | − | |
| R ZH736R | | | | | | DQB1*03:03;DQB1*03:01;DQA1*03:01;DQA1*05:01 | 3714 | − | |
| **GBM23** | ♀ | WT | 20 | 21 | 24 | A*03:01;A*23:01;B*35:02;B*47:01;C*04:01;C*06:02 | | | − /− |
| P ZH763 | 70 | + | | | | DRB1*07:01;DRB1*11:04;DRB3*02:02;DRB4*01:01; | 1981 | − | |
| R ZH945 | | | | | | DQB1*02:01;DQB1*03:01;DQA1*02:01;DQA1*05:01 | 542 | + | |
| Unpaired tumor samples | | | | | | | | | − |
| P ZH613 | ♂ 74 | WT − | n.d. | n.d. | n.d. | A*02:05;A*31:01;B*51:01;B*58:01;C*05:01;C*07:01 DRB1*13:01;DRB1*13:02;DRB3*02:02;DRB3*03:01; DQB1*06:09;DQB1*06:03;DQA1*01:03;DQA1*01:02 | 840 | − | |
| P ZH616 | ♀ 86 | WT − | n.d. | n.d. | n.d. | A*02:01;A*29:02;B*07:02;B*44:02;C*05:01;C*07:02 DRB1*12:01;DRB1*15:01;DRB3*02:02;DRB5*01:01; DQB1*03:01;DQB1*06:02;DQA1*01:02;DQA1*05:01 | 1160 | − | |
| P ZH617 | ♀ 74 | WT + | n.d. | n.d. | n.d. | A*01:01;A*02:01;B*08:01;B*44:02;C*05:01;C*07:01 DRB1*03:01;DRB1*03:01;DRB3*01:01;DRB3*01:01; DQB1*02:01;DQB1*02:01;DQA1*05:01;DQA1*05:01 | 600 | − | |
| P ZH631 | ♂ 60 | WT + | n.d. | n.d. | n.d. | A*11:01;A*32:01;B*44:02;B*51:01;C*03:03;C*07:04 DRB1*01:01;DRB1*08:01;DQB1*04:02;DQB1*05:01; DQA1*01:01;DQA1*04:01 | 370 | − | |
| P ZH645 | ♂ 66 | WT − | n.d. | n.d. | n.d. | A*01:01;A*02:01;B*49:01;B*51:01;C*01:02;C*07:01 DRB1*07:01;DRB1*13:02;DRB3*03:01;DRB4*01:01; DQB1*02:01;DQB1*06:04;DQA1*01:02;DQA1*02:01 | 1037 | − | |
| P ZH654 | ♂ 72 | WT + | n.d. | n.d. | n.d. | A*01:01;A*31:01;B*51:01;B*57:01;C*06:02;C*15:06 DRB1*04:04;DRB1*07:01;DRB4*01:03;DRB4*01:01; DQB1*03:03;DQB1*03:02;DQA1*02:01;DQA1*03:01 | 2759 | − | |
| P ZH678 | ♂ 66 | WT − | n.d. | n.d. | n.d. | A*02:01;A*11:01;B*44:03;B*51:01;C*04:01;C*15:02 DRB1*11:01;DRB1*11:01;DRB3*02:02;DRB3*02:02; DQB1*03:01;DQB1*03:01;DQA1*05:01;DQA1*05:01 | 2619 | − | |
| P ZH681 | ♂ 78 | WT + | n.d. | n.d. | n.d. | A*02:01;A*02:01;B*07:02;B*57:01;C*06:02;C*07:02 DRB1*07:01;DRB1*13:02;DRB3*03:01;DRB4*01:03; DQB1*03:03;DQB1*06:04;DQA1*01:02;DQA1*02:01 | 1244 | − | |
| P ZH720 | ♂ 70 | WT − | n.d. | n.d. | n.d. | A*02:01;A*02:01;B*18:01;B*39:10;C*07:01;C*12:03 DRB1*13:01;DRB1*13:01;DRB3*01:01;DRB3*01:01; DQB1*06:03;DQB1*06:03;DQA1*01:03;DQA1*01:03 | 960 | − | |
| P ZH750 | ♀ 60 | WT − | n.d. | n.d. | n.d. | A*02:01;A*24:02;B*15:17;B*44:02;C*07:01;C*07:04 DRB1*11:01;DRB1*13:02;DRB3*02:02;DRB3*03:01; DQB1*03:01;DQB1*06:04;DQA1*01:02;DQA1*05:01 | 1019 | − | |
| P ZH757 | ♂ 70 | WT − | n.d. | n.d. | n.d. | A*02:01;A*02:01;B*07:02;B*27:05;C*01:02;C*07:02 DRB1*08:01;DRB1*15:01;DRB5*01:01;DQB1*04:02; DQB1*06:02;DQA1*01:02;DQA1*04:01 | 645 | − | |
| P ZH761 | ♂ 80 | WT + | n.d. | n.d. | n.d. | A*02:01;A*03:01;B*44:02;B*51:26;C*01:02;C*16:01 DRB1*01:01;DRB1*12:01;DRB3*02:02;DQB1*03:01; DQB1*05:01;DQA1*01:01;DQA1*05:01 | 1193 | − | |
| P ZH791 | ♂ 63 | WT − | n.d. | n.d. | n.d. | A*03:01;A*11:01;B*07:02;B*13:02;C*06:02;C*07:02 DRB1*11:01;DRB1*15:01;DRB3*02:02;DRB5*01:01; DQB1*03:01;DQB1*06:02;DQA1*01:02;DQA1*05:01 | 751 | − | |
| P ZH802 | ♀ 70 | WT − | n.d. | n.d. | n.d. | A*31:01;A*33:01;B*14:02;B*44:02;C*05:01;C*08:02 DRB1*04:04;DRB1*13:02;DRB3*03:01;DRB4*01:01; DQB1*03:02;DQB1*06:04:01;DQA1*01:02;DQA1*03:01 | 1712 | − | |
| P ZH810 | ♀ 53 | WT + | n.d. | n.d. | n.d. | A*24:02;A*24:02;B*44:03;B*57:01;C*04:01;C*06:02 DRB1*07:01;DRB1*11:01;DRB3*02:02;DRB4*01:01; DQB1*03:03;DQB1*03:01;DQA1*02:01;DQA1*05:01 | 1158 | − | |

| P ZH829 | ♀ 70 | WT – | n.d. | n.d. | n.d. | A*02:01;A*02:01;B*44:05;B*55:01;C*01:02;C*02:02 DRB1*13:01;DRB1*16:01;DRB3*02:02;DRB5*02:02; DQB1*05:02;DQB1*06:03;DQA1*01:03;DQA1*01:02 | 276 | – |
| R ZH753 | ♀ 59 | WT + | n.d. | n.d. | n.d. | A*02:01;A*02:01;B*08:01;B*51:01;C*07:01;C*15:02 DRB1*01:01;DRB1*03:01;DRB3*01:01;DQB1*02:01; DQB1*05:01;DQA1*01:01;DQA1*05:01 | 2483 | – |
| R ZH784 | ♂ 63 | WT + | n.d. | n.d. | n.d. | A*01:01;A*02:01;B*07:02;B*08:01;C*07:01;C*07:02 DRB1*11:01;DRB1*15:01;DRB3*02:02;DRB5*01:01; DQB1*03:01;DQB1*06:02;DQA1*01:02;DQA1*05:01 | 287 | – |
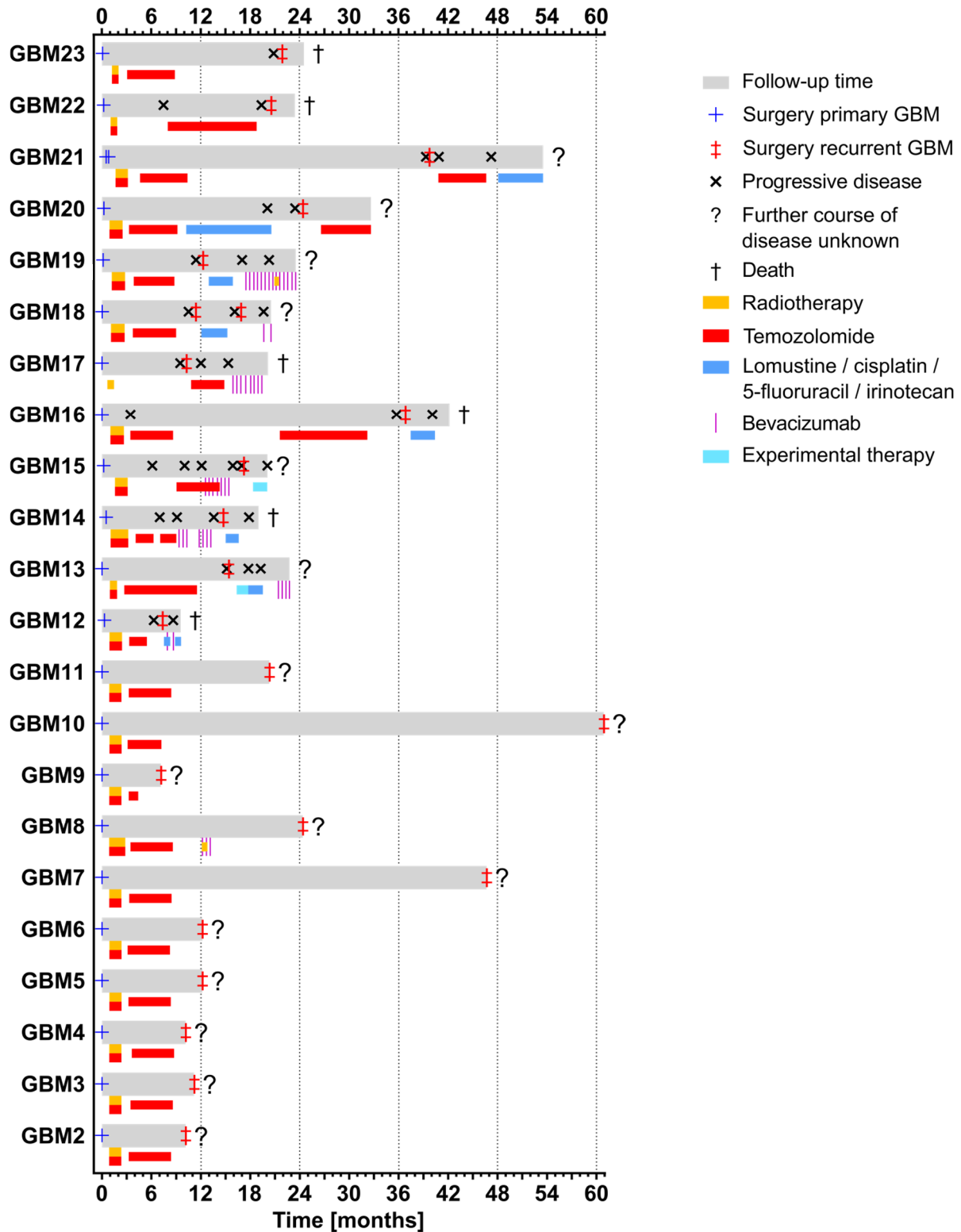


**Figure 9. Clinical timeline for the 22 glioblastoma patients with both primary and recurrent tumors being analyzed.** Median follow-up time accounted for 20 [7-61] months with patients 2-11

lacking clinical information subsequent to surgery for recurrent glioblastoma. All patients excepting GBM17 received radiochemotherapy and adjuvant temozolomide following surgical resection of primary glioblastoma. The time between surgery of primary and recurrent glioblastoma came up to 14 [6-60] months in median. Experimental therapies as part of clinical trials comprise the CDK2 / Janus kinase 2 (JAK2) / FMS-like tyrosine kinase 3 (FLT3) inhibitor TG02 (EORTC-1608; GBM13) and the fibroblast growth factor receptor inhibitor BGJ398 (CBGJ398X2201; GBM15). Prior to surgical resection of recurrent tumors, patients GBM4 and GBM10 were treated with an anti-EGFR antibody or bevacizumab (AVAglio study), respectively – precise dates of treatments were, however, not available.

### HLA-IP and subsequent LC-MS/MS to identify HLA-presented peptides

HLA class I- and II-presented peptides were isolated from primary human tissue and analyzed by LC-MS/MS as described in 3.2. To handle the pronounced high degree of intratumoral heterogeneity characteristic of glioblastoma, a pool of frozen chopped tissue was created from which samples for HLA-IP, whole exome, and RNA sequencing were gathered. All peptide eluates were analyzed in three technical replicates each consuming 20% sample share on an Orbitrap Fusion Lumos. From autologous pairs of primary und recurrent tumors suitable for LFQ-MS, five adjusted technical replicates starting with 17% sample share in the dose-finding run were acquired. Two additional technical replicates consuming 17% sample share each were measured of samples heavily diluted for relative quantitation purposes. The maximum available number of LC-MS/MS runs were subjected to comparative profiling upon co-processing. Direct injection of several peptide eluates was performed as indicated in Table 6.

### HLA typing, whole exome, and RNA sequencing

HLA class I and II allotypes of all primary glioblastomas without autologous recurrent tumors being available as well as of ZH753, ZH784, and patients GBM13, 17, 18, 19, 22, and 23 were determined on 4-digit level by next-generation sequencing at HistoGenetics (New York, USA). For remaining patients, 4-digit HLA class I typing was retrieved from whole exome sequencing data of genomic DNA isolated from blood or PBMCs using the OptiType[20] algorithm by Marie Gauder and Dr. Stefan Czemmel (Quantitative Biology Center, University of Tübingen).

Whole exome and RNA sequencing was performed for all 22 patients with paired primary and recurrent glioblastoma as well as for ZH613, ZH616, ZH654, ZH681, ZH720, ZH750, ZH753, ZH757, and ZH802 at CeGaT GmbH (Tübingen). Genomic DNA was isolated from PBMCs using the DNeasy 96 Blood & Tissue Kit (Qiagen). For patients GBM2-11, isolated genomic DNA had already been provided by Dr. med. Malte Mohme. DNA and RNA were isolated from fresh frozen tumor tissue using the AllPrep DNA/RNA Mini Kit (Qiagen). Whole exome sequencing libraries were prepared from 50 ng DNA using the Twist Human Core Exome Kit (Twist Bioscience) and subsequent data acquisition was performed by paired-end sequencing and at 100 bp read length on a NovaSeq 6000 sequencing system (Illumina). For genomic DNA, 10 giga bases (Gb) were acquired per sample, whereas sequencing depth of tumor samples came up to 18 Gb. RNA was only isolated from tumor tissues. Following quality control for RNA integrity, sequencing libraries were prepared from 10 ng RNA using the SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian (Takara Bio), whereby ribosomal RNA was removed. Paired-end sequencing at 100 bp read length was performed for 10 Gb on a NovaSeq 6000. Sequencing reads were demultiplexed employing the Illumina bcl2fastq 2.19 followed by Adapter trimming with Skewer 0.2.2[21] (exome sequencing) or cutadapt 1.12[22]

(RNA sequencing). FASTQ[23]-formatted files were uploaded to qPortal[24] (Quantitative Biology Center, University of Tübingen) for further data analysis.

Variant calling and calculation of expression values at a time when only 13 primary tumors had been sequenced, was performed by Christopher Mohr and Silvia Morini (Quantitative Biology Center, University of Tübingen). BWA v0.7.17-r1188[25] was employed to map exomic reads to the human reference genome GRCh37 (Ensembl release 75). Somatic variants comprising insertions or deletions (indels) as well as single nucleotide variants (SNVs) were called separately by Strelka v2.9.3[26] with variants being annotated with SnpEff v4.3t[27]. Variant allele frequencies (VAFs) representing the percentage of glioblastoma cells harboring the respective mutation were computed by division of the number of variant reads by the number of WT reads acquired from the same genomic region. Of note, this calculation assumes relatively high tumor cell content in the glioblastoma sample from which DNA was extracted.[28] Reported mutational loads (both indels and SNVs) refer to variants passing confidence filtering criteria of the variant caller Strelka v2.9.3 (filter 'PASS'). The probability $p$ of an incorrect variant call is calculated from a phred-scale empirical variant scoring (EVS) step: $p = 10^{\frac{-somaticEVS}{10}}$. The EVS model is based on a supervised random forest classifier and provides a quality score for every variant.[29,30] This quality score takes into account '(1) the genotype probability computed by the core variant probability model, (2) root-mean-square mapping quality, (3) strand bias, (4) the fraction of reads consistent with locus haplotype model, and (5) the complexity of the reference context as measured by metrics such as homopolymer length and compressibility'[29]. Applying the 'PASS' filter to variants excludes variants with a read depth < 2 in the glioblastoma sample and sets an EVS threshold of ≥ 7 (equivalent to $p < 20\%$) or of ≥ 6 (equivalent to $p < 25\%$) for SNVs and indels, respectively.[29,30] Finer selection for confident somatic variants was based on filtering for mutations with an EVS ≥ 13 corresponding to a probability of ≤ 5% that the respective variant is incorrect.

Analysis of RNA data was based on the Nextflow pipeline release 1.3[31] and the Sarek pipeline release 2.3[32]. Quality control of FASTQ files was accomplished by FASTQC[33], whereas MultiQC v1.73[34] aggregated the Bioinformatic workflow. Before read alignment to the human reference genome GRCh37 (Ensembl release 75) with STAR v2.6.1d[35], another adapter trimming step was conducted *via* Trim Galore v0.5.05[36]. RNA sequencing data was evaluated using RSeQC v3.0.0[37] with features (transcripts) being counted by featureCounts v1.6.4[38]. Besides reporting raw counts per gene, reads per kilobase per million mapped reads (RPKM) were calculated as a measure of expression strength[39] by (1) dividing the total number of reads in the respective sample by 1,000,000, (2) normalizing the read counts of a gene by the factor calculated in (1), and (3) dividing the result of (2) by the number of kilobases spanned by the respective gene.

For GBM 17, 18, 22, and 23, whole exome and RNA sequencing data of primary tumors and genomic DNA will be re-analyzed by Marie Gauder and Dr. Stefan Czemmel to ensure comparability with data obtained from autologous tumor recurrence.

### *Search for neo-antigenic HLA-presented peptides*

From the initial unfiltered variant calling performed for 13 primary glioblastomas, FASTA-formatted files were computed by translation of whole exome data with FRED 2[40]. Patient-

specific proteomes including mutated protein sequences arising from SNVs concatenated with a human reference proteome (Swiss-Prot release UP000005640, 20,416 reviewed protein sequences) as well as common laboratory contaminants served as database for annotation of MS$^2$ spectra using the MHCquant pipeline[41]. MHCquant was built within the OpenMS 2.4 framework[42] employing the search engine Comet 2016.01 rev. 3[43] for peptide identification and Percolator 3.1.1[44] for FDR estimation. The FDR threshold was set to ≤ 5% on peptide level and only the best sequence annotation (rank 1) was reported per fragment spectrum. Oxidation of methionine residues was the only permitted dynamic modification and no enzymatic restriction was set. Database search was performed at 5 ppm precursor ion and 0.02 Da fragment ion tolerance. HLA class I ligands were restricted to span 8 to 12 AA, to have a molecular weight of 800 to 2,500 Da, and to carry two or three positive charges. In contrast, the search space for HLA class II-restricted peptides was set to a length of 8 to 25 AA, a precursor mass of 800 to 5,000 Da, and a number of 2 to 5 positive charges. Identifications mapping to common laboratory contaminants were automatically excluded from reported peptide lists. Neo-antigenic peptides were predicted by shifting a window of 8 to 12 AA (HLA class I) or 8 to 25 AA (HLA class II) around the mutated amino acid, whereby no prediction of HLA binding probability was performed. Peptides identified by the MHCquant pipeline were designated as mutated when uniquely mapping to the list of predicted mutated 8- to 12-mers or 8- to 25-mers, respectively.

Potential neo-epitopes as identified by Leon Bichmann using the MHCquant pipeline underwent an in-house validation step by standard data processing with the SEQUEST search engine embedded in Proteome Discoverer 1.4 against patient-specific customized databases. This especially allowed assessing the spectral quality by viewing fragment spectra and corresponding scores. Moreover, a prediction of binding to the patient's HLA class I allotypes was performed as described in 3.2.2 and peptide sequences (with all possible exchanges of the isobaric AAs leucine and isoleucine) were queried against the UniProt[45] and dbSNP[46] databases to exclude that the identified peptide mapped to a WT sequence or a single nucleotide polymorphism. The VAF of the mutation-of-interest was reviewed with VAFs ≥ 5% being considered as confident. In addition, the expression of the corresponding gene (independent of whether WT or variant transcripts were sequenced) was verified based on RNA data, whereby a minimum of 10 raw counts was set as threshold. Finally, heavy isotope-labeled peptides were synthesized by solid-phase peptide synthesis (Wirkstoffpeptidlabor, Department of Immunology, University of Tübingen), whereby heavy isotope-labeled isoleucine [I($^{13}C_6$; $^{15}N$)] was incorporated at one position each. Peptides were first completely dissolved in 100% DMSO, facilitated by vortexing, with subsequent addition of MS grade $H_2O$. Peptides were diluted with $A_{Load}$ and spiked at ~100 fmol/µl into a complex matrix of HLA class I peptides eluted from JY cells. Following LC-MS/MS (standard HLA class I method as described in 3.2.2) on an Orbitrap Fusion Lumos, raw files were processed using a concatenated FASTA-formatted file consisting of the Swiss-Prot release from September 27$^{th}$ 2013 (20,279 reviewed protein sequences) and the mutant protein sequences. Heavy isotope labels (+7.017 Da) were permitted as additional dynamic modification for isoleucine residues and processing was otherwise performed as described in 3.2.2 for HLA class I peptidome data.

Somatic mutations of confirmed HLA-presented neo-antigenic peptides were queried against the COSMIC database[47] to investigate whether these are recurrent or even known driver mutations. In addition, effects of these mutations on protein phosphorylation were assessed using the PhosphoMotif Finder of the Human Protein Reference Database[48]. To search for threonine and tyrosine phosphorylation within the confirmed mutated peptide of ZH681, phosphorylation (+79.966 Da) was permitted as dynamic modification at these residues for standard data processing with the SEQUEST search engine against the patient-specific customized database.

# 4 Results

## 4.1 Peptide yields of HLA-IPs and HLA class I allotype coverage

To define candidate targets for glioblastoma immunotherapy naturally presented on HLA, we analyzed 38 primary and 24 recurrent tumor specimens derived from 40 different patients. The study cohort included 55 distinct HLA class I allotypes covering 99.95% of the world population, whereby 92.17% of all individuals are expected to be positive for at least three allotypes (Supplementary Figure 1). The most frequent allotypes among glioblastoma patients were HLA-A*02:01 (63%), -A*03:01 (28%), -A*01:01 (25%), -B*07:02 (33%), -B*44:02 (28%), -B*08:01 (15%), -B*51:01 (9%), -C*07:01 (30%), -C*07:02 (30%), -C*01:02 (20%), and -C*06:02 (20%) (Supplementary Table 1).

A median of 3742 [450-7007] and 3710 [65-6333] HLA class I ligands were identified from primary or recurrent glioblastomas, respectively. All HLA class I peptide eluates except that of GBM5R exhibited a purity of at least 80% and none of the samples were censored for low peptide yield or low percentage of HLA class I ligands. Concerning HLA class II, median peptide yields came up to 2527 [293-8812] and 3203 [333-8287]. The total number of unique HLA class I and II peptides, HLA class I ligands as well as the purity of HLA class I peptide eluates are given in Figure 10 A for every specimen. The length distribution of HLA class I ligands clearly peaked at 9 AA length, whereas HLA class II-presented peptides were typically 13- to 18-mers. A fraction of samples showed a broadened length distribution curve elevated either for shorter (≤ 12 AA) or longer (≥ 19 AA) peptides, whereby no difference between primary and recurrent tumors was observed (Figure 10 B). Taking tissue masses subjected to HLA-IP into account (Table 6), enabled us to investigate whether peptide yields correlate with sample quantities used. The amount of glioblastoma tissue correlated with the number of HLA class I ligand identifications, whereas this was not the case for HLA class II-presented peptides (Figure 10 C). Considering the relative number of identified unique peptides per one mg of tissue input revealed a slightly increased yield of HLA class I as compared with HLA class II peptides eluted from primary glioblastomas and of HLA class II-restricted peptides identified on recurrent *versus* primary tumors (Figure 10 D).
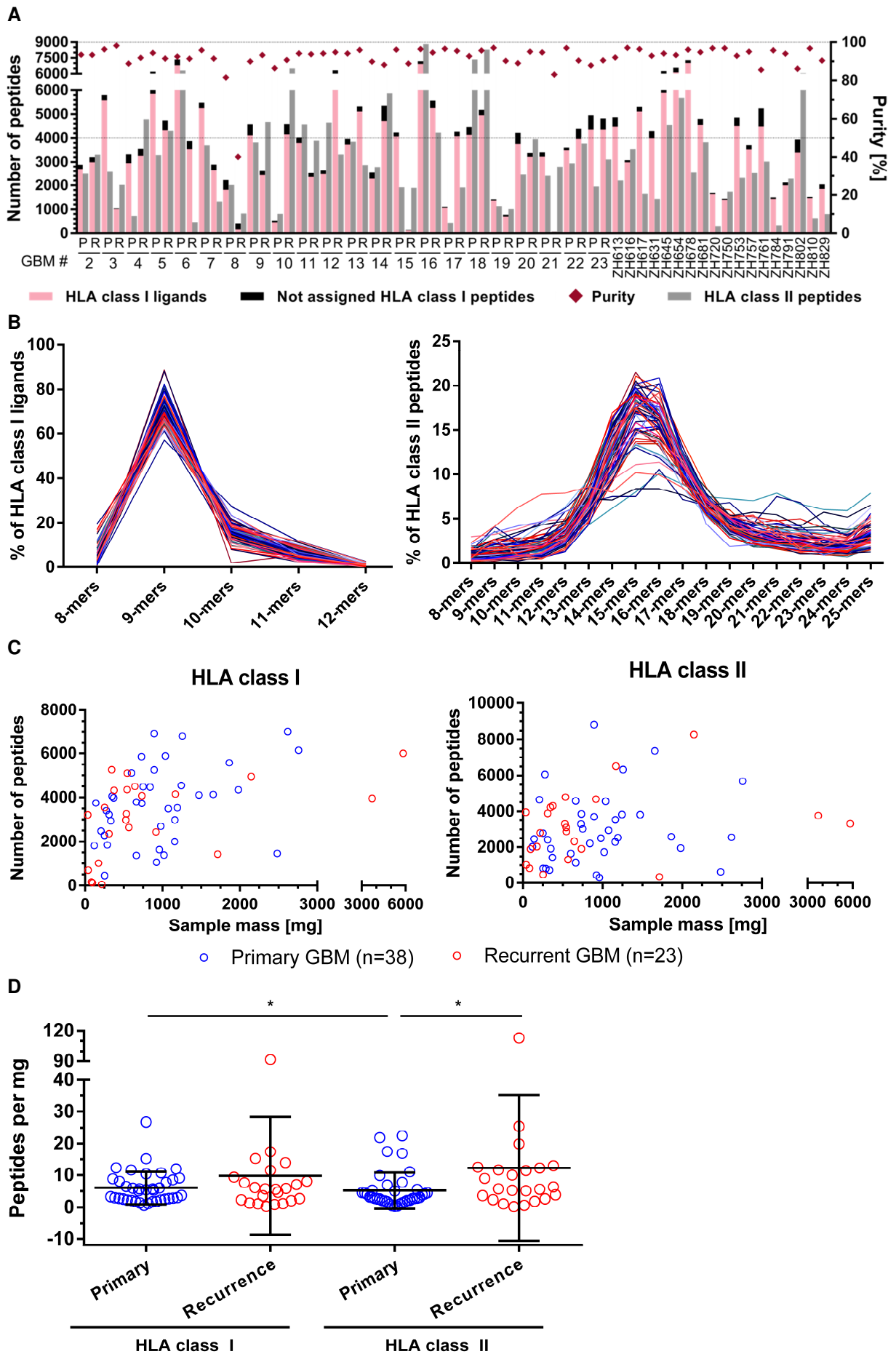
**Figure 10. Number and length distribution of identified HLA class I- and II-presented peptides. (A) HLA class I and II peptide yields of primary and recurrent glioblastomas.** Calculated purities refer to the proportion of HLA class I peptides annotated to an HLA allotype of the respective patient. **(B) Length distribution of HLA class I ligands and HLA class II peptides.** Across the entire dataset, 9-mers constituted 71% of HLA class I ligands, whereas 73% of HLA class II-presented peptides had a length between 13 and 18 AA. Each line represents data of one sample with primary and recurrent tumors being illustrated in blueish and redish shades, respectively. While the entire HLA class II dataset included 9% of peptides with ≤ 12 AA and 19% of peptides with ≥ 19 AA, these shares were distorted in a fraction of patients (GBM2R, GBM4P, GBM8P, GBM8R, GBM17R, ZH720, and ZH802: 16-28% 8- to 12-mers; GBM4P, GBM6R, GBM10P, ZH679, ZH720, and ZH829: 30-49% 19- to 25-mers). **(C) Unique peptides per sample *versus* amount of tissue subjected to HLA-IP.** The total amount of unique HLA class I ligands or HLA class II-restricted peptides was set in relation to the amount of tissue subjected to HLA-IP, whereby GBM14R was excluded owing to sample loss during HLA-IP. By non-linear regression (one-phase association) exponential functions with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) were fitted. The goodness of fit was poor for all these models: $R^2$ = 0.13 (primary HLA class I) / 0.40 (recurrence HLA class I) / 0.02 (primary HLA class II) / 0.09 (recurrence HLA class II). However, a correlation analysis across normally distributed HLA class I ligand identifications (according to D'Agostino & Pearson omnibus normality test) identified a significant correlation of sample quantities used and the number of identified HLA class I-restricted peptides: two-tailed *p*-values = 0.0169 (primary) / 0.0222 (recurrence). In turn, HLA class II peptide yields were not found to correlate with sample masses subjected to HLA-IP: two-tailed *p*-values = 0.1854 (primary HLA class I; Spearman correlation for non-Gaussian data) / 0.2840 (recurrence HLA class I; Pearson correlation for parametric data). **(D) Peptide yields normalized to one mg of tissue input.** To investigate, whether the relative number of peptide identifications differs between primary and recurrent glioblastomas (21/23 recurrent samples are autologous to primary tumors) as well as between HLA class I- and II-IPs of the same sample, Wilcoxon matched-pairs signed rank tests were performed (normalized peptide yields – excepting recurrence HLA class II – did not have Gaussian distributions according to D'Agostino & Pearson omnibus normality test). Narrowly significant differences were observed between the number of HLA class I and II peptides eluted from primary tumors (two-tailed *p*-value = 0.0429) as well as between the number of HLA class II-presented peptides on primary *versus* recurrent glioblastomas (two-tailed *p*-value = 0.0290). One outlier, GBM20R, showed both increased relative HLA class I and II peptide yields.

## 4.2 Definition of glioblastoma-associated antigens

By immunoaffinity chromatography and LC-MS/MS, the repertoire of HLA class I and II peptides and corresponding antigens naturally presented on 62 glioblastomas was mapped. To define glioblastoma-associated antigens and peptides, an in-house benign database comprising 30 distinct primary human organs (n=418 HLA class I and n=364 HLA class II datasets) was subtracted. The term glioblastoma-associated was assigned to peptides and antigens that were never identified on CNS-related tissues (brain, cerebellum, and spinal cord) and for which a maximum of one non-CNS-related sample was positive. Moreover, the robust presentation on both primary and recurrent tumors was defined as a prerequisite for all candidate immunotherapeutic targets reported herein. The frequency of positive primary human malignancies other than glioblastoma (n=824 samples for HLA class I; n=585 samples for HLA class II) encompassing 36 cancer entities was evaluated as well. As additional criterium to select targets for cancer immunotherapies, RNA expression data acquired across a large set of benign human tissues and deposited in the GTEx database[15] was reported for every candidate antigen. Further, this allowed the identification of antigens not known to be expressed in any tissue (defined as less than ten transcripts per million (TPM) in any tissue), such with a classical CTA-like expression profile (not exceeding ten TPM in other organs than

testis) as well as to exclude antigens with brain-specific expression representing the tissue of tumor origin.

### *Glioblastoma-associated HLA class I-presented antigens and peptides*

HLA class I peptidome analyses of primary (n=38) and recurrent glioblastoma (n=24) allowed the identification of 12,182 and 10,646 distinct source proteins represented by HLA class I ligands on primary or recurrent tumor tissue, respectively. These represent between 92% (recurrence) and 96% (primary) of the estimated maximum attainable amount of distinct source proteins (Figure 11 C). Despite the vast overlap of glioblastomas with benign samples, 381 antigens were exclusively presented on tumors of at least two different patients (Figure 11 A). Following manual curation of the underlying peptides for HLA motifs as well as multi-mapping to several source proteins, a set of 62 glioblastoma-associated antigens and corresponding peptides naturally presented on 5-50% of primary as well as on 4-63% of recurrent tumors was created. Among these, TANC2, FA2H, and BEST1 were the most frequent ones. Despite being shared by primary and recurrent glioblastomas, several antigens were rather associated with primary tumors (e.g. SHISA4), while others were preferentially presented at disease recurrence (e.g. LHFPLS; Figure 11 B). Peptide sequences and their HLA restriction, a listing of positive samples, and the GTEx profile of the corresponding source protein can be retrieved from Supplementary Table 2. It is noteworthy that two of these tumor-associated HLA class I antigens (HSF2BP, E2F1) exhibited a CTA-like expression profile and were not listed in the CTDatabase[49].

**A**
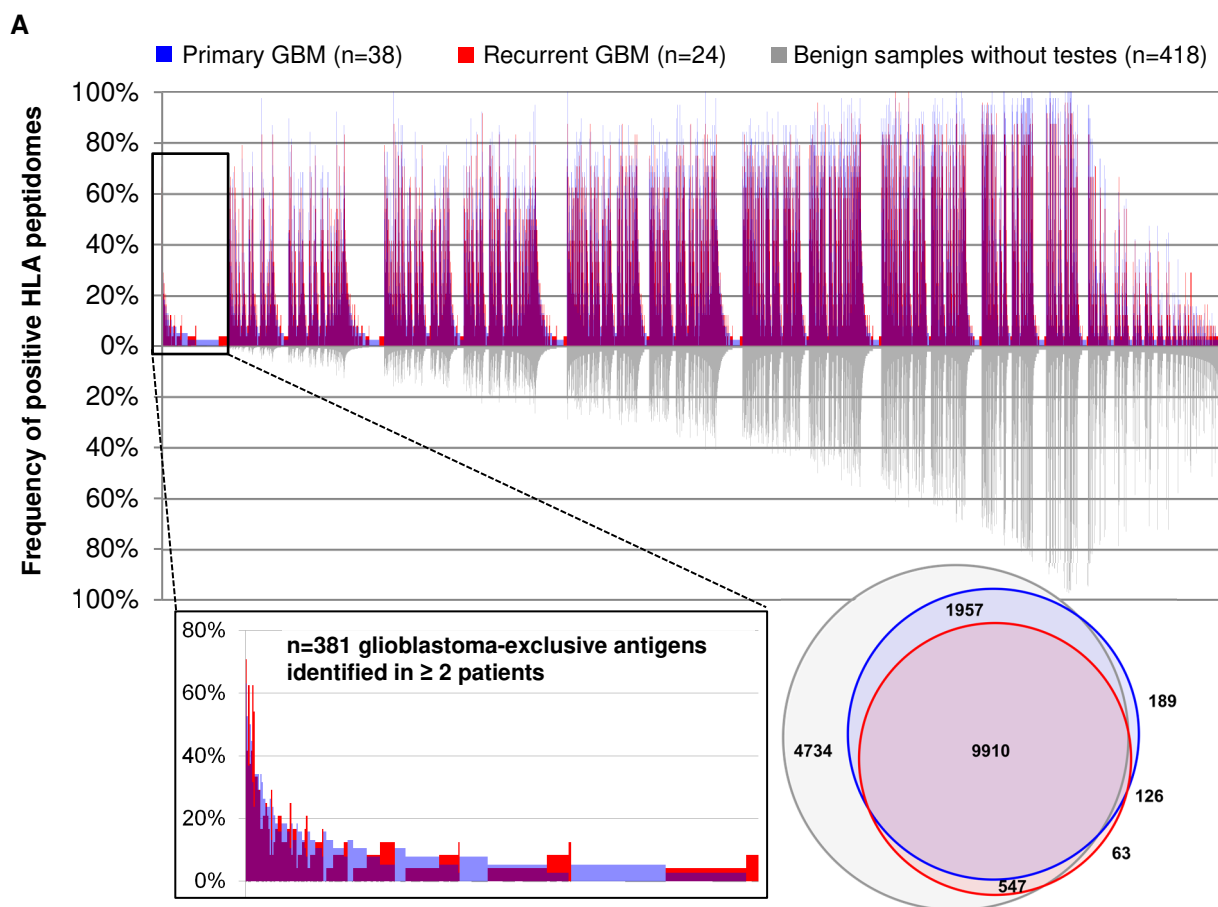
**B**

G patch domain-containing protein 11(GPATCH11)

growth arrest and DNA damage-inducible proteins-interacting protein 1(GADD45GIP1)    T-box transcription factor TBX15(TBX15)

85/88 kDa calcium-independent phospholipase A2(PLA2G6) uncharacterized protein C7orf50 (C7orf50)

CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 4 (ST3GAL4)    IGF-like family receptor 1(IGFLR1)

lipoma HMGIC fusion partner-like 2 protein(LHFPL2) mitochondrial 39S ribosomal protein L35(MRPL35)

zinc finger and BTB domain-containing protein 47 (ZBTB47)   cannabinoid receptor 1(CNR1)   glycosyltransferase-like domain-containing protein 2 (GTDC2)

protein shisa-4 (SHISA4) transmembrane protein 255A(TMEM255A)

5'-AMP-activated protein kinase subunit beta-2(PRKAB2)    neuronal migration protein doublecortin(DCX)

SAC3 domain-containing protein 1(SAC3D1)    protein shisa-9(SHISA9)    lysyl oxidase homolog 2 (LOXL2)

sugar phosphate exchanger 3(SLC37A3)

collagen alpha-2(IX) chain(COL9A2) solute carrier family 15 member 2 (SLC15A2)

insulin-like growth factor-binding protein 2(IGFBP2)    transmembrane protein 87A(TMEM87A)

fatty acid 2-hydroxylase (FA2H)

transcription factor SOX-21(SOX21)    transcription factor HES-4 (HES4)

protein Wnt-5a(WNT5A) protein TANC2 (TANC2)

transcription factor SOX-2 (SOX2)    heat shock factor 2-binding protein (HSF2BP)
uroporphyrinogen-III synthase (UROS)

6-pyruvoyl tetrahydrobiopterin synthase(PTS) Bestrophin-1 (BEST1)

T-box transcription factor TBX18(TBX18)

embryonal Fyn-associated substrate (EFS)    protein pitchfork (PIFO)    probable G-protein coupled receptor 75(GPR75)

NADH dehydrogenase [ubiquinone] 1 alpha    transcription factor E2F1(E2F1)

protein Dos (DOS)    transmembrane protein 237(TMEM237)

Frizzled-3(FZD3) subcomplex subunit 4-like 2 (NDUFA4L2) peroxisome biogenesis factor 10 (PEX10)

POU domain, class 3, transcription factor 3(POU3F3) leucine-rich repeat neuronal protein 3(LRRN3)

uncharacterized protein C2orf72(C2orf27) blood vessel epicardial substance (BVES)
transcription factor E2F7 (E2F7)

sodium- and chloride-dependent creatine transporter 1(SLC6A8)   uncharacterized protein C21orf62 (C21orf62)   olfactory receptor 51A7 (OR51A7)

immunoglobulin superfamily member 11(IGSF11)integral membrane protein DGCR2/IDD(DGCR2)

uncharacterized aarF domain-containing protein kinase 1(ADCK1)    solute carrier family 25 member 53(SLC25A53)

exostosin-like 3 (EXTL3)    low-density lipoprotein receptor class A domain-containing protein 3 (LDLRAD3)

leucine-rich repeats and immunoglobulin-like domains protein 1(LRIG1)    BTB/POZ domain-containing protein 17(BTBD17)

oligodendrocyte transcription factor 3(OLIG3)

**C**
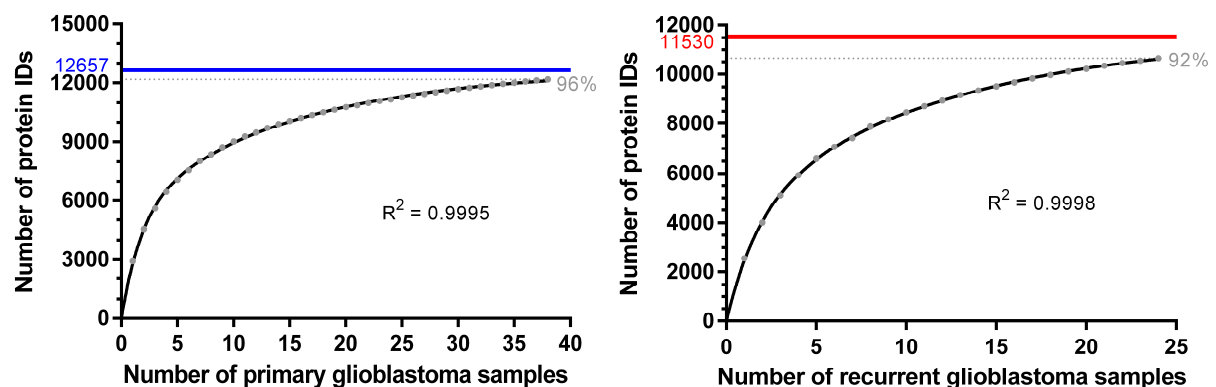


**Figure 11. Definition of glioblastoma-associated antigens based on class I immunopeptidome analyses. (A) Comparative profiling of the HLA class I peptidome of glioblastoma *versus* an in-house benign database.** Each bar in this waterfall plot (associated with the x-axis) represents a single source protein, whereas the frequency of positive HLA peptidomes is shown on the y-axis, separately

for primary glioblastoma (n=38), recurrent glioblastoma (n=24), and benign samples without testes (n=418 covering 29 different human tissues). Those source proteins detected on a maximum of one non-CNS-related tissue were designated as glioblastoma-exclusive, whereby n=361 were identified on primary and/or recurrent tumors of at least two patients (highlighted as enlarged view on the left). The Venn diagram on the right illustrates the number of distinct HLA class I-presented antigens per group, however, the overlaps cannot map the permission of one positive non-CNS-related sample within the benign dataset. **(B) Word cloud of glioblastoma-associated antigens.** Based on comparative profiling and subsequent quality control of underlying peptides (HLA motifs as well as multi-mapping to several source proteins), a set of 62 glioblastoma-associated antigens naturally presented on 5-50% of primary as well as on 4-63% of recurrent tumors was defined. The font size in the word cloud is proportional to the frequency of positive primary (blue) and recurrent (red) glioblastomas. **(C) Saturation analysis for the identification of antigens represented by HLA class I ligands on primary or recurrent glioblastoma tissue.** For each source count, the mean number of antigens was calculated by 1,000 random samplings. Using non-linear regression, exponential functions with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) were fitted. For both models, the goodness of fit was in the uppermost range ($R^2$ = 0.9995 and $R^2$ = 0.9998). Based on these curves, the maximum attainable number of distinct source proteins was estimated (highlighted as solid lines). With the available number of 38 primary and 24 recurrence samples, 96% or 92% of the estimated maximum attainable amount of unique HLA class I-presented proteins had been identified, respectively. Considering primary and recurrent tumors jointly as 62 glioblastomas, these achieve 98% coverage of the estimated maximum attainable number of distinct source proteins (Supplementary Figure 2).

**A**

**B**
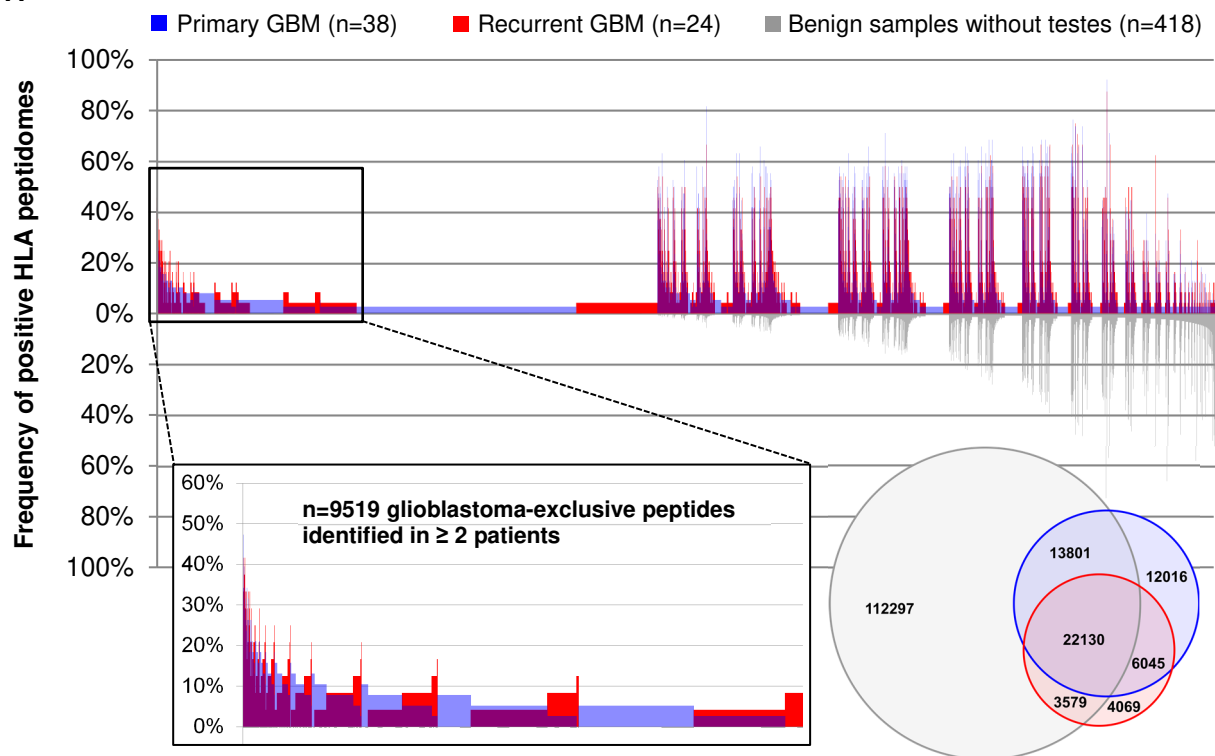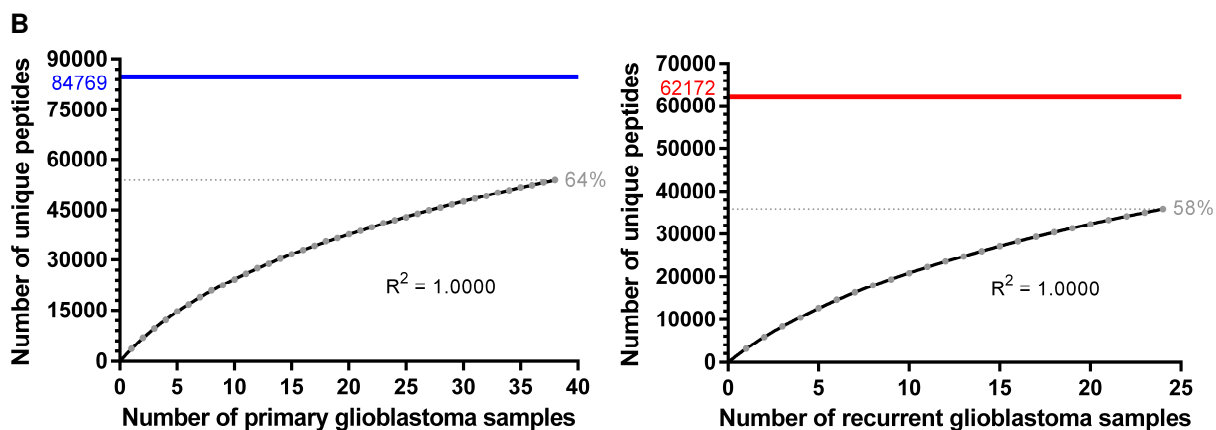


**Figure 12. HLA class I peptidomics to define glioblastoma-associated peptides. (A) Comparative profiling of HLA class I ligands presented on glioblastoma *versus* an in-house benign database.** Every peptide evaluated for tumor association is represented by a bar in the waterfall plot (associated with the x-axis), whereas the y-axis shows the frequency of positive HLA peptidomes, separately for primary glioblastoma (n=38), recurrent glioblastoma (n=24), and benign samples without testes (n=418 covering 29 different human tissues). Peptides were designated as glioblastoma-exclusive, when detected on a maximum of one non-CNS-related tissue, whereby n=9,519 were identified on primary and/or recurrent tumors of at least two patients (highlighted as enlarged view on the left). The number of distinct HLA class I ligands per group is illustrated by the Venn diagram on the right, whereby the overlaps cannot map the permission of one positive non-CNS-related sample within the benign dataset. **(B) Saturation analysis for the identification of HLA class I ligands in primary or recurrent glioblastoma tissue.** For each source count, the mean number of peptides was calculated by 1,000 random samplings. Using non-linear regression, exponential functions with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) were fitted. For both models, the goodness of fit was in the uppermost range ($R^2$ = 1.0000). Based on these curves, the maximum attainable number of distinct peptides was estimated (highlighted as solid lines). With the available number of 38 primary and 24 recurrence samples, 64% and 58% of the estimated maximum attainable amount of unique HLA class I ligands had been identified, respectively. Considering primary and recurrent tumors jointly as 62 glioblastomas, these achieve 74% coverage of the estimated maximum attainable number of distinct peptides (Supplementary Figure 2).

On the peptide level, 53,992 and 35,823 distinct HLA class I ligands were eluted from primary (n=38) and recurrent glioblastoma (n=24), obtaining 64% and 58% of the estimated maximum attainable coverage, respectively (Figure 12 B). Comparative profiling identified 9,519 glioblastoma-exclusive peptides presented on primary and/or recurrent tumors of at least two different patients (Figure 12 A). Subsequent to manual curation, a set of 155 peptides derived from 158 antigens and presented on 16-55% of primary as well as on 17-46% of recurrent tumors was defined. Among these, HLA ligands derived from GFAP, CERS1, CARNS1, CHI3L2, and PTPRZ were the most frequent ones. Peptide sequences and their HLA restriction, a listing of positive samples, and the corresponding source protein are given in Supplementary Table 3.

Combining the list of peptides derived from HLA class I-presented glioblastoma-associated antigens (Supplementary Table 2) with that of HLA class I ligands designated as glioblastoma-associated (Supplementary Table 3) gave n=357 candidate target peptides for cancer immunotherapy. These cover 99.93% of the world population (Supplementary Figure 3), whereby an average of 84 peptides are expected to match per patient. The population coverage on a per-country basis is shown in Figure 13.
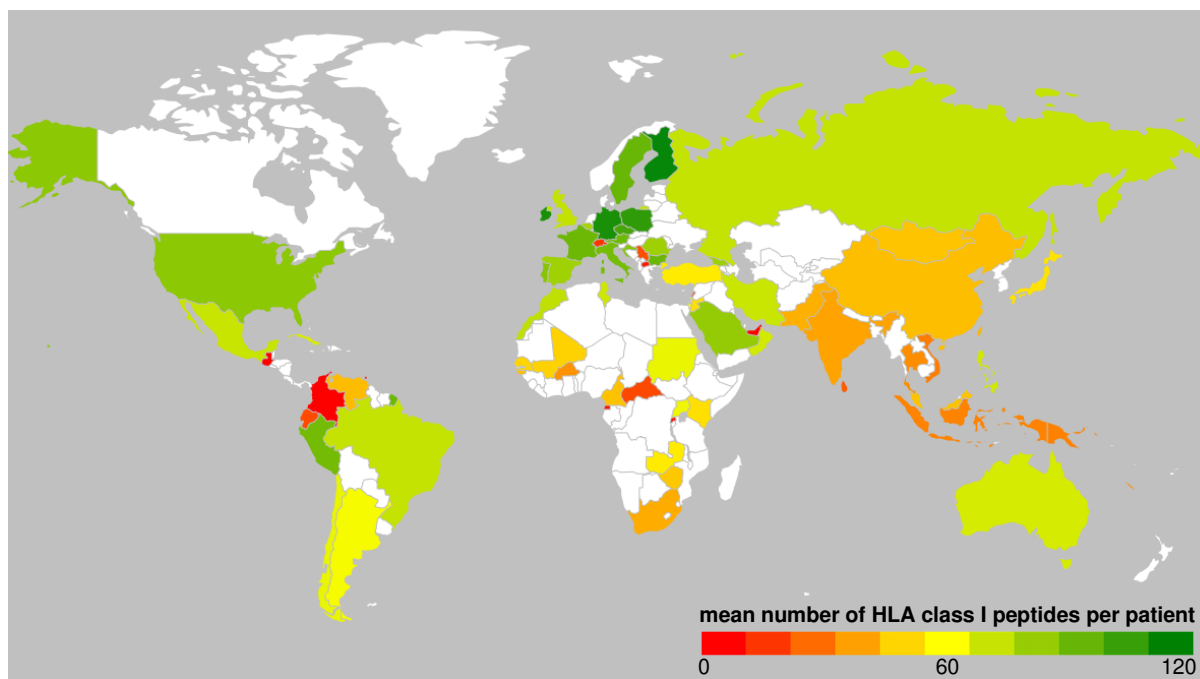
**Figure 13. Population coverage of glioblastoma-associated HLA class I peptides.** Using the population coverage tool provided by the IEDB Analysis Resource[50], the population coverage of the n=357 candidate target peptides for glioblastoma immunotherapy was calculated on a per-country basis. On average, 84 HLA class I peptides match per patient worldwide. For visualization of the United Kingdom, the individual values of England, Northern Ireland, Scotland, and Wales were multiplied with the relative area portion. Countries not included in the IEDB tool or not covered by the geographic heat map add-on of Microsoft Excel are colorless.

### *Glioblastoma-associated HLA class II antigens*

HLA class II peptidome analyses of primary (n=38) and recurrent tumors (n=24) allowed the identification of 7,792 and 7,225 distinct source proteins giving rise to HLA class II-restricted peptides on glioblastoma tissue, respectively. These represent between 72% (recurrent glioblastoma) and 79% (primary glioblastoma) of the estimated maximum attainable amount of distinct source proteins (Figure 14 C). Despite the vast overlap of glioblastomas with benign samples, 413 antigens were exclusively presented on primary and/or recurrent tumors of at least two different patients (Figure 14 A). Following manual curation of the underlying peptides for peptide length and the presence of length variants as well as multi-mapping to several source proteins, a set of 26 glioblastoma-associated antigens and corresponding peptides naturally presented on 5-32% of primary as well as on 4-46% of recurrent tumors was created. Among these, GPC5, COLGALT2, NOP16, ESCO1, and BBOX1 were the most frequent ones. Despite being shared by primary and recurrent glioblastomas, several antigens were rather associated with primary tumors (e.g. NOP16), while others were preferentially presented at disease recurrence (e.g. CDC26; Figure 14 B). Peptide sequences, a listing of positive patients, and the GTEx profile of the corresponding source protein can be retrieved from Supplementary Table 2. Of note, one of these glioblastoma-associated HLA class II antigens (RAD51) exhibited a CTA-like expression profile and was not listed in the CTDatabase[49].

**A**



**B**



**C**
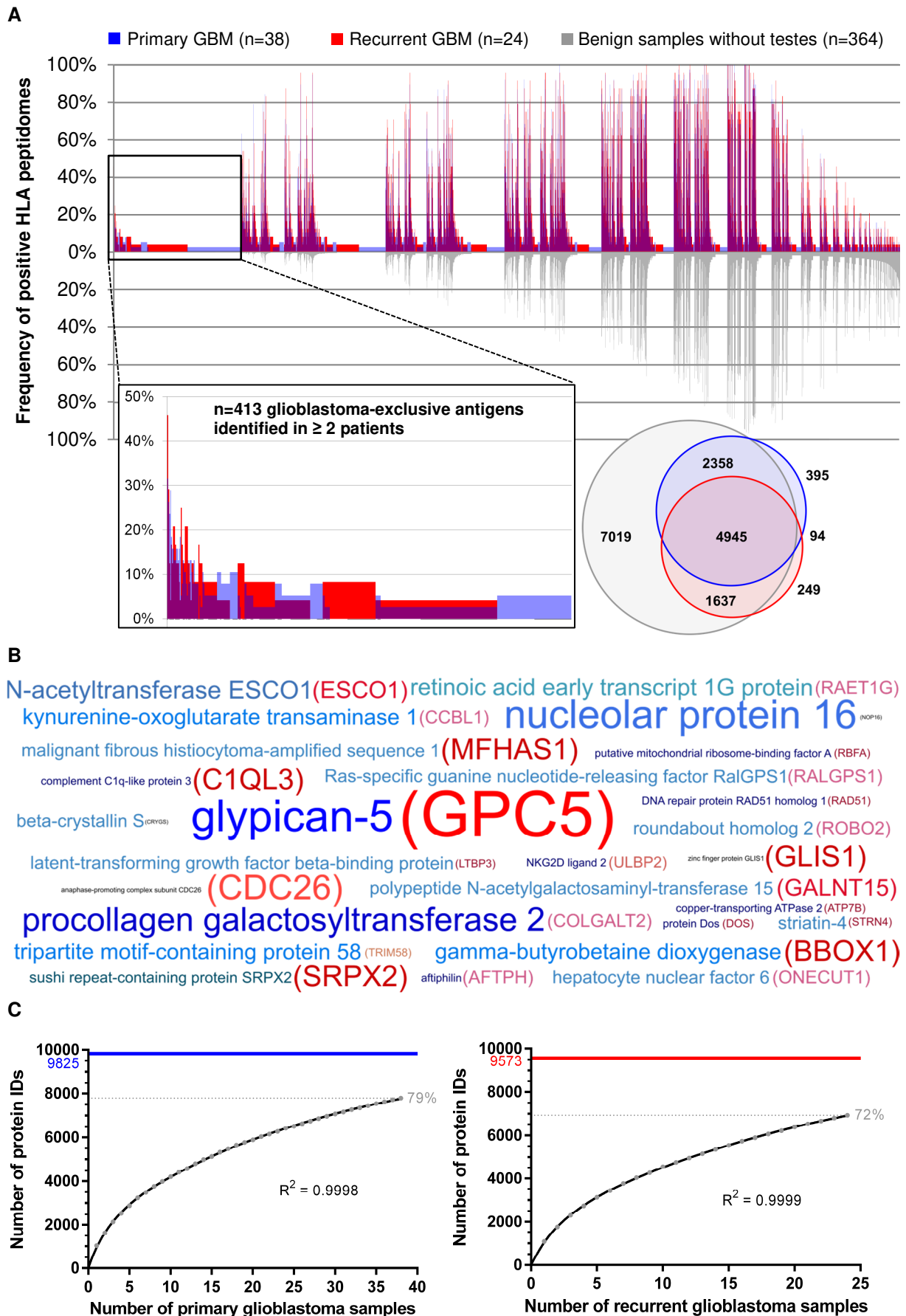


**Figure 14. Definition of glioblastoma-associated antigens based on class II immunopeptidome analyses. (A) Comparative profiling of the HLA class II peptidome of glioblastoma *versus* an in-**

**house benign database.** Each bar in this waterfall plot (associated with the x-axis) represents a single source protein, whereas the frequency of positive HLA peptidomes is shown on the y-axis, separately for primary glioblastoma (n=38), recurrent glioblastoma (n=24), and benign samples without testes (n=364 covering 30 different human tissues). Those source proteins detected on a maximum of one non-CNS-related tissue were designated as glioblastoma-exclusive, whereby n=413 were identified on primary and/or recurrent tumors of at least two patients (highlighted as enlarged view on the left). The Venn diagram on the right illustrates the number of distinct HLA class II-presented antigens per group, however, the overlaps cannot map the permission of one positive non-CNS-related sample within the benign dataset. **(B) Word cloud of glioblastoma-associated antigens.** Based on comparative profiling and subsequent quality control of underlying peptides (peptide length and/or presence of length variants as well as multi-mapping to several source proteins), a set of 26 glioblastoma-associated antigens naturally presented on 5-32% of primary as well as on 4-46% of recurrent tumors was defined. The font size in the word cloud is proportional to the frequency of positive primary (blue) and recurrent (red) glioblastomas. **(C) Saturation analysis for the identification of antigens represented by HLA class II peptides on primary or recurrent glioblastoma tissue.** For each source count, the mean number of antigens was calculated by 1,000 random samplings. Using non-linear regression, exponential functions with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) were fitted. For both models, the goodness of fit was in the uppermost range ($R^2 = 0.9998$ and $R^2 = 0.9999$). Based on these curves, the maximum attainable number of distinct source proteins was estimated (highlighted as solid lines). With the available number of 38 primary and 24 recurrence samples, 79% or 72% of the estimated maximum attainable amount of distinct HLA class II-presented proteins had been identified, respectively. Considering primary and recurrent tumors jointly as 62 glioblastomas, these achieve 83% coverage of the estimated maximum attainable number of distinct source proteins (Supplementary Figure 2).

On the peptide level, 48,149 and 40,375 distinct HLA class II-presented peptides were eluted from primary (n=38) and recurrent glioblastoma (n=24), obtaining 59% (primary) and 47% (recurrence) of the estimated maximum attainable coverage (Figure 15 B). Subsequent to comparative profiling, all antigens represented by at least one glioblastoma-exclusive HLA class II-presented peptide were subjected to hotspot analysis (Figure 15 A). Glioblastoma-associated HLA class II presentation hotspots were defined to have a minimum length of eight AA and to be covered by peptides identified in at least six primary as well as four recurrent tumors of a minimum of eight different patients, while not having matching sequences in benign samples. This identified a set of 21 antigens harboring regions uniquely presented on malignant tissue with peptide-specific frequencies reaching up to 39% and 29% of positive HLA peptidomes for primary and recurrent glioblastomas, respectively. Peptide sequences, a listing of positive patients, and the corresponding source protein are given in Supplementary Table 4.

Comparing glioblastoma-associated HLA class I- (n=62) and II-presented antigens (n=26) as well as glioblastoma-associated HLA class I- (n=158 source proteins) and II-restricted peptides (n=21 source proteins) delineated seven antigens (DOS, COLGALT2, PTPRZ1, NRCAM, LRP1, TNC, and CHI3L1) represented on both HLA class I and II molecules. Remaining glioblastoma-associated candidate target antigens and peptides were uniquely identified *via* either HLA class I or II peptidome analysis.

**A**



**B**
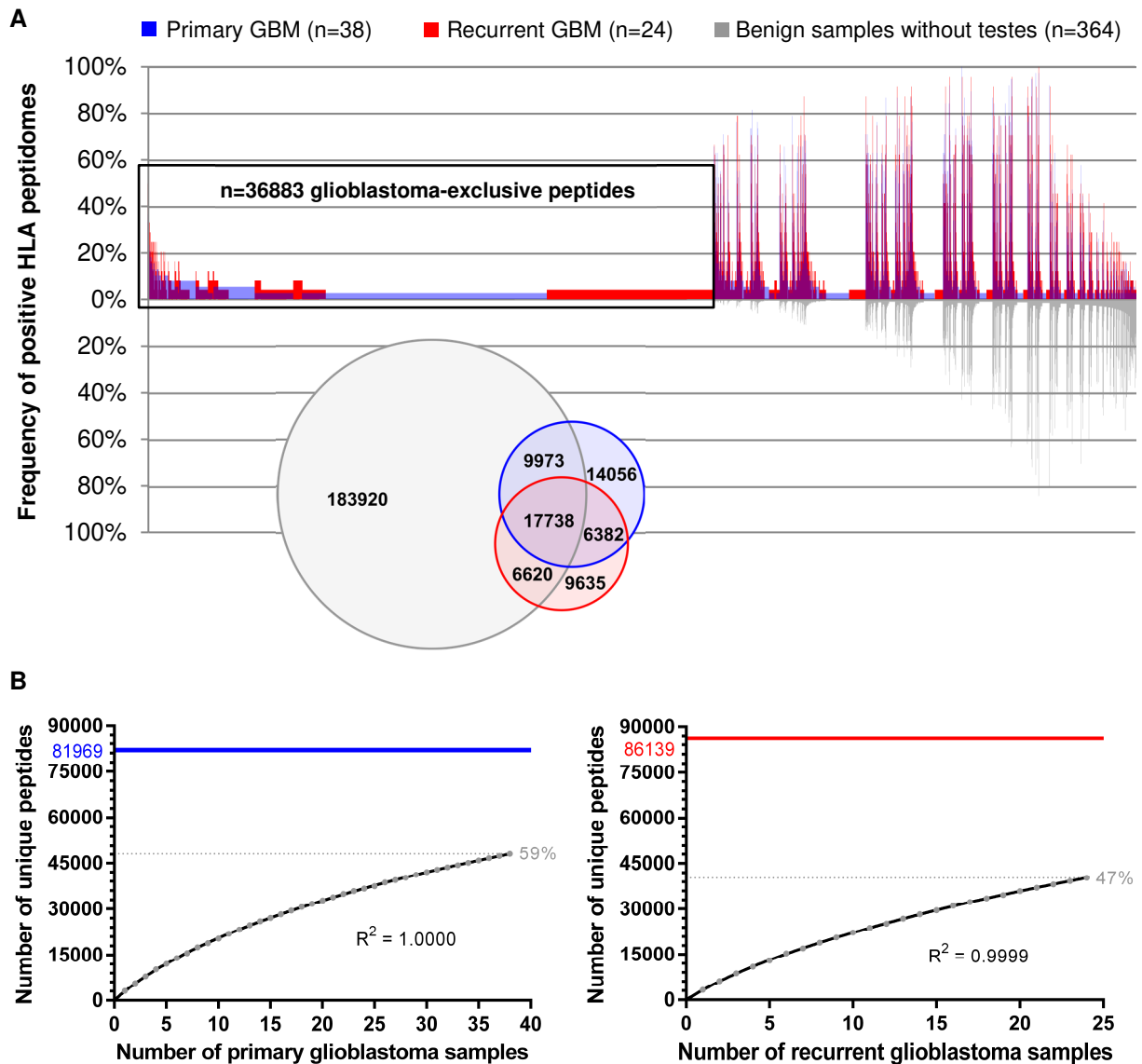


**Figure 15. HLA class II peptidomics to define glioblastoma-associated peptides (A) Comparative profiling of HLA class II peptides presented on glioblastoma *versus* an in-house benign database.** Every peptide evaluated for tumor association is represented by a bar in the waterfall plot (associated with the x-axis), whereas the y-axis shows the frequency of positive HLA peptidomes, separately for primary glioblastoma (n=38), recurrent glioblastoma (n=24), and benign samples without testes (n=364 covering 30 different human tissues). Being detected on a maximum of one non-CNS-related tissue, n=36,883 peptides were designated as glioblastoma-exclusive. Corresponding source proteins were subjected to hotspot analysis. The number of distinct HLA class II-restricted peptides per group is illustrated by the Venn diagram on the left, whereby the overlaps cannot map the permission of one positive non-CNS-related sample within the benign dataset. **(B) Saturation analysis for the identification of HLA class II-presented peptides in primary or recurrent glioblastoma tissue.** For each source count, the mean number of peptides was calculated by 1,000 random samplings. Using non-linear regression, exponential functions with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) were fitted. For both models, the goodness of fit was in the uppermost range ($R^2$ = 1.0000 and $R^2$ = 0.9999). Based on these curves, the maximum attainable number of distinct peptides was estimated (highlighted as solid lines). With the available number of 38 primary and 24 recurrence samples, 59% or 47% of the estimated maximum attainable amount of unique HLA class II-presented peptides had been identified, respectively. Considering primary and recurrent tumors jointly as 62 glioblastomas, these achieve 63% coverage of the estimated maximum attainable number of distinct peptides (Supplementary Figure 2).

## HLA ligands derived from established CTAs and TAAs

**A**
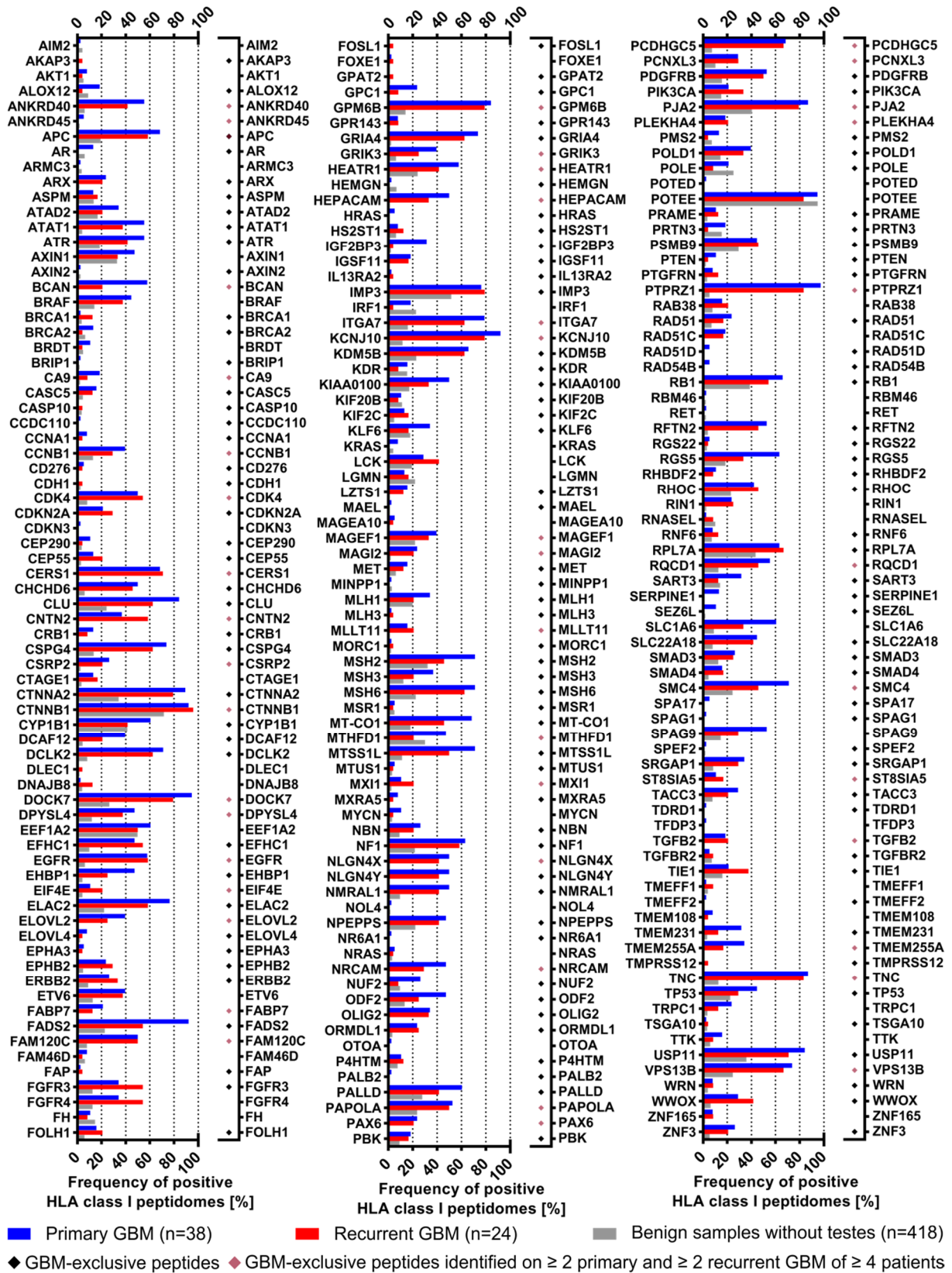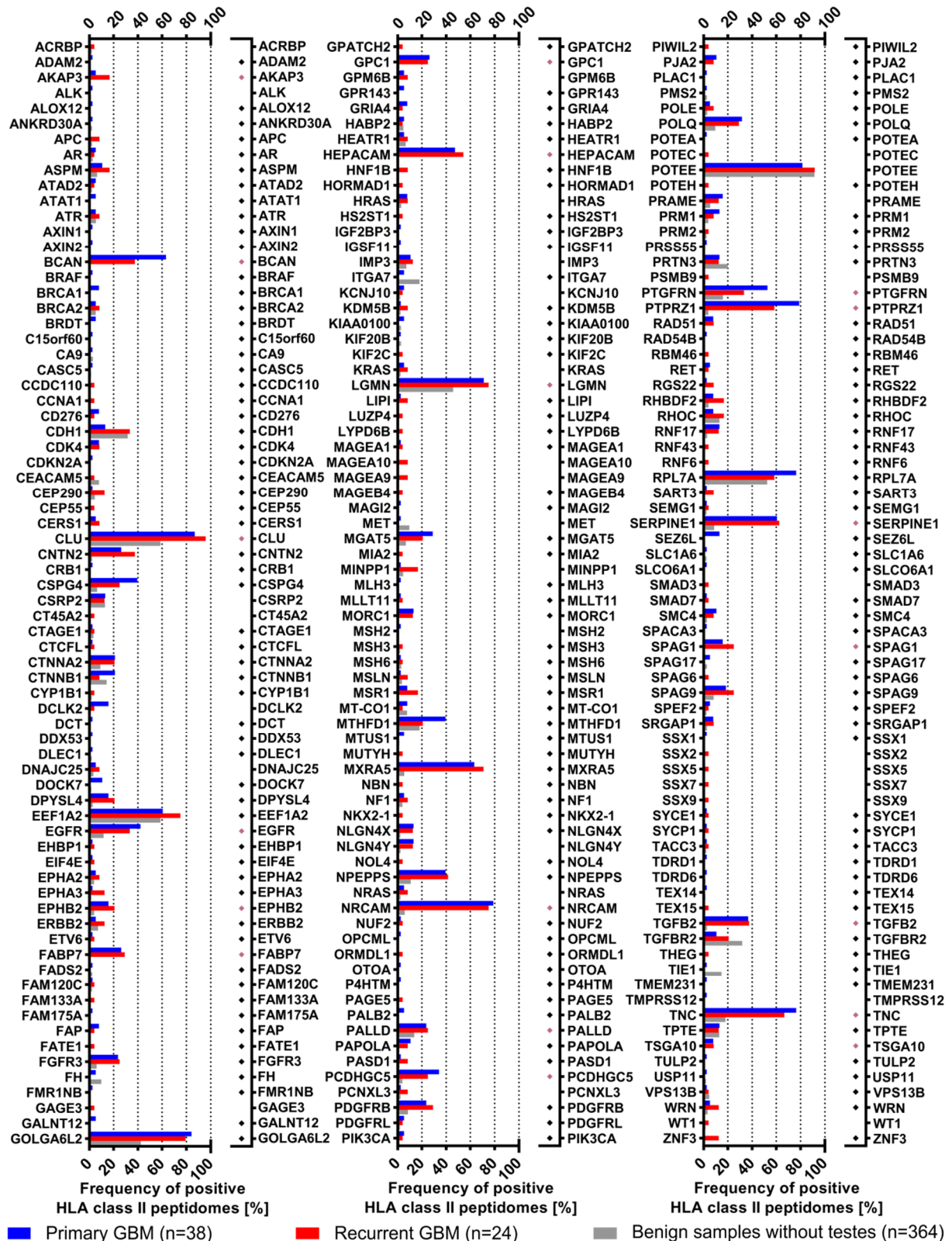


**Frequency of positive HLA class I peptidomes [%]**

**Frequency of positive HLA class I peptidomes [%]**

**Frequency of positive HLA class I peptidomes [%]**

■ Primary GBM (n=38)   ■ Recurrent GBM (n=24)   ■ Benign samples without testes (n=418)

◆ GBM-exclusive peptides   ◆ GBM-exclusive peptides identified on ≥ 2 primary and ≥ 2 recurrent GBM of ≥ 4 patients

**B**



Figure 16. Identification of established TAAs, CTAs, and glioblastoma-associated antigens across the present HLA peptidome dataset.

**Figure 16. Identification of established TAAs, CTAs, and glioblastoma-associated antigens across the present HLA peptidome dataset.** While peptides mapping to multiple source proteins were considered to calculate the frequency of positive HLA peptidomes, these were excluded to report the representation by glioblastoma-exclusive peptides. CTAs and TAAs exclusively identified on benign samples were not listed. **(A) CTAs and TAAs naturally presented on HLA class I molecules of**

**human primary and/or recurrent glioblastoma tissue.** The present immunopeptidomic dataset included HLA class I ligands derived from 217 TAAs and CTAs, with 44 sharing tumor-exclusive peptides in a minimum of two primary and two recurrent glioblastomas of at least four different patients (highlighted with red diamonds). The frequency of positive HLA peptidomes was assessed based on HLA class I ligands for tumor samples, whereby benign hits were reported independent of HLA binding probabilities of the underlying peptide identifications. **(B) TAAs and CTAs represented in the HLA class II peptidomes of primary and/or recurrent glioblastoma tissue.** Among 216 naturally presented CTAs and TAAs, 19 were represented by glioblastoma-exclusive peptides on at least two primary as well as two recurrent tumors of a minimum of four different patients (highlighted with red diamonds).

Considering a total number of 366 established CTAs and TAAs (3.2.1) as well as 83 antigens reported to be associated with glioblastoma (2.3.2; n=16 overlapped with the general list of CTAs and TAAs), the present HLA peptidome dataset acquired from primary and recurrent tumors was screened for previously published tumor antigens. Of these, n=217 and n=216 were represented by HLA class I ligands and HLA class II-presented peptides, respectively. Despite these high identification rates, presentation frequencies of CTAs and TAAs were in general low, especially of those exclusively identified on malignant tissues. Among 16 HLA class I-presented TAAs and CTAs fulfilling the aforementioned criteria to be designated as glioblastoma-exclusive antigen, OLIG2, TMEM255A, and PAX6 were the most frequent ones (24-34% positive primary as well as 17-33% positive recurrent tumors). 41 additional TAAs and CTAs were represented by tumor-exclusive HLA class I ligands on at least two primary as well as on two recurrent glioblastomas obtained from a minimum of four different patients (Figure 16 A, Supplementary Table 5). On HLA class II, 44 antigens were exclusively identified in the peptidome of glioblastomas, with FABP7, CDK4, RAD51, and CERS1 being the most frequent ones (5-26% positive primary as well as 5-29% positive recurrent tumors). 18 further CTAs and TAAs were represented by glioblastoma-exclusive HLA class II-restricted peptides eluted from at least two primary as well as two recurrent neoplastic specimens originating from a minimum of four different patients (Figure 16 B, Supplementary Table 5).

## 4.3  The HLA peptidome of primary *versus* recurrent glioblastoma

*Primary- and recurrence-exclusive antigens*

Using the same HLA class I and II peptidome datasets as in 4.2 while applying a different evaluation enabled the identification of fundamental differences of the antigenic landscape of glioblastoma presented at primary or recurrent disease, respectively. A total of 2,146 HLA class I- and 2,753 HLA class II-presented antigens were identified in primary tumors only, whereas these numbers came up to 610 and 1,886 recurrence-exclusive antigens, respectively (Figure 17 A and B). It was striking that the number of primary- and recurrence-exclusive antigens was inverse for HLA class I and II: at a frequency threshold of 17%, a total of three (HLA class I) and 15 (HLA class II) recurrence-exclusive antigens were identified while 20 (HLA class I) and five (HLA class II) antigens were exclusively presented on ≥ 18% of primary glioblastomas. This distorted ratio was maintained at lower thresholds (e.g. ≥ 13% of positive HLA peptidomes: 19/52 recurrence-exclusive and 122/29 primary-exclusive HLA class I/II antigens). Subsequent to manual curation of the corresponding peptides including multi-mapping to multiple source proteins, HLA class I binding motifs as well as the length and the presence of length variants of HLA class II-restricted peptides, a set of 19/5 and 3/14 HLA

class I/II antigens associated with primary or recurrent disease in glioblastoma was defined. PDZD2, ROBO1, and PTPRG constituted the most frequent primary-associated antigens, whereas THY1 and HEATR3 were highly associated with recurrent glioblastoma (Supplementary Table 6).
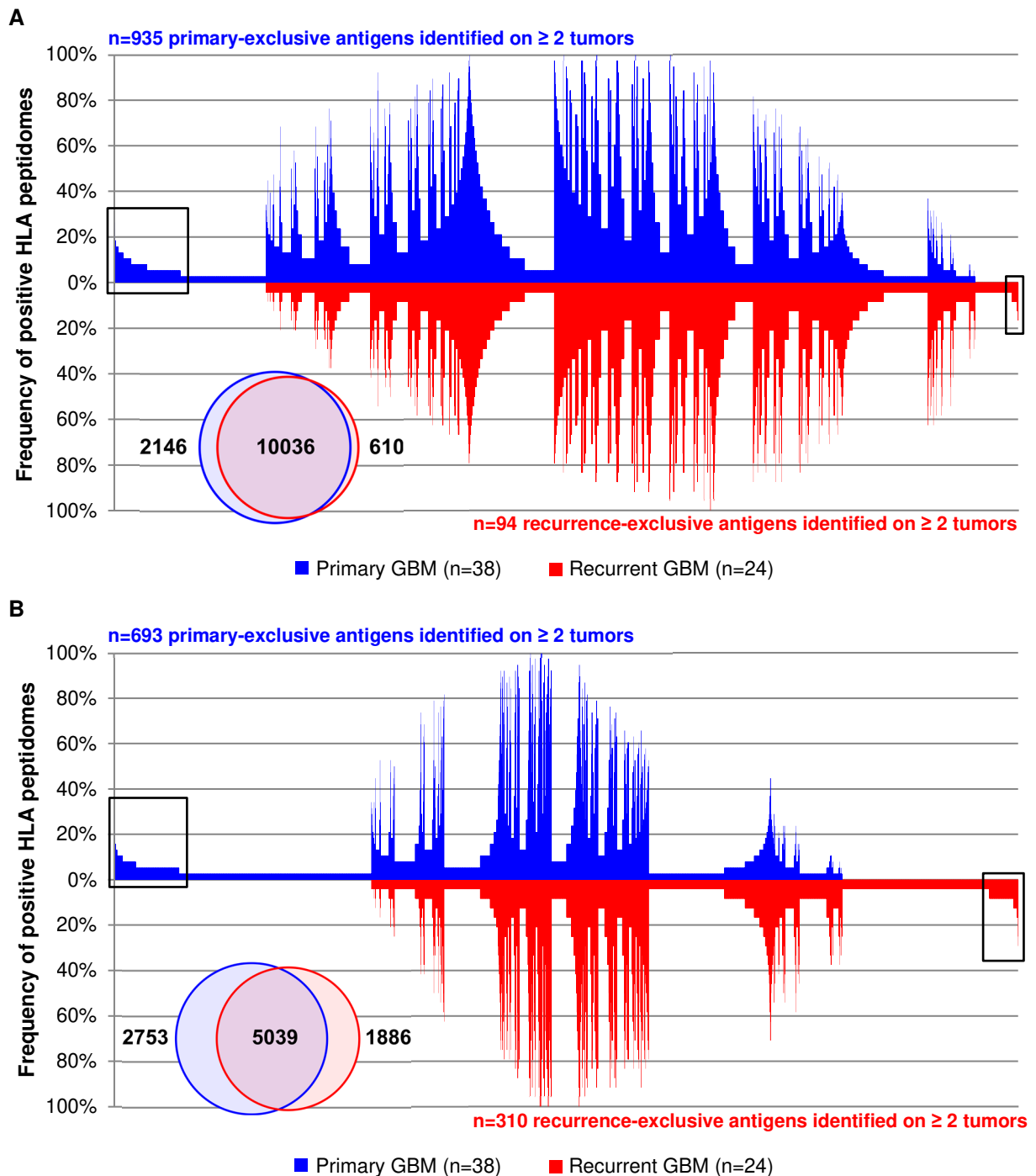


**Figure 17. (A) HLA class I- and (B) HLA class II-presented antigens associated with primary or recurrent disease in glioblastoma.** Each protein evaluated by comparative profiling is represented by a bar in the waterfall plots, whereas the frequency of positive HLA peptidomes is shown on the y-axis, separately for primary (n=38; blue) and recurrent glioblastoma (n=24; red). Primary- and recurrence-exclusive antigens detected on at least two tumors each are highlighted with boxes. The Venn diagrams in the left lower corner of the waterfall plots illustrate the number of unique antigens per group and the overlap between the two groups.

### *Relative HLA ligand abundances on primary versus recurrent glioblastoma*

LFQ-MS to identify HLA class I ligands significantly up- and down-modulated at disease recurrence was possible for eleven glioblastoma patients and based on a mean number of 3,809 ± 1,056 peptides evaluated for relative abundance per patient. On average, 9.76 ± 7.19% and 8.46 ± 3.21% of the patients' HLA class I peptidomes were subject to significant up- and down-modulation, respectively (Figure 18 A). Comparative profiling to identify common patterns of modulated HLA presentation was performed on source protein level. In total, 260 and 259 antigens were exclusively represented by up- or down-modulated peptides, respectively. Following manual curation of the underlying peptides for HLA motifs as well as multi-mapping to several source proteins, a set of nine antigens represented by up-modulated peptides on recurrent *versus* primary glioblastoma in two out of eleven of patients was defined. Conversely, 21 proteins were under-represented in the HLA class I peptidome acquired from recurrent as compared with autologous primary disease (18-27% of patients). Among these, SEZ6 was the most frequent one (Figure 18 B, Supplementary Table 7).

**A**



**Log₂ fold change (recurrent / primary glioblastoma)**

○ down-modulated          ○ not modulated          ○ up-modulated

**B**
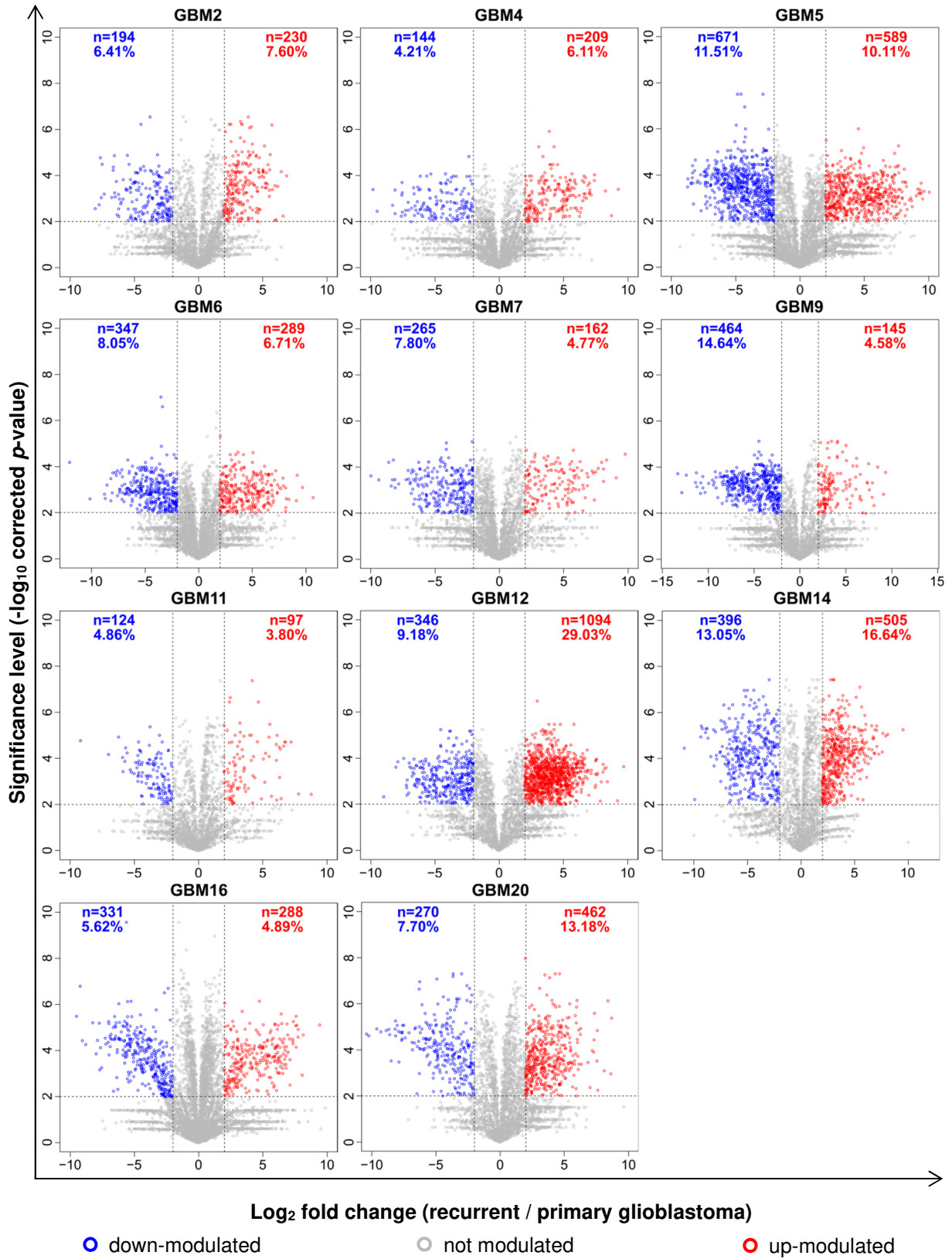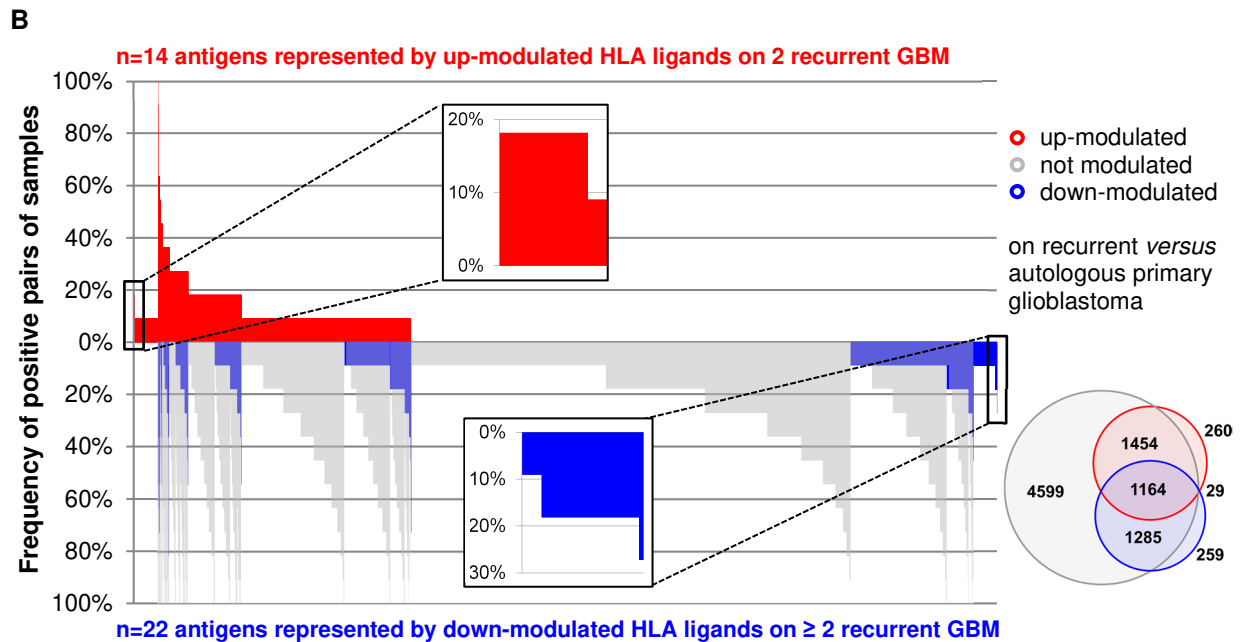


**Figure 18. Patterns of modulated HLA class I presentation in glioblastoma across eleven patients. (A) Volcano plots of relative HLA ligand abundances on recurrent *versus* autologous primary glioblastoma.** By LFQ-MS, relative abundances of HLA class I ligands, each of which is represented by a dot, were compared. The x-axis indicates changes of abundance as $\log_2$ fold change and corresponding significance levels (after BH correction for multiple testing) are associated with the y-axis. Significant modulation was defined by a corrected *p*-value ≤ 0.01 and a fold change of mean AUC in (recurrence / primary) ≥ 4 or ≤ 0.25 regarding up- (highlighted in red) or down-modulated (highlighted in blue) peptides, respectively. The total number of up- and down-modulated peptides as well as their proportion in the patient's HLA class I peptidome are indicated in quadrants of each Volcano plot. **(B) Comparative profiling of antigens corresponding to peptides displayed in Volcano plots.** Each bar in this waterfall plot (associated with the x-axis) represents a single protein, whereas the frequency of positive pairs of samples is shown on the y-axis. Comparing the source proteins of peptides underlying significant up- or down-modulation as well as of those not being modulated allowed the identification of exclusively and recurrently over- (n=14) or under-represented (n=22) antigens across eleven LFQ datasets acquired from primary and autologous recurrent glioblastoma.

In twelve glioblastoma patients, a mean number of 3,894 ± 1,202 HLA class II-presented peptides were evaluated for relative abundance on recurrent *versus* primary tumors by LFQ-MS. On average, 14.66 ± 9.22% and 17.27 ± 7.50% of the patients' HLA class II peptidomes were subject to significant up- or down-modulation, respectively (Figure 19 A). Comparative profiling to identify common patterns of modulated HLA presentation was performed on source protein level, since length variants with common core sequences cannot be adequately addressed across patients. In total, n=194 and n=256 antigens were exclusively represented by up- or down-modulated peptides, respectively. Subsequent to manual curation of multi-mapping peptides, 8 and 12 antigens were found to be over-represented in the HLA class II peptidome of recurrent or primary glioblastoma (Figure 19 B, Supplementary Table 7).

**A**



Log₂ fold change (recurrent / primary glioblastoma)

○ down-modulated       ○ not modulated       ○ up-modulated

**B**
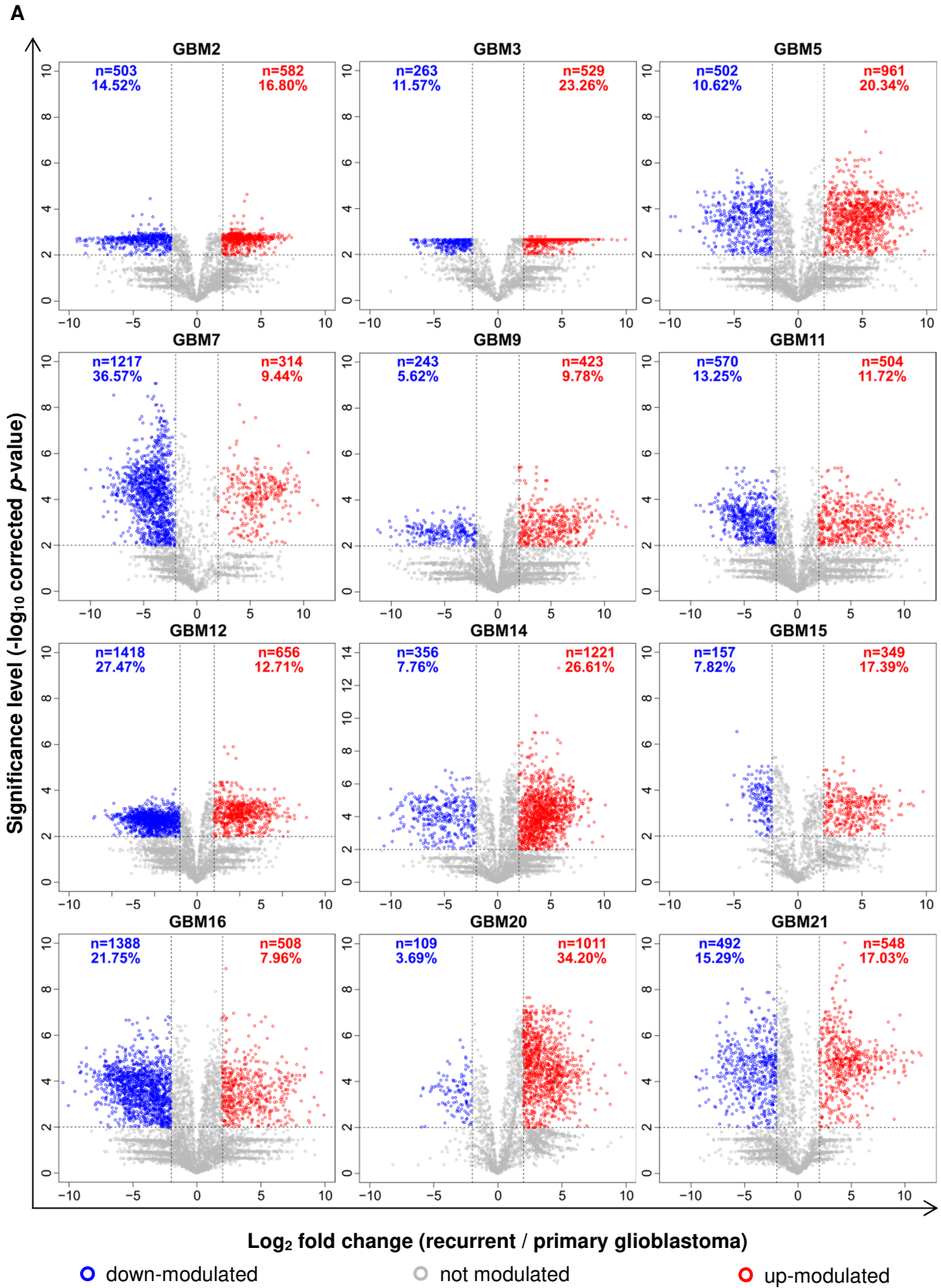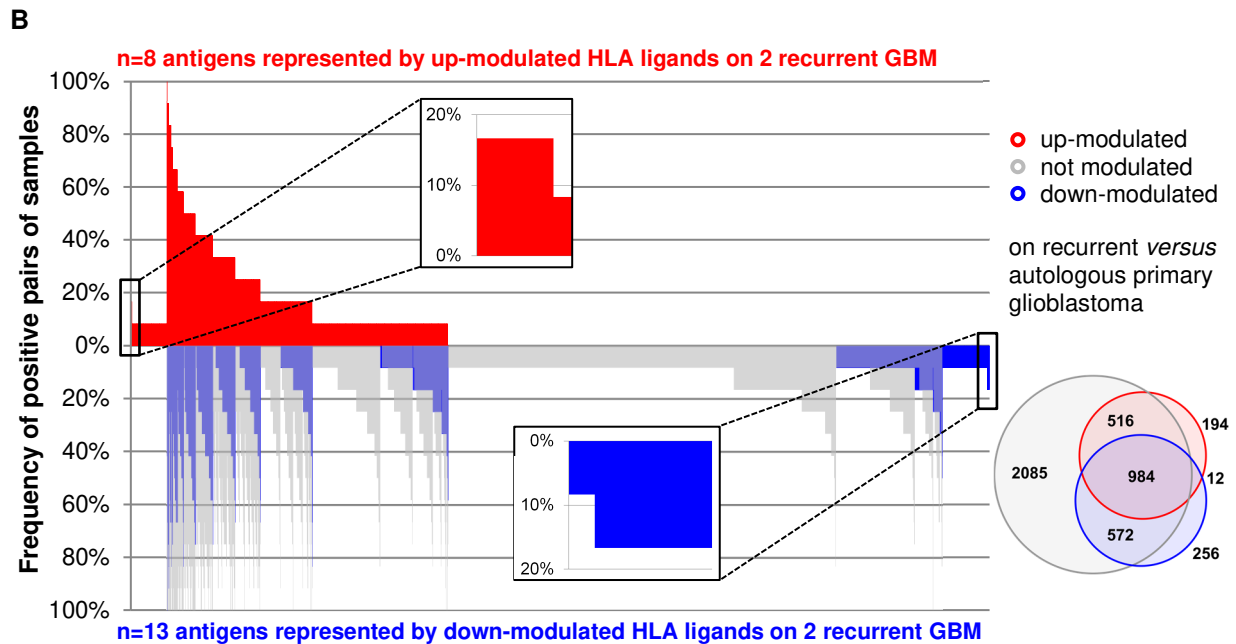


**Figure 19. Patterns of modulated HLA class II presentation in glioblastoma across twelve patients. (A) Volcano plots of relative HLA class II-presented peptide abundances on recurrent *versus* autologous primary glioblastoma.** By LFQ-MS, relative abundances of HLA class II-restricted peptides, each of which is represented by a dot, were compared. The x-axis indicates changes of abundance as $\log_2$ fold change and corresponding significance levels (after BH correction for multiple testing) are associated with the y-axis. Significant modulation was defined by a corrected p-value ≤ 0.01 and a fold change of mean AUC in (recurrence / primary) ≥ 4 or ≤ 0.25 regarding up- (highlighted in red) or down-modulated (highlighted in blue) peptides, respectively. The total number of up- and down-modulated peptides as well as their proportion in the patient's HLA class II peptidome are indicated in quadrants of each Volcano plot. **(B) Comparative profiling of antigens corresponding to peptides displayed in Volcano plots.** Each bar in this waterfall plot (associated with the x-axis) represents a single protein, whereas the frequency of positive pairs of samples is shown on the y-axis. Comparing the source proteins of peptides underlying significant up- or down-modulation as well as of those not being modulated allowed the identification of exclusively and recurrently over- (n=8) or under-represented (n=13) antigens across twelve LFQ datasets acquired from primary and autologous recurrent glioblastoma.

## *Functional annotation of antigens associated with primary and recurrent disease*

All proteins exclusively identified on primary or recurrent tumors as well as antigens exclusively represented by down- or up-modulated peptides at disease recurrence were subjected to functional annotation clustering, separately for HLA class I and II. Top three clusters associated with primary disease comprised amino acid transport, metal ion transport, and intraspecies interaction for antigens presented on HLA class I as well as organelle organization, microtubule cytoskeleton organization, and histone H4 acetylation for antigens presented on HLA class II molecules. Conversely, source proteins associated with tumor recurrence were mainly involved in the negative regulation of fatty acid metabolism, humoral immune response, and lipid metabolism or in the positive regulation of hydrolase activity, gene expression, and mitotic cell cycle considering HLA class I- or II-presented antigens, respectively.

**A**
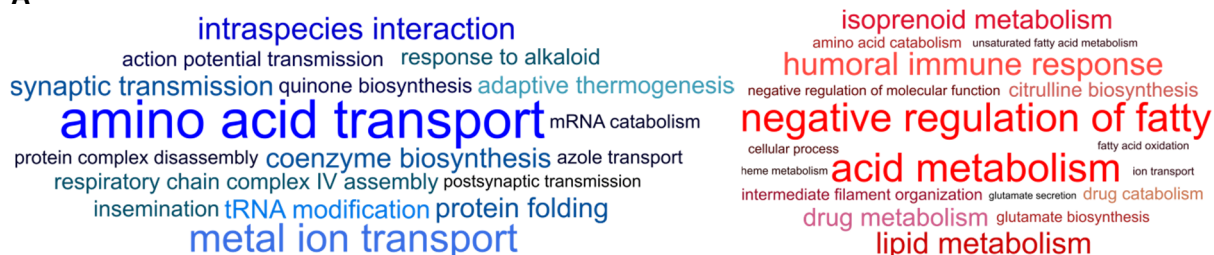


**B**
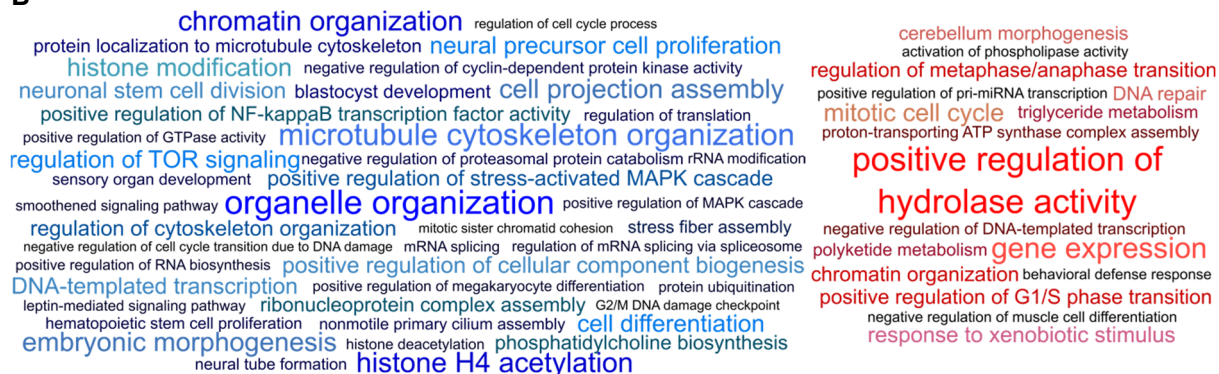


**Figure 20. Functional annotation of (A) HLA class I- and (B) HLA class II-presented antigens associated with primary (blue) and recurrent disease (red).** All proteins exclusively identified on primary or recurrent tumors as well as antigens exclusively represented by down- or up-modulated peptides at disease recurrence were subjected to functional annotation clustering. Enrichment scores are proportional to the font size in the word clouds.

## 4.4 Natural HLA presentation of neo-antigens

### *Mutational burden of primary glioblastomas*

From a total of 13 primary glioblastomas, somatic variants comprising insertions, deletions, and single nucleotide alterations were determined. Primary glioblastomas carried a median of 409 [175-3,417] SNVs as well as 1 [0-8] indels. Intronic variants constituted the majority of SNVs, which is why the median number of non-synonymous mutations was drastically smaller than that of SNVs (52 [16-772]; Figure 21). All indels were located up- or downstream of genes, in intergenic regions or introns, as well as in splice regions of non-coding transcripts.

One patient, ZH613, with strongly elevated mutational load was found to carry a missense mutation affecting the MMR protein MLH1 (ENST00000231790 / ENSG00000076242 c.1643A>G; ENSP00000231790 p.548 Y>C). The COSMIC database included two entries of missense mutations affecting the identical AA position (glioblastoma: c.1643A>T / p.548 Y>F; endometrioid carcinoma c.1643A>G / p.548 Y>C).
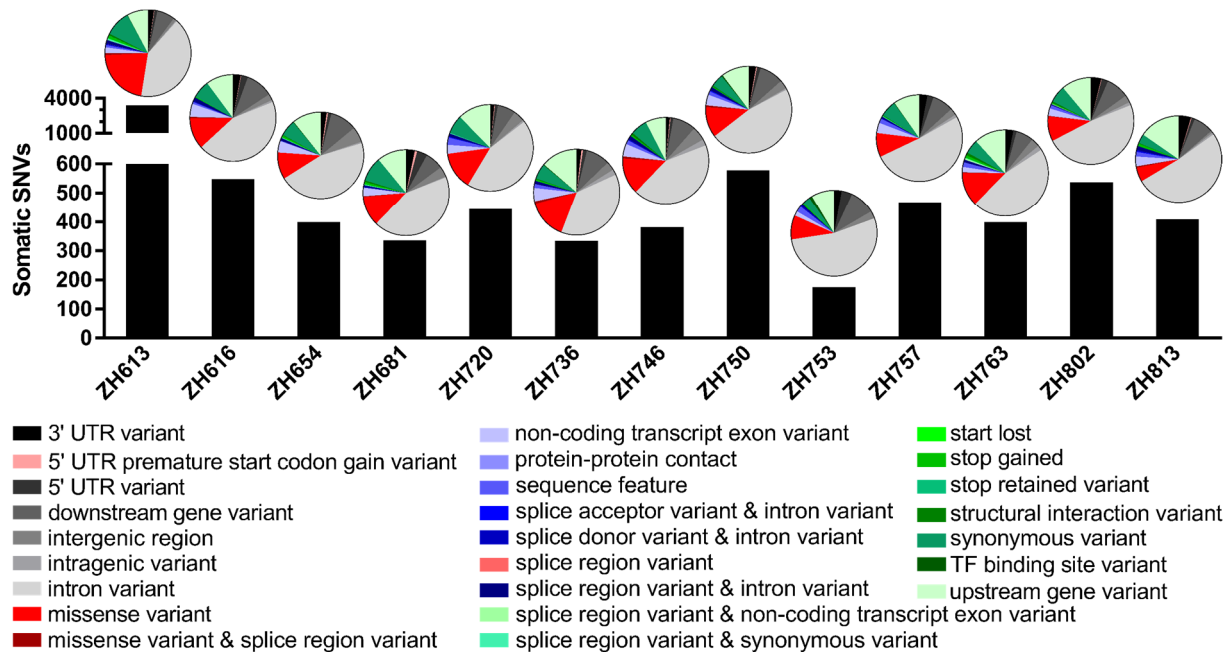
**Figure 21. Composition of SNVs in primary glioblastomas.** While the majority of SNVs affected non-coding regions such as introns, non-synonymous mutations (5' UTR premature start codon gain variants, splice region variants, missense variants, missense and splice region variants; marked in red) constituted on average only 13 ± 4% of SNVs. The SNV load of ZH613 (3,417 SNVs) was eightfold increased as compared with the average number of SNVs in ZH616-813 (417 SNVs).

## *Identification of mutated HLA ligands*

Unfiltered SNVs served as base to search immunopeptidomic data for neo-antigenic HLA ligands. For HLA class I, this search yielded one candidate each for patient ZH613 and ZH681 – two samples with pronounced high peptide yields (4,474 and 4,546 annotated ligands). The HLA-A*02:05 ligand NVITDGVTSV derived from the SZT2 subunit of KICSTOR complex (ENST00000562955 / ENSG00000198198 c.779T>A; ENSP00000457168 p.260I>N) had promising spectrum-specific scores (based on SEQUEST and MHCquant database search; 0.1 – 1.5% FDR) and middle-range spectral quality by means of manual assessment (Figure 22 A). Further, the mutation had a pronounced high VAF of 71.90% and the gene was expressed at 10.97 RPKM (5,568 raw counts). A total of five (four by both search engines) WT HLA class I ligands derived from other positions within the source protein SZT2 were detected within the immunopeptidome of this patient: SSNPALALR (HLA-A*31:01), RLFNEHLVSA (HLA-A*02:05), GQAGPEITDEL (annotated to *HLA-A*02:05*), FQPEIYVTI (HLA-A*02:05), and YQSLIKVLL (HLA-A*02:05). The HLA-B*57:01 ligand RAYTPPRISW arising from mutant PDGFRα (ENST00000257290 / ENSG00000134853 c.1027C>A; ENSP00000257290 p.343P>T) was identified at 2.5% FDR using the MHCquant pipeline, but at > 20% FDR by SEQUEST. Despite poor spectral quality according to SEQUEST scores and manual assessment (Figure 22 B), this peptide underwent validation by a synthetic heavy isotope-labeled peptide due to strong expression (35,434 raw counts equivalent to 98.65 RPKM) and a VAF of 15.27%. Moreover, eight HLA class I-presented non-mutated peptides derived from PDGFRα were identified within the same sample both by MHCquant and SEQUEST: KYSDIQRSL (HLA-C*07:02), TRSYVILSF (HLA-C*06:02; -C*07:02), SQLEAVNLHEV (annotated to *HLA-A*02:01*), KQADTTQYV (HLA-A*02:01), STFLPVKW (HLA-B*57:01),

GSTFLPVKW (HLA-B*57:01), TLIENLTEI (HLA-A*02:01), and RPASYKKKSML (HLA-B*07:02). Both candidates were included in filtered high confidence somatic variant lists and were validated by searching the dbSNP database and querying the sequences (with all possible exchanges of the isobaric AAs leucine and isoleucine) against the UniProt database. Synthesis of heavy isotope-labeled peptides spiked into a complex matrix of HLA class I peptides eluted from JY cells and subsequent LC-MS/MS finally proved the neo-antigenic sequence identity by comparison of experimental and synthetic peptide fragment spectra (Figure 22).

Interestingly, the position 343 of PDGFRα was found to be affected by recurrent mutations in glioblastoma. Two different patients were listed with p.343P>S mutations in the COSMIC database, whereby the mutation to serine generates – like the mutation to threonine of patient ZH681 – a phosphorylatable AA. Both mutations were found to alter the phosphorylation state of PDGFRα by generating one neo-phosphorylation motif each for tyrosine residues as well as eight (p.343P>T) or 13 (p.343P>S) neo-phosphorylation motifs for serine or threonine residues. As a consequence, both the mutated AAs themselves and the tyrosine residue at p.342 may become phosphorylated creating binding motifs for protein domains. One and two neo-phospho-serine/threonine binding motifs recognized by WW or 14-3-3 domains were identified for p.343P>T and p.343P>S, respectively. Remarkably, the two neo-phospho-tyrosine binding motifs identified in both protein variants represent binding sites for Src homology 2 (SH2) domains of the Crk adapter molecule and for the C-terminal SH2 domain of the Ras GTPase activating protein (RasGAP; Supplementary Table 9). RAYTPPRISW, the neo-antigenic HLA-B*57:01 ligand verified in ZH681, was not found to carry a tyrosine or threonine phosphorylation. The SZT2 p.260I>N variant was neither listed in the COSMIC database nor found to have a direct impact on SZT2 protein phosphorylation.

For HLA class II, a single candidate was identified in patient ZH750: SARGPSTPGVLSNCTSPLPG derived from autophagy-related protein 9 (ATG9B) ENST00000377974 / ENSG00000181652 c.2294A>G; UPI00015E055A p.756E>G. However, this peptide did not undergo further validation, since the fragment spectrum was annotated with better scores to another WT protein by SEQUEST (Supplementary Figure 5).

**A**
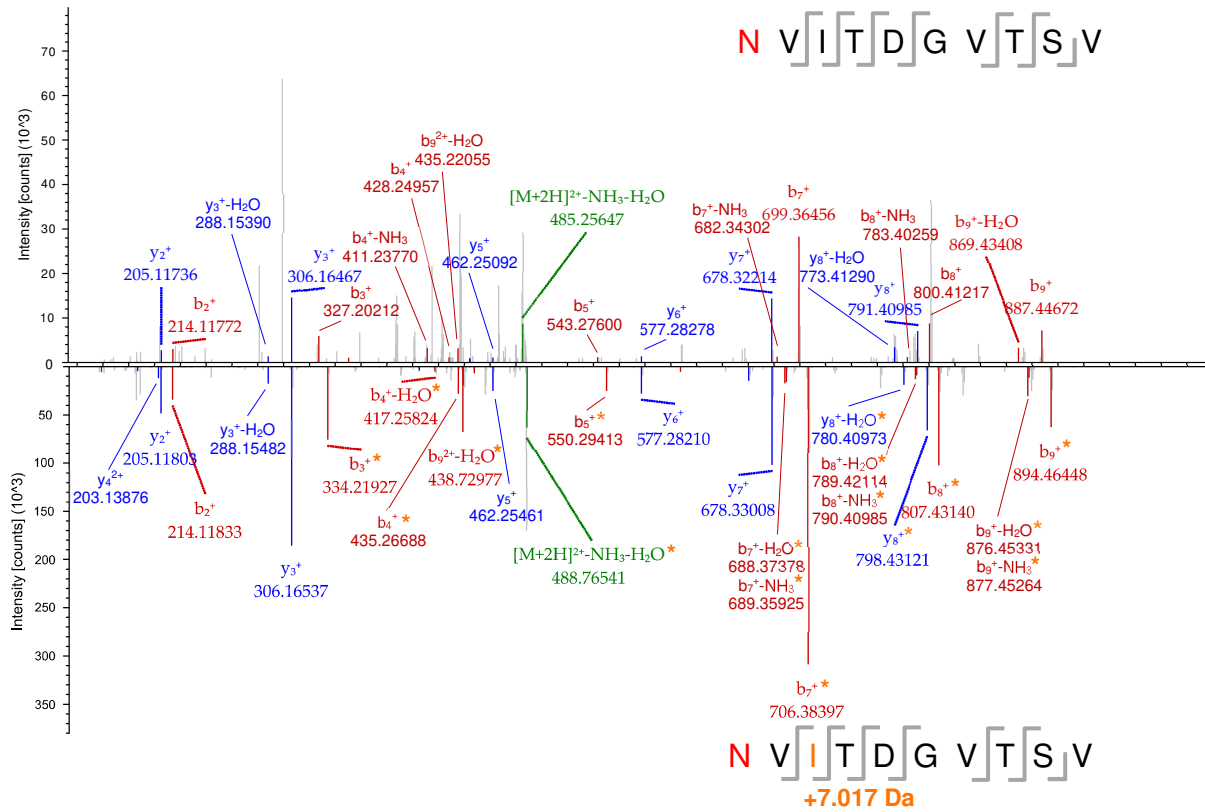


**B**
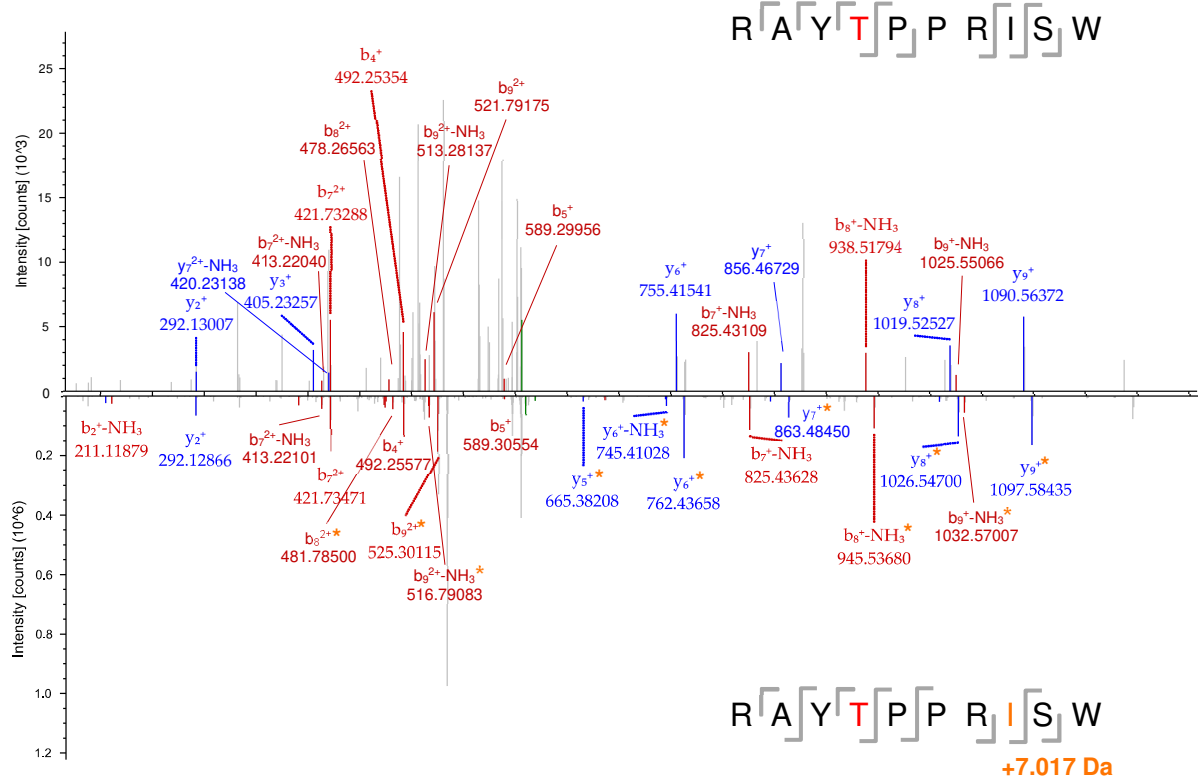


**Figure 22. Fragment spectra of neo-antigenic HLA class I ligands identified in primary glioblastomas.** The upper spectrum per panel was acquired from glioblastoma tissue, whereas the lower one represents the validation spectrum of a heavy isotope-labeled synthetic peptide. Mutated amino acids are marked in red within the sequence, b and y ions are indicated as red and blue peaks

in fragment spectra, and fragments harboring heavy isotope-labeled isoleucine (+7.017 Da) are marked with an orange asterisk. **(A) HLA-A*02:05-restricted peptide derived from SZT2 subunit of KICSTOR complex (ENST00000562955 / ENSG00000198198 c.779T>A; ENSP00000457168 p.260I>N) detected in ZH613.** NVITDGVTSV was identified at -1.2 ppm precursor mass deviation, 1.5% FDR (q value = 0.015; MHCquant: 0.1% FDR / q value = 0.001), and a cross-correlation of theoretical and measured spectrum of 1.44 (XCorr = 1.44). Despite several interfering signals, the entire sequence was covered by a sufficient number of both b and y ions. The second-best sequence annotated to this spectrum by SEQUEST scored 38.89% worse (dScore = 0.3889). The experimental fragment spectrum is mirrored by that of the synthetic peptide with most peaks being shared. **(B) HLA-B*57:01-restricted peptide arising from mutant PDGFRα (ENST00000257290 / ENSG00000134853 c.1027C>A; ENSP00000257290 p.343P>T) detected in patient ZH681.** RAYTPPRISW was identified at 2.41 ppm precursor mass deviation, 21.3% FDR (q value = 0.213; MHCquant: 2.5% FDR / q value = 0.025), and poor spectral correlation (XCorr = 0.96). Despite poor signal-to-noise ratio, more than ten b and y ions contributed to sequence annotation. The next-best sequence annotation by SEQUEST fitted only 8.33% worse (dScore 0.0833). The experimental fragment spectrum is mirrored by that of the synthetic peptide with most peaks being shared.

The number of unique HLA class I peptides, non-synonymous mutational loads, and the amount of validated neo-antigenic peptide identifications obtained from 13 primary glioblastomas were supplemented with comparable published HLA class I peptidome datasets. Taking all samples presented in Figure 23 into account, one neo-antigenic peptide identification resulted on average from $5.7 \times 10^5$ unique HLA class I peptides and $1.2 \times 10^3$ non-synonymous mutations, respectively. For the calculation of mutated peptides in relation to the number of non-synonymous mutations, 15 glioblastoma patients of the GAPVAC-101 trial[12] were considered additionally. From these, no neo-antigenic peptides could be verified with mutational loads being available while lacking peptide yields.
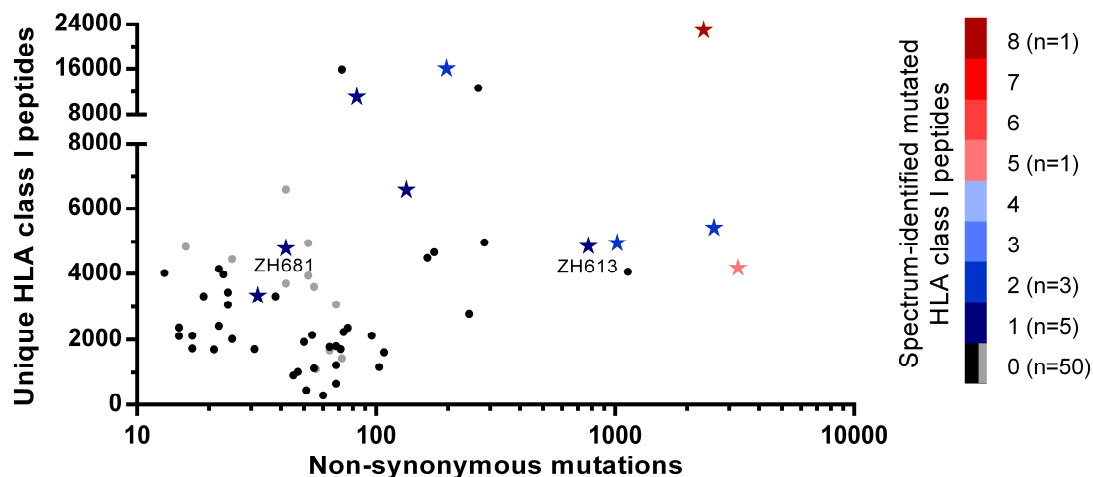


**Figure 23. Identification of neo-antigenic peptides naturally presented on HLA class I by LC-MS/MS.** The number of unique HLA class I peptides was plotted against the number of non-synonymous mutations on a per-sample basis (n=60). Data as shown in Freudenmann *et al.*[51] was complemented by that of the 13 glioblastoma patients presented herein (marked in grey), by hepatocellular carcinomas (n=16) published by Löffler *et al.*[16], as well as by colorectal cancer organoids (n=5) analyzed by Newey *et al.*[52] Color-coded stars depict validated mutated peptide identifications, whereby those samples with more than one neo-antigenic peptide are clearly separated from those devoid of mutated HLA ligands.

# 5 Discussion

Glioblastoma is the most aggressive and most frequent malignant brain tumor accounting for an average annual incidence of almost 12,000 cases in the US. Despite extensive research, prognosis has remained poor with a 5-year relative survival rate of only 6.8%.[1] Cancer immunotherapy may meet the urgent demand for innovative treatments.[2-6] Few studies addressing the naturally presented antigenic landscape of glioblastoma have already been published, however, none has focused on disease recurrence. Likewise, HLA class II-restricted targets have not been covered sufficiently.[10-14] Herein, we present a large-scale multi-omics study with immunopeptidomics as core element examining the similarities and differences of primary and recurrent glioblastomas and defining candidate targets for immunotherapeutic intervention compatible with both disease conditions.

The present study population comprising 40 glioblastoma patients had a representative age distribution from 33 to 86 years with a median age of onset of 67 years and a slightly increased proportion of females (male : female ratio of 1.22).[1] The cohort included 55 distinct HLA class I allotypes covering 99.95% of the world population, whereby 92.17% of all individuals are expected to be positive for at least three allotypes. HLA class I and II ligands were isolated in considerable numbers from a total of 38 primary and 24 recurrent – almost exclusively IDH1$^{WT}$ – tumors. This large immunopeptidomic dataset strongly refutes significant down-regulation or even loss of HLA expression to be a common phenomenon in glioblastoma, which is in line with findings in many other cancer entities.[10,51,53-55] Nevertheless, there were two recurrent tumor specimens (GBM15R, GBM21R) with low HLA class I ligand identifications while yielding remarkable numbers of HLA class II-restricted peptides. In these selected cases, reduced HLA expression appears plausible, since technical artefacts affecting lysate preparation, peptide purification, or mass spectrometry can be ruled out. We observed marginally increased yields of HLA class I as compared with HLA class II peptides eluted from primary glioblastomas and of HLA class II-restricted peptides identified on recurrent *versus* primary tumors. The fact that the two compared groups had overlapping error bars each, however, weakens the confidence of the underlying statistical calculation. High numbers of HLA class II peptide identifications can originate from tumor cells themselves, but also from infiltrating macrophages and microglia.[10,56-58] The latter have been postulated to constitute 30-50% of bulk tumor mass in glioma[56,58-60] and to be tumor-supportive by creating an immunosuppressive microenvironment, releasing cytokines and growth factors thus promoting glioma cell migration, proliferation, and survival.[56,57,61-63]

On the basis of the present dataset comprising 38 primary and 24 recurrent tumors with 22 representing autologous pairs gathered from the same patient, we have learned that the HLA peptidome of glioblastoma undoubtedly underlies significant dynamics during the progression from primary to recurrent disease. At recurrence, 20-30% of the patients' HLA class I and II peptidomes were subject to modulated HLA presentation as compared with autologous primary disease. Moreover, almost 5,000 and 2,500 antigens including tumor-exclusive ones were only presented on primary or recurrent neoplasms, respectively. Thus, we draw the conclusion that radiochemotherapy and clonal evolution during disease progression[8,9] drastically alter the antigenic landscape, which has to be considered when defining targets for glioblastoma immunotherapy. Consequently, we focused on those antigens and peptides

exhibiting robust and frequent presentation independent of disease state. This not only renders them broadly applicable to a large number of patients, but also reduces the risk of lacking clinical efficacy when targeting antigens lost following initial surgical resection and radiochemotherapy.

We found glioblastoma to be a pronounced rich source of candidate targets for cancer immunotherapy. With the currently available number of 23 benign brain and cerebellum samples, we expect to achieve less than 100% saturation of HLA class I and II protein identifications. Transcriptome data acquired from a large set of benign human tissues were therefore integrated as orthogonal level into target selection to exclude antigens with a highly CNS-associated expression profile. This way, we aim at minimizing on-target off-tumor toxicities, which is of utmost importance as antigen-specific T cells directed against glioblastoma cross the blood-brain barrier.[64-67] Nevertheless, all candidate targets reported herein require a strict immunological validation process to warrant a good safety profile. Despite selecting tumor-exclusive antigens with a non-CNS-specific expression profile, it is conceivable that antigens such as GFAP – a prominent astrocytic marker[68] – may not be unrestrictedly specific for malignant cells. The search for glioblastoma-associated antigens frequently and robustly presented on both primary and recurrent neoplasms uncovered 62 and 26 for HLA class I and II, respectively. Among these, TANC2, FA2H, BEST1, GPC5, COLGALT2, NOP16, ESCO1, and BBOX1 were the most frequent ones. TANC2 has been described as driver gene amplified and overexpressed in breast cancer. In such cells, TANC2 knockdown induces cell cycle arrest resulting in halving of cell viability.[69] The synthesis of sphingolipids including 2-hydroxy fatty acids, which are ubiquitous in the CNS as they are an essential component of myelin, is catalyzed by FA2H.[70,71] Upregulation of FA2H and aberrant sphingolipid composition have been reported for several cancer entities including ovarian and lung carcinoma, neuroblastoma, and schwannoma.[70,72-74] In the latter, a cell cycle inhibitory function has been attributed to FA2H, but the tumor-promoting function of this enzyme has so far not been clarified.[70,72] The calcium-activated chloride channel BEST1 is expressed in epithelial and nervous cells.[75-78] Besides fulfilling physiological functions, BEST1 is also involved in tumorigenesis by promoting EMT and cancer cell proliferation upon overexpression.[76-79] Glioma cells hijack the calcium-chloride axis *via* BEST1 to induce regulatory volume decrease as survival mechanism and to promote infiltration.[80] Glypicans (GPC1-6) are cell surface-resident heparan sulfate proteoglycans with multifaceted roles in growth factor signaling and neoplastic cell proliferation.[81,82] Members of the glypican family have already been identified as oncoproteins and suggested as targets for glioblastoma (GPC1), neuroblastoma (GPC2), and hepatocellular carcinoma (GPC3) immunotherapy.[10,83,84] COLGALT2 accomplishes glycosylation of collagen[85] and is strongly expressed in glioma, but also in healthy brain tissue.[86] Extracellular matrix modification is a key process in the course of cancer progression facilitating tumor cell migration and proliferation as well as establishment of a tumor-protective niche.[87] Little is known about the nucleolar protein 16 (five PubMed entries[88]), which exhibits estrogen- and c-Myc-responsive expression and is associated with poor prognosis in breast cancer.[86,89] Glioblastoma cells may benefit from expressing the acetyltransferase ESCO1 at high levels, as it promotes DNA replication by establishing sister chromatid cohesion.[90-92] BBOX1 catalyzes L-carnitine synthesis, which is essential for fatty acid oxidation.[93,94] Besides that, BBOX1 antisense RNA 1 binds and inhibits the tumor-

suppressive microRNA miR-3940-3p resulting in up-regulation of Survivin thus inhibiting apoptosis and enhancing proliferation.[95] Of note, this set of HLA class I- and II-presented antigens comprised three with CTA-like expression profiles (HSF2BP, E2F1, RAD51), which had so far not been listed in the CTDatabase[49]. When targeting glioblastoma-associated proteins by peptide-specific immunotherapy, one would – if possible – preferentially select such peptides presented on both primary and recurrent tumors or in accordance with the patient's indication (e.g. APFDGSRLVF derived from FA2H was eluted from recurrent glioblastomas only).

Apart from defining targets being tumor-exclusive on the level of the entire protein, we have also focused on glioblastoma-associated peptides which are shared by specimens gathered at primary and recurrent disease and potentially arise from differential antigen processing in malignant cells. This unveiled a set of 155 HLA class I ligands derived from 158 antigens presented on 16-55% of primary as well as on 17-46% of recurrent tumors and of 21 antigens harboring glioblastoma-associated HLA class II presentation hotspots giving rise to peptides naturally presented on up to 15 primary and seven recurrent malignant samples. Taking together glioblastoma-associated antigens and peptides presented on HLA class I molecules (n=357 candidate target peptides), these achieve a world population coverage of 99.93% with an average number of 84 peptides matching per patient. Assuming that two third of these candidates will be excluded during the course of immunogenicity testing, which appears overestimated according to our previous experience with immunopeptidomics-based target definition approaches,[96] the number of peptides would still be enough to achieve sufficient coverage of the world population. Overlapping glioblastoma-associated HLA class I- and II-presented antigens as well as glioblastoma-associated HLA class I- and II-restricted peptides with each other, it become evident that each HLA class presents a unique repertoire of tumor antigens to T cells. This warrants HLA class I and II to be considered as equal partners in comprehensive target definition approaches. The present immunopeptidomic dataset comprising 62 primary human glioblastoma samples has, moreover, demonstrated once more that established TAAs and CTAs are not prime targets for cancer immunotherapy as being either broadly presented across both malignant and benign tissues or lacking frequent presentation on HLA molecules.[96] Hereby, we did not observe a fundamental difference between primary and recurrent glioblastomas.

Neo-epitopes arising from mutant proteins are designated as ideal tumor antigens offering maximum tumor specificity.[51,97,98] However, proving these to be naturally presented on HLA in native human tumor tissue has remained a major obstacle questioning their clinical relevance when contributing in amounts below the detection limit of modern highly resolving LC-MS/MS systems to the tumor HLA peptidome.[12,16,17,51] In a recent clinical trial, GAPVAC-101, tissues of 15 primary glioblastomas were evaluated for naturally presented neo-antigens – without any hit.[12] We performed whole exome sequencing from 13 primary glioblastomas unveiling a mutational burden comparable to that of the GAPVAC-101 patients with the exception of one patient, ZH613, with strongly elevated mutational load reasoned by a mutation of the MMR protein MLH1. Indeed, we here provide the first evidence of two neo-antigenic HLA class I ligands to be naturally presented on glioblastoma cells: NVITDGVTSV (HLA-A*02:05) derived from SZT2 subunit of KICSTOR complex (p.260I>N) in ZH613 and RAYTPPRISW (HLA-

B*57:01) derived from PDGFRα (p.343P>T) in ZH681. From both antigens, we also eluted five (SZT2) or eight (PDGFRα) different WT sequence peptides, which might be a general indicator for the presence of mutated peptides in malignancies ensuring presentability of the antigen of interest. Remarkably, the position 343 of PDGFRα was found to be recurrently mutated in glioblastoma creating both a neo-phosphorylation site and neo-phosphorylation motifs within this growth factor receptor. This allows the presumption that the p.343P>T and p.343P>S mutations take part in rendering the growth factor receptor constitutively active thus promoting tumor cell proliferation.[47,48] PDGFRα is known to drive glioblastoma cell proliferation and small molecules for the treatment of PDGFRα-activated glioblastoma are currently being developed.[8,99-101] Hence, we speculate that we might even have detected a mutated peptide suitable not only for fully individualized application but for a larger number of patients carrying the respective mutation. Immunological characterization of both neo-antigenic peptides including T-cell priming and/or IFN-γ enzyme-linked immunospot assay (ELISpot) employing PBMCs and TILs of the patients and – if possible – killing assays with autologous tumor cell lines is currently being performed by Dr. med. Julia Velz and Gioele Medici in the Laboratory for Molecular Neuro-Oncology at the University of Zürich. Despite proving natural HLA presentation of mutated peptides in brain tumors for the first time, we conclude that the translation of non-synonymous mutations into neo-antigenic HLA ligands remains inefficient. Considering 60 class I immunopeptidome datasets acquired from various cancer entities, one neo-antigenic peptide identification resulted on average from $5.7 \times 10^5$ unique HLA class I peptides and $1.2 \times 10^3$ non-synonymous mutations. Thus, we are convinced that, for the large majority of glioblastoma patients, detection of neo-epitopes remains impossible rendering non-mutated antigens the first choice for antigen-specific immunotherapies.

Once analysis of RNA and whole exome sequencing as well as DNA methylation data has been completed for all patients with both primary and recurrent tumors being available, we aim to evaluate expression levels of defined candidate target antigens, to screen the cohort for tumors exhibiting a hypermutation phenotype following radiochemotherapy, and to search for neo-antigenic HLA ligands in this extended dataset. A pilot dataset acquired from GBM3 on a meanwhile obsolete sequencing system indicated a vast increase in the proportion of missense mutations in the hypermutated recurrent as compared with the autologous primary tumor (GBM3P: n=21 / 6%; GBM3R: n=4,569 / 38%; data not shown). This might identify a small subset of patients in which the search for mutated peptides is more promising paving the way for stratified target discovery and treatment attempts in recurrent glioblastoma. Most importantly, these data will provide a deeper insight into clonal evolution and transcriptional changes during glioblastoma progression. We aim to map quantitative changes in primary *versus* recurrent glioblastoma with a special focus on immunobiological markers (e.g. CTLA4, PD1/PDL1, FOXP3, CD3, CD4, CD8, CD68, CD70, CD163)[102] as well as the entire antigen processing machinery. Transcriptional, mutational, and DNA methylation phenotypes as well as the repertoire of presented antigens will be evaluated for correlation with each other and with clinical parameters (e.g. MGMT promotor methylation, progression-free or overall survival).

Herein, we investigated the immunopeptidomic landscape of glioblastoma in an unprecedented depth addressing for the first time both primary and recurrent tumors as well

as HLA class I- and II-presented targets. We defined a large, novel set of non-mutated tumor antigens robustly presented at primary and recurrent disease and provide the first evidence for two neo-antigens naturally presented on HLA molecules of brain tumor cells. This study paves the way for future antigen-specific immunotherapies including DC or peptide vaccination as well as T cell-based concepts which may contribute to meet the urgent need for therapeutic options to manage disease recurrence and to improve dismal survival rates.

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# CHAPTER 3

## HLA peptidome analysis of medulloblastoma uncovers potential targets for cancer immunotherapy

# 1 Abstract

Neoplasms of the brain and CNS are the most common pediatric tumors – with medulloblastoma representing the malignant entity with the highest incidence – and the leading cause of cancer-related mortality in children. Multimodal therapy achieves cure rates of up to 75%, whereas recurrent medulloblastoma remains largely incurable. Conversely, those patients surviving face severe long-term sequelae in consequence of irradiation of the developing brain. Cancer immunotherapy may contribute to the development of innovative therapeutic regimens, whereby the definition of appropriate targets represents a fundamental pillar. Herein, we present the first investigation of the immunopeptidomic landscape of 28 medulloblastomas for target antigens and peptides naturally presented on HLA class I and II molecules. While established tumor-associated and cancer-testis antigens did not fulfill the requirement of both frequent and tumor-exclusive presentation, immunopeptidome analysis unveiled a novel set medulloblastoma-associated antigens and peptides. Remarkably, a significant fraction was shared by childhood and adult medulloblastomas and, most importantly, identified across WNT-activated, non-WNT/non-SHH, and SHH-activated tumors. In conclusion, we defined a set of tumor-associated antigens and peptides characterized by natural, frequent, and exclusive HLA presentation on native medulloblastoma tissue. These represent prime candidates for application in antigen-specific immunotherapeutic approaches, which may replace radiotherapy in multimodal therapeutic regimens and represent an option for the management of disease recurrence.

# 2 Introduction

Brain and CNS tumors are the most common pediatric neoplasms, the leading cause of death due to pediatric cancer, and the seventh most common cause of child mortality in general.[1,2] Medulloblastoma is *per se* graded with WHO grade IV and constitutes the most frequent intracranial malignancy in children, whereas only around 7% of total cases occur in adults older than 40 years.[1,3] Therapeutic regimens for medulloblastomas are guided by both the age and the present subgroup, whereby radiotherapy is standard-of-care in patients older than three to five years and in infants suffering from high- or very high-risk tumors.[4,5] Overall, multimodal therapy comprising surgical resection, chemotherapy, and radiotherapy – when indicated – achieves good cure rates of 70-75%.[4-6] Management of disease recurrence is still an open question and recurrent medulloblastoma has remained largely incurable.[4,7] Those patients surviving face long-term sequelae predominantly caused by irradiation of the developing brain. These comprise neurological, developmental, psychosocial, and neuroendocrine deficits, or even secondary neoplasms such as glioblastoma or meningioma.[4-6,8,9] Hence, the development of novel therapies offering therapeutic options for recurrent medulloblastomas and, most importantly, replacing radiotherapy in the treatment of primary tumors is urgently required.

The antigenic landscape of medulloblastoma has never been investigated and this tumor entity is characterized by a profound lack of potential targets for cancer immunotherapy. Consequently, we aimed to define tumor-associated peptides and antigens that are naturally presented on HLA class I or II molecules of primary medulloblastomas, but not on benign

human tissues. In addition, candidate target antigens were queried against the GTEx database containing RNA expression data acquired across a large set of benign human tissues in order to examine whether they exhibit CTA-like expression profiles, to exclude such with brain- or cerebellum-specific expression, and to identify antigens not known to be expressed in any tissue.[10]

# 3  Methods

## *Patient collective*

Written informed consent of the 28 patients included in the present study and/or their legal representative was obtained in accordance with the Declaration of Helsinki protocol and the local review boards (Kantonale Ethikkommission Zürich / KEK-ZH-Nr. 2015-0163; Ethik-Kommission der Ärztekammer Hamburg / PV4904; authorized vote-free usage of residual tissue collected for other purposes in Sankt Augustin and Würzburg) before surgery. Patients underwent surgery at the Department of Neurosurgery of the University Hospital Zürich, Asklepios Hospital Sankt Augustin, Altona Children's Hospital, or at the University Children's Hospital Würzburg. Tissue samples were snap frozen in liquid nitrogen, Bambanker medium, or Tissue-Tek® O.C.T.™ and stored at -80°C until use. Medulloblastoma specimens along with clinical metadata were kindly provided by PD Dr. med. Marian Christoph Neidert and Dr. med. Julia Velz (University Hospital Zürich, Department of Neurosurgery), Prof. Dr. med. Matthias Eyrich (University Children's Hospital Würzburg, Department of Pediatric Oncology), Prof. Dr. med. Manfred Westphal and Dr. med. Malte Mohme (University Medical Center Hamburg-Eppendorf, Department of Neurosurgery) as well as by Prof. Dr. med. Martina Messing-Jünger, PD Dr. med. Harald Reinhard, and Dr. med. Andreas Röhrig (Asklepios Hospital Sankt Augustin). All patients had histopathologically confirmed medulloblastoma, whereby the collective comprised tumors of all subgroups: n=4 WNT-activated, n=7 SHH-activated, n=2 Group 3, n=2 Group 4, n=8 non-WNT/non-SHH (not differentiated into Group 3 and Group 4), and n=5 without annotation to a subgroup. Two samples were obtained during surgery at disease recurrence. The present study population had a female-to-male ratio of 2:3 and a median age of onset of 8 [1-37] years including two cases of adolescent and adult medulloblastoma each. The median available amount of tissue for HLA-IP accounted to 320 [120-1511] mg. Individual patient and sample characteristics are listed in Table 7, whereby a closer look on HLA allotype frequencies in the study cohort is provided in CHAPTER 5.

**Table 7. Clinical and experimental metadata of the 28 medulloblastoma patients included in the present study.** Letters in internal sample IDs indicate the location, where patients underwent surgery: HH – Altona Children's Hospital, SA – Asklepios Hospital Sankt Augustin, Wü – University Children's Hospital Würzburg, ZH – University Hospital Zürich. Age of onset was defined as the age at initial diagnosis, whereby two cases of adolescent and adult medulloblastoma each were included in the study cohort (marked in grey). Several Non-WNT/non-SHH tumors were not differentiated into Group 3 and Group 4 medulloblastomas. Abbreviation not introduced in the text above: not determined (n.d.). #Sample mass contains few Bambanker medium or Tissue-Tek® O.C.T.™ and is considered separately for calculations of e.g. median amount of available tissue or relative peptide yields.

| Internal sample ID | Gender Age of onset [years] | Subgroup | HLA typing | Sample mass HLA-IP [mg] |
|---|---|---|---|---|
| HH-01 | ♀ 7 | SHH | A*02:01;A*32:01;B*13:02;B*27:02;C*02:02;C*06:02 | 345 |

| | | | | |
|---|---|---|---|---|
| HH-02 | ♂ 13 | Group 3 | A*02:05;A*11:01;B*35:03;B*50:01;C*06:02;C*12:03 | 207 |
| HH-03 | ♂ 4 | Non-WNT/non-SHH | A*02:01;A*68:01;B*51:01;B*51:01;C*14:02;C*15:02 | 209 |
| HH-04 | ♂ 12 | WNT | A*03:01;A*24:02;B*07:02;B*44:02;C*07:02;C*16:04 | 167 |
| HH-05 | ♀ 11 | WNT | A*11:01;A*24:02;B*35:01;B*44:02;C*04:01;C*05:01 | 178 |
| HH-06 | ♂ 5 | Non-WNT/non-SHH | A*11:01;A*68:01;B*41:02;B*52:01;C*12:02;C*17:01 | 386 |
| SA1 Tissue from 1st recurrence at 4 years after initial diagnosis | ♀ 4 | Most likely Non-WNT/non-SHH | A*02:01;A*68:01;B*15:01;B*51:01;C*03:03;C*15:02 | 186 |
| SA2 | ♂ 7 | n.d. | A*01:01;A*11:01;B*07:02;B*08:01;C*07:01;C*07:02 | 290 |
| SA3 Tissue from 1st recurrence at 1 year after initial diagnosis | ♀ 8 | n.d. | A*02:01;A*32:01;B*57:01;B*40:02;C*02:02;C*06:02 | 222 |
| SA4 | ♂ 9 | n.d. | A*01:01;A*02:01;B*07:02;B*37:01;C*06:02;C*07:02 | 120 |
| SA5 | ♀ 1 | n.d. | A*11:01;A*26:01;B*15:08;B*27:05;C*01:02;C*02:02 | 295 |
| Wü-N391/17 | ♂ 19 | n.d. | A*02:01;A*02:11;B*41:01;B*52:01;C*07:01;C*12:02 | 776[#] |
| Wü-N526/17 | ♀ 12 | Group 4 | A*03:01;A*26:01;B*44:03;B*50:01;C*04:01;C*06:02 | 513[#] |
| Wü-N998/13 | ♂ 2 | WNT | A*03:01;A*29:02;B*44:03;B*51:01;C*14:02;C*16:01 | 1066[#] |
| ZH513 | ♂ 8 | Non-WNT/non-SHH | A*03:01;A*11:01;B*27:05;B*35:01;C*01:02;C*04:01 | 1461 |
| ZH680 | ♀ 7 | Non-WNT/non-SHH | A*25:01;A*31:01;B*15:18;B*27:05;C*01:02;C*07:04 | 1554[#] |
| ZH703 | ♂ 4 | Group 4 | A*02:02;A*03:01;B*13:02;B*18:01;C*06:02;C*07:04 | 1302 |
| ZH713 | ♂ 3 | Group 3 | A*03:01;A*26:01;B*38:01;B*49:01;C*07:01;C*12:03 | 932[#] |
| ZH718 | ♂ 1 | SHH/TP53[WT] | A*02:01;A*25:01;B*44:02;B*18:01;C*05:01;C*05:01 | 823[#] |
| ZH732 | ♂ 5 | Non-WNT/non-SHH | A*03:01;A*80:01;B*40:02;B*58:01;C*02:02;C*07:01 | 607 |
| ZH741 | ♀ 8 | WNT | A*02:01;A*24:02;B*18:01;B*44:05;C*02:02;C*07:01 | 1668[#] |
| ZH859 | ♂ 16 | SHH/TP53[mut] | A*03:01;A*24:02;B*35:01;B*35:02;C*04:01;C*04:01 | 863 |
| ZH868 | ♀ 2 | SHH/TP53[WT] | A*01:01;A*02:01;B*44:02;B*57:01;C*05:01;C*06:02 | 1165 |
| ZH872 | ♂ 6 | SHH/TP53[mut] | A*03:01;A*68:02;B*44:02;B*57:01;C*06:02;C*07:04 | 1511 |
| ZH890 | ♀ 9 | Non-WNT/non-SHH | A*01:01;A*31:01;B*08:01;B*35:03;C*04:01;C*07:01 | 3218[#] |
| ZH913 | ♂ 29 | SHH/TP53[WT] | A*29:02;A*68:01;B*44:02;B*44:03;C*07:04;C*16:01 | 869 |
| ZH919 | ♀ 8 | Non-WNT/non-SHH | A*02:01;A*02:01;B*35:08;B*51:01;C*04:01;C*15:02 | 402 |
| ZH937 | ♂ 37 | Most likely SHH/TP53[WT] | A*02:01;A*30:01;B*13:02;B*44:02;C*05:01;C*06:02 | 278 |

### *HLA typing and RNA sequencing*

HLA class I allotypes were retrieved from whole RNA sequencing using the OptiType[11] algorithm by Dr. Gurpreet Kaur, whereas HLA class II allotypes are currently being determined by Marie Gauder and Dr. Stefan Czemmel (Quantitative Biology Center, University of Tübingen).

RNA sequencing was performed for all 28 patients at CeGaT GmbH (Tübingen). Since genomic DNA was not available, we forwent whole exome or genome sequencing. However, DNA was isolated from tumor tissue as well for future DNA methylation profiling. RNA and DNA were isolated from fresh frozen tumor tissue (≥ 20 mg) – containing residual Bambanker medium or Tissue-Tek® O.C.T.™ in some cases – using the AllPrep DNA/RNA Mini Kit (Qiagen). Following quality control for RNA integrity, sequencing libraries were prepared from

78 ng RNA using the KAPA RNA HyperPrep Kit with RiboErase (HMR; Roche), whereby ribosomal RNA was depleted. Paired-end sequencing at 100 bp read length was performed on a NovaSeq 6000 sequencing system (Illumina). Sequencing reads were demultiplexed employing the Illumina bcl2fastq 2.20 and untrimmed FASTQ[12]-formatted files were uploaded to qPortal[13] (Quantitative Biology Center, University of Tübingen) for further evaluation. Analysis of transcriptome data beyond HLA typing has not yet been completed, but is currently being performed by Marie Gauder and Dr. Stefan Czemmel (Quantitative Biology Center, University of Tübingen).

### HLA-IP and subsequent LC-MS/MS to identify HLA-presented peptides

HLA class I- and II-presented peptides were isolated from primary human tissue and analyzed by LC-MS/MS as described in 3.2. As far as possible, Tissue-Tek® O.C.T.™ and Bambanker medium were cut off from embedded samples, while keeping specimens frozen during this step. Both HLA-IP and RNA sequencing were performed from cleaned up tissue to reduce interferences with embedding material during experimental sample processing. All peptide eluates were analyzed in four technical replicates each consuming 14% sample share on an Orbitrap Fusion Lumos. Direct injection was performed for all samples excepting HH-01, HH-02, ZH513, ZH680, ZH703, ZH718, and ZH732.

# 4 Results

## 4.1 HLA class I allotype coverage and peptide yields of HLA-IPs

Aimed at defining medulloblastoma-associated antigens naturally presented on HLA as candidate targets for cancer immunotherapy, 28 primary tumors were analyzed. The study cohort comprised 60 different HLA class I allotypes with HLA-A*02:01 (39%), -A*03:01 (32%), -A*11:01 (21%), -B*44:02 (25%), -B*51:01 (14%), -B*07:02 (11%), -B*13:02 (11%), -B*18:01 (11%), -B*27:05 (11%), -B*35:01 (11%), -B*44:03 (11%), -B*57:01 (11%), -C*06:02 (32%), -C*04:01 (21%), -C*07:01 (21%), and -C*02:02 (18%) being the most frequent ones (Supplementary Table 10). All HLA class I allotypes cover 99.95% of the world population, whereby 92.08% of all individuals are expected to be positive for at least three allotypes (Supplementary Figure 6).

A median of 2016 [88-5867] HLA class I ligands and 1347 [400-4164] HLA class II-presented peptides were identified from medulloblastoma samples. HLA class I peptide eluates exhibited a purity of at least 70% and none of the samples were censored for low peptide yield or low percentage of HLA class I ligands. It was striking that the average purity of samples collected in Zürich (93.3 ± 1.6%; n=14) was superior compared to tissue specimens received from Hamburg (85.6 ± 7.2%; n=6) or Sankt Augustin (84.4 ± 8.8%; n=5) and slightly increased in comparison with tissues from Würzburg (90.3 ± 1.8%; n=3). The total number of unique HLA class I and II peptides, HLA class I ligands as well as the purity of HLA class I peptide eluates are given in Figure 24 A for every specimen. The length distribution of HLA class I ligands clearly peaked at 9 AA length, whereas HLA class II-presented peptides were typically 13- to 18-mers. A fraction of patients showed a broadened length distribution curve elevated either for shorter (≤ 12 AA) or longer (≥ 19 AA) peptides (Figure 24 B). Taking tissue masses

subjected to HLA-IP into account (Table 7), enabled us to investigate whether peptide yields correlate with sample quantities used. This revealed a significant or indicated correlation of the amount of snap frozen medulloblastoma tissue with the number of identified HLA class I ligands or HLA class II-restricted peptides, respectively. In turn, no correlation of employed sample quantities and peptide yields was observed for tissues that had previously been embedded in Bambanker medium or Tissue-Tek® O.C.T.™ (Figure 24 C). Considering the relative number of identified unique peptides per one mg of tissue input revealed a significantly increased yield of HLA class I and HLA class II peptides eluted from snap frozen *versus* embedded tissues (Figure 24 D).



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**D**
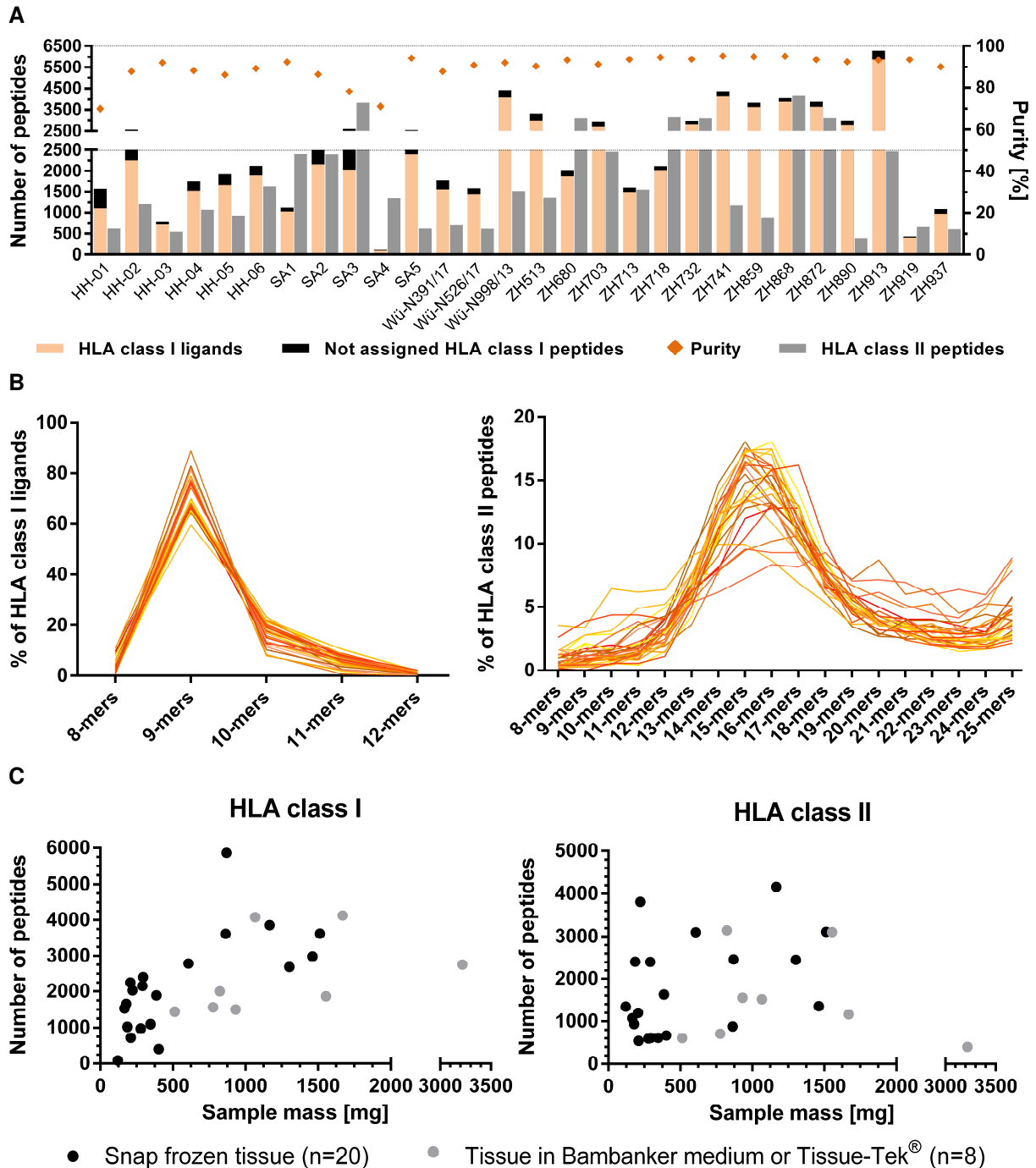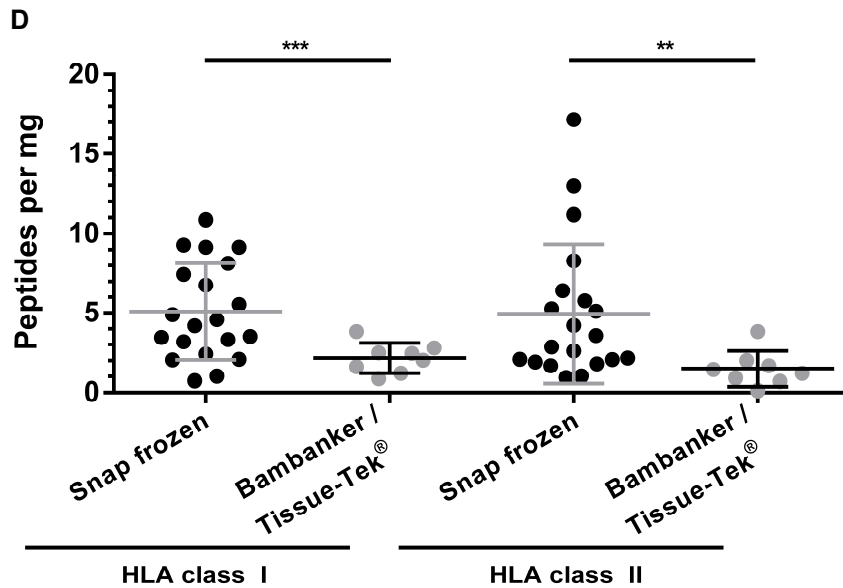


**Figure 24. Number and length distribution of identified HLA class I- and II-presented peptides.**
**(A) HLA class I and II peptide yields obtained from medulloblastoma tissue.** Calculated purities
refer to the proportion of HLA class I peptides annotated to an HLA allotype of the respective patient.
**(B) Length distribution of HLA class I ligands and HLA class II peptides.** Across the entire dataset,
9-mers constituted 71% of HLA class I ligands, whereas 67% of HLA class II-presented peptides had a
length between 13 and 18 AA. Each line represents data of a single sample. While the entire HLA class II
dataset included 9% of peptides with ≤ 12 AA and 24% of peptides with ≥ 19 AA, these shares were
distorted in a fraction of patients (HH-02, Wü-N998/13, ZH713, and ZH937: 13-26% 8- to 12-mers;
HH-04, HH-05, and ZH919: 39-48% 19- to 25-mers). **(C) Unique peptides per sample *versus* amount
of tissue subjected to HLA-IP.** The total amount of unique HLA class I ligands or HLA class II-restricted
peptides identified by analyzing four technical replicates was set in relation to the amount of tissue
subjected to HLA-IP. For this purpose, samples were differentiated into snap frozen tissues and such
embedded in Bambanker medium or Tissue-Tek® O.C.T.™. By non-linear regression (one-phase
association) exponential functions with a forced y-intercept of 0 (internal data created by Daniel
Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free
lysis buffer to HLA-IP does not result in peptide identifications) were fitted. However, the goodness of fit
was poor for all models with the exception of HLA class I ligands eluted from snap frozen tissue:
$R^2 = 0.5527$ (snap frozen HLA class I) / 0.2888 (embedded HLA class I) / 0.1328 (snap frozen HLA
class II) / 0.03929 (embedded HLA class II). A correlation analysis across these normally distributed
data (according to D'Agostino & Pearson omnibus normality test) identified a significant correlation of
snap frozen sample quantities and the number of identified HLA class I ligands (two-tailed $p$-
value = 0.0011) and an indicated correlation with the number of HLA class II-restricted peptides not
reaching statistical significance (two-tailed $p$-value = 0.0639). The amount of tissue obtained after cutting
off Tissue-Tek® O.C.T.™ and removing Bambanker medium as far as possible did neither correlate with
the number HLA class I nor with HLA class II peptide identifications: two-tailed $p$-values = 0.3662 (HLA
class I) / 0.5748 (HLA class II). **(D) Peptide yields normalized to one mg of tissue input.** It was
intended to investigate, whether the relative number of peptide identifications differs between snap
frozen samples and tissues embedded in Bambanker medium or Tissue-Tek® O.C.T.™ as well as
between HLA class I- and II-IPs. An unpaired t test (normalized HLA class I ligand yields had Gaussian
distributions according to D'Agostino & Pearson omnibus normality test) with Welch's correction for
unequal standard deviation (according to F test, $p$-value = 0.0042) revealed a significant difference
between relative HLA class I ligand yields obtained from snap frozen *versus* embedded tissues (two-
tailed $p$-value = 0.0007). Likewise, a significantly increased number of HLA class II peptides was isolated
from snap frozen in comparison to embedded tissues (two-tailed $p$-value = 0.0026), as identified by
Mann-Whitney testing for unpaired non-parametric data. In turn, no significant differences were
observed between relative HLA class I and II peptide yields obtained from the same type of sample:
two-tailed $p$-values = 0.2774 (snap frozen tissues) / 0.2500 (embedded tissues); Wilcoxon matched-

pairs signed rank tests for paired non-Gaussian data (according to D'Agostino & Pearson omnibus normality test).

# 4.2 Identification of medulloblastoma-associated antigens

Employing immunoaffinity chromatography and subsequent LC-MS/MS, we mapped the antigenic repertoire naturally presented on HLA class I and II molecules of 28 medulloblastomas. To define medulloblastoma-associated antigens and peptides, an in-house benign database comprising 30 distinct primary human organs (n=418 HLA class I and n=364 HLA class II datasets) was subtracted. The term medulloblastoma-associated was assigned to peptides and antigens that were never identified on CNS-related tissues (brain, cerebellum, and spinal cord) and for which a maximum of one non-CNS-related sample was positive. Moreover, the frequency of positive primary human malignancies other than medulloblastoma (n=874 samples for HLA class I; n=626 samples for HLA class II) encompassing 37 cancer entities was evaluated. As additional criterium to select targets for cancer immunotherapies, RNA expression data acquired across a large set of benign human tissues and deposited in the GTEx database[10] was reported for every candidate antigen. Further, this allowed the identification of antigens not known to be expressed in any tissue (defined as less than ten TPM in any tissue), such with a classical CTA-like expression profile (not exceeding ten TPM in other organs than testis) as well as to exclude antigens specifically expressed in the brain and/or cerebellum, which represent the tissue of tumor origin.

***Medulloblastoma-associated HLA class I-presented antigens and peptides***

HLA class I peptidome analysis of medulloblastoma (n=28) allowed the identification of 9,821 distinct source proteins represented by HLA class I ligands on native tumor tissue. These represent 91% of the estimated maximum attainable amount of distinct source proteins (Figure 25 C). Despite the vast overlap of medulloblastomas with benign samples, 77 antigens were exclusively presented on at least two neoplasms (Figure 25 A). Following manual curation of the underlying peptides for HLA motifs as well as multi-mapping to several source proteins, a set of 15 medulloblastoma-associated antigens and corresponding peptides naturally presented on 11-29% of tumors was created. Among these, SPINK8, WNT5A, and SHISA9 were the most frequent ones (Figure 25 B). We found a total of seven medulloblastoma-associated antigens to be presented by both childhood and adult tumors (WNT5A, INSM2, NDUFA4L2, OLIG3, BTBD17, EFS, SERTAD4) as well as five presented across all subgroups (SPINK8, WNT5A, NDUFA4L2, BTBD17, EFS). Three antigens were exclusively identified in the non-WNT/non-SHH subgroup, whereas five and two ones were shared by non-WNT/non-SHH and SHH-activated (INSM2, HES4, IGFBPL1, DCX, OLIG3) or by WNT- and SHH-activated neoplasms (VMA21, SERTAD4), respectively. Peptide sequences and their HLA restriction, a listing of positive patients, and the GTEx profile of the corresponding source protein can be retrieved from Supplementary Table 11. Of note, none of these medulloblastoma-associated HLA class I antigens exhibited a CTA-like expression profile.

**A**



**B**

mitochondrial coenzyme Q-binding protein COQ10 homolog A (COQ10A)

transcription factor HES-4 (HES4)   oligodendrocyte transcription factor 3 (OLIG3)

embryonal Fyn-associated substrate (EFS)   insulin-like growth factor-binding protein-like 1 (IGFBPL1)

protein shisa-9 (SHISA9)   SERTA domain-containing protein 4 (SERTAD4)

# serine protease inhibitor Kazal-type 8 (SPINK8)

solute carrier family 22 member 1 (SLC22A1)   neuronal migration protein doublecortin (DCX)

BTB/POZ domain-containing protein 17 (BTBD17)   protein Wnt-5a (WNT5A)

insulinoma-associated protein 2 (INSM2)   vacuolar ATPase assembly integral membrane protein VMA21 (VMA21)

NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4-like 2 (NDUFA4L2)

**C**



**Figure 25. Definition of medulloblastoma-associated antigens based on class I immunopeptidome analyses. (A) Comparative profiling of the HLA class I peptidome of medulloblastoma *versus* an in-house benign database.** Each bar in this waterfall plot (associated with the x-axis) represents a single source protein, whereas the frequency of positive HLA peptidomes is shown on the y-axis, separately for medulloblastoma (n=28) and benign samples without testes

(n=418 covering 29 different human tissues). Those source proteins detected on a maximum of one non-CNS-related tissue were designated as medulloblastoma-exclusive, whereby n=77 were identified on at least two tumors (highlighted as enlarged view on the left). The Venn diagram on the right illustrates the number of distinct HLA class I-presented antigens per group, however, the overlap cannot map the permission of one positive non-CNS-related sample within the benign dataset. **(B) Word cloud of medulloblastoma-associated antigens.** Based on comparative profiling and subsequent quality control of underlying peptides (HLA motifs as well as multi-mapping to several source proteins), a set of 15 medulloblastoma-associated antigens naturally presented on 11-29% of tumors was defined. The font size in the word cloud is proportional to the frequency of positive specimens. **(C) Saturation analysis for the identification of antigens represented by HLA class I ligands on medulloblastoma tissue.** For each source count, the mean number of antigens was calculated by 1,000 random samplings. Using non-linear regression, an exponential function with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) was fitted. The goodness of fit was in the uppermost range ($R^2$ = 0.9999). Based on this curve, the maximum attainable number of distinct source proteins was estimated (highlighted as solid line). With the available number of 28 medulloblastoma samples, 91% of the estimated maximum attainable amount of distinct HLA class I-presented proteins had been identified.

On the peptide level, 32,199 distinct HLA class I ligands were eluted from medulloblastomas (n=28), obtaining 75% of the estimated maximum attainable coverage (Figure 26 B). Although the HLA class I peptidome of medulloblastoma showed a pronounced high overlap with that of benign samples, 1,963 medulloblastoma-exclusive peptides presented on at least two tumors were identified (Figure 26 A). Subsequent to manual curation, a set of 34 peptides derived from 38 antigens and presented on 18-29% of tumors was defined. Among these, HLA ligands derived from GFAP, HNRNPK, SNX14, AGRN, DNMT3A, KIF1A, and GABRG2 were the most frequent ones. Although only two adults were part of the cohort, twelve medulloblastoma-associated peptides were eluted from both childhood and adult tumors. Considering the different subgroups, 13 peptides proved to be pan-medulloblastoma targets, whereas WNT- and SHH-activated or WNT-activated and non-WNT/non-SHH shared two peptides each. One HLA class I ligand was exclusively part of the immunopeptidome of non-WNT/non-SHH medulloblastomas. Peptide sequences and their HLA restriction, a listing of positive patients, and the corresponding source protein are given in Supplementary Table 12.

Combining the list of peptides derived from HLA class I-presented medulloblastoma-associated antigens (Supplementary Table 11) with that of HLA class I ligands designated as medulloblastoma-associated (Supplementary Table 12) gave n=66 candidate target peptides for cancer immunotherapy. These cover 98.64% of the world population (Supplementary Figure 7), whereby an average of 14 peptides are expected to match per patient. The population coverage on a per-country basis is shown in Figure 27.

**A**



**B**
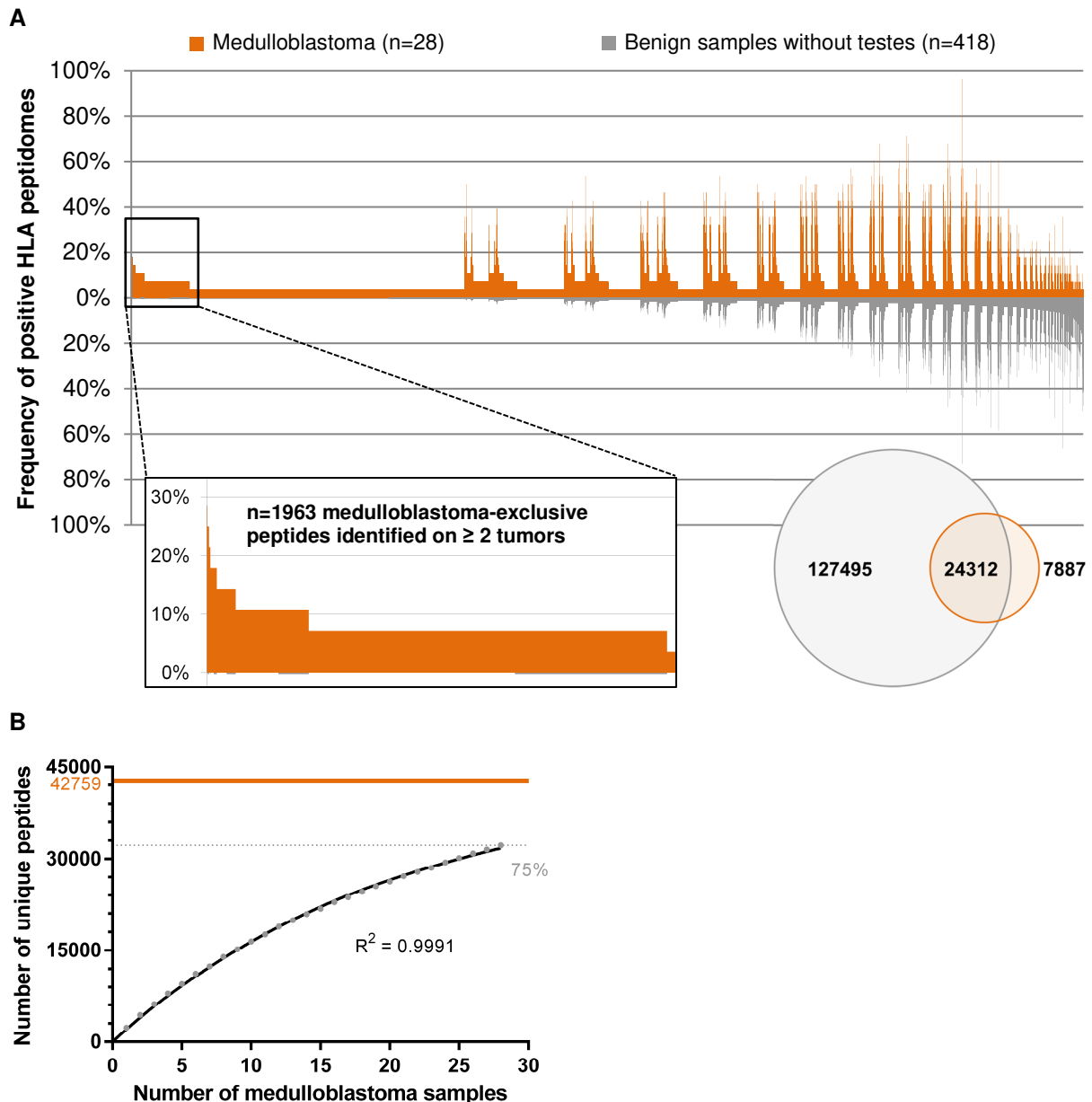


**Figure 26. HLA class I peptidomics to define medulloblastoma-associated peptides. (A) Comparative profiling of HLA class I ligands presented on medulloblastoma *versus* an in-house benign database.** Every peptide evaluated for tumor association is represented by a bar in the waterfall plot (associated with the x-axis), whereas the y-axis shows the frequency of positive HLA peptidomes, separately for medulloblastoma (n=28) and benign samples without testes (n=418 covering 29 different human tissues). Peptides were designated as medulloblastoma-exclusive, when detected on a maximum of one non-CNS-related tissue, whereby n=1,963 were identified on at least two neoplasms (highlighted as enlarged view on the left). The number of distinct HLA class I ligands per group is illustrated by the Venn diagram on the right, whereby the overlap cannot map the permission of one positive non-CNS-related sample within the benign dataset. **(B) Saturation analysis for the identification of HLA class I ligands in medulloblastoma tissue.** For each source count, the mean number of peptides was calculated by 1,000 random samplings. Using non-linear regression, an exponential function with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) was fitted. The goodness of fit was in the uppermost range ($R^2 = 0.9991$). Based on this curve, the maximum attainable number of distinct peptides was estimated (highlighted as solid lines). With the available number of 28 medulloblastoma samples, 75% of the estimated maximum attainable amount of distinct HLA class I ligands had been identified.
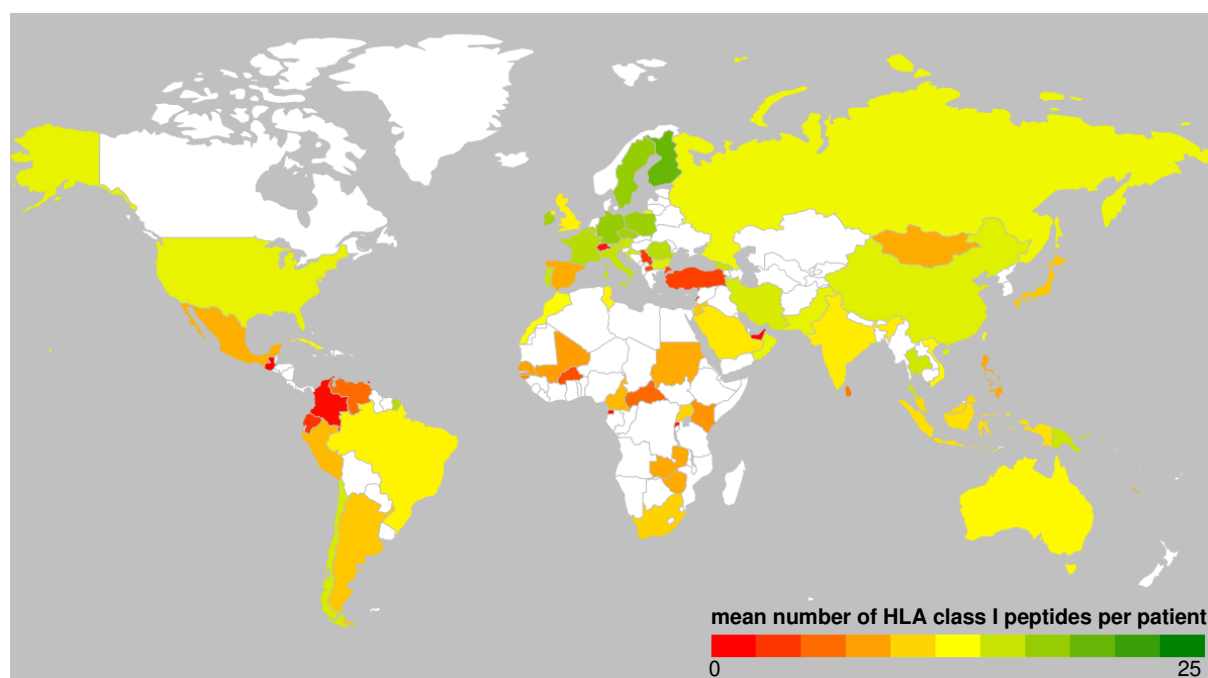
**Figure 27. Population coverage of medulloblastoma-associated HLA class I peptides.** Using the population coverage tool provided by the IEDB Analysis Resource[14], the population coverage of the n=66 candidate target peptides for medulloblastoma immunotherapy was calculated on a per-country basis. On average, 14 HLA class I peptides match per patient worldwide. For visualization of the United Kingdom, the individual values of England, Northern Ireland, Scotland, and Wales were multiplied with the relative area portion. Countries not included in the IEDB tool or not covered by the geographic heat map add-on of Microsoft Excel are colorless.

### *Medulloblastoma-associated HLA class II antigens*

HLA class II peptidome analyses of medulloblastoma allowed the identification of 4,922 distinct source proteins giving rise to HLA class II-restricted peptides. These represent 71% of the estimated maximum attainable amount of distinct antigens (Figure 28 C). Although medulloblastomas largely overlapped with benign samples, 60 antigens were exclusively presented by at least two tumors (Figure 28 A). Following manual curation of the underlying peptides for peptide length and the presence of length variants as well as multi-mapping to several source proteins, a set of ten medulloblastoma-associated antigens and corresponding peptides naturally presented on 11-36% of tumors was created. Among these, IGFBPL1, CBX4, ESCO1, SYCP3, and CCDC59 were the most frequent ones (Figure 28 B). Three of the medulloblastoma-associated antigens were identified across all subgroups (CBX4, ESCO1, LLPH) and five ones were shared by non-WNT/non-SHH and SHH-activated tumors (IGFBPL1, SYCP3, PLA2G7, PCDH20, CGGBP1). WNT- and SHH-activated neoplasias or WNT-activated and non-SHH/non-WNT tumors had one antigen each in common. Six out of ten tumor-associated antigens were found to be presented on HLA class II in both adult und childhood medulloblastomas (IGFBPL1, CBX4, ESCO1, CCDC59, PCDH20, CGGBP1), although the present study population included only two adults. Peptide sequences, a listing of positive patients, and the GTEx profile of the corresponding source protein can be retrieved from Supplementary Table 11. It is noteworthy that one of these tumor-associated HLA class II antigens (SYCP3) exhibited a CTA-like expression profile with the related SYCP1 being listed in the CTDatabase[15].

**A**
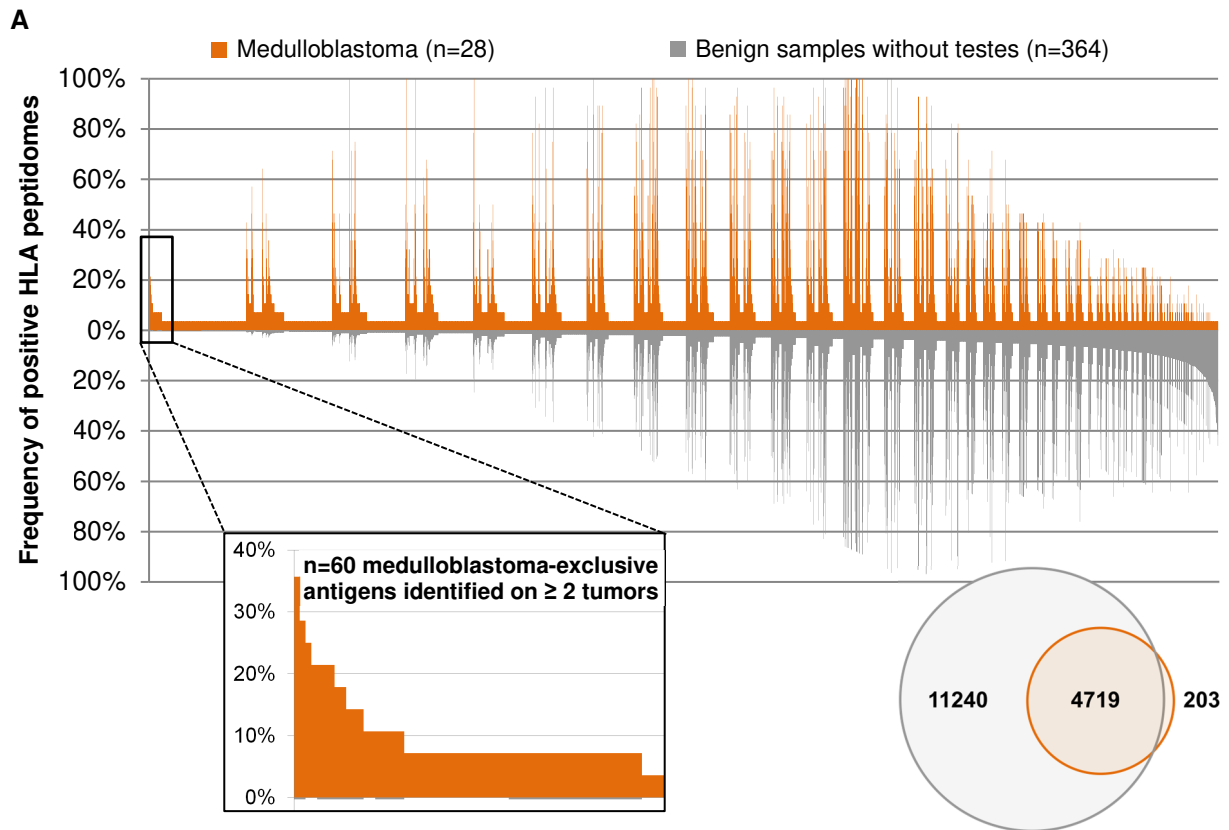


**B**

E3 SUMO-protein ligase CBX4 (CBX4)    CGG triplet repeat-binding protein 1 (CGGBP1)

something about silencing protein 10 (UTP3)    synaptonemal complex protein 3 (SYCP3)

# insulin-like growth factor-binding

protein LLP homolog (LLPH)                            protocadherin-20 (PCDH20)

## protein-like 1 (IGFBPL1)

thyroid transcription factor 1-associated protein 26 (CCDC59)

platelet-activating factor acetylhydrolase (PLA2G7)  N-acetyltransferase ESCO1 (ESCO1)

**C**
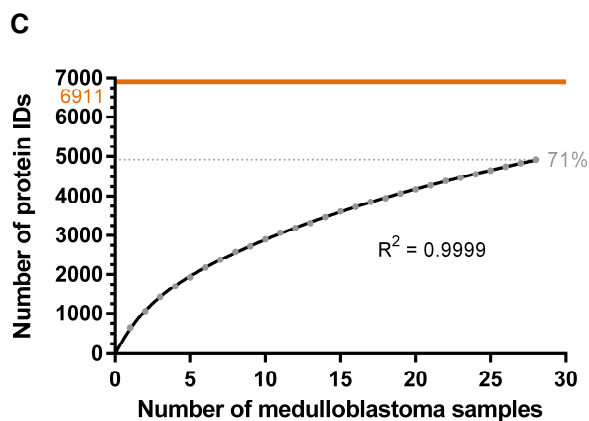


**Figure 28. Definition of medulloblastoma-associated antigens based on class II immunopeptidome analyses. (A) Comparative profiling of the HLA class II peptidome of medulloblastoma *versus* an in-house benign database.** Each bar in this waterfall plot (associated with the x-axis) represents a single source protein, whereas the frequency of positive HLA peptidomes is shown on the y-axis, separately for medulloblastoma (n=28) and benign samples without testes

(n=364 covering 30 different human tissues). Those source proteins detected on a maximum of one non-CNS-related tissue were designated as medulloblastoma-exclusive, whereby n=60 were identified on at least two neoplasms (highlighted as enlarged view on the left). The Venn diagram on the right illustrates the number of distinct HLA class II-presented antigens per group, however, the overlap cannot map the permission of one positive non-CNS-related sample within the benign dataset. **(B) Word cloud of medulloblastoma-associated antigens.** Based on comparative profiling and subsequent quality control of underlying peptides (peptide length and/or presence of length variants as well as multi-mapping to several source proteins), a set of ten tumor-associated antigens naturally presented on 11-36% of medulloblastomas was defined. The font size in the word cloud is proportional to the frequency of positive tumors. **(C) Saturation analysis for the identification of antigens represented by HLA class II peptides on medulloblastoma tissue.** For each source count, the mean number of antigens was calculated by 1,000 random samplings. Using non-linear regression, an exponential function with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) was fitted. The goodness of fit was in the uppermost range ($R^2 = 0.9999$). Based on this curve, the maximum attainable number of distinct source proteins was estimated (highlighted as solid line). With the available number of 28 medulloblastoma samples, 71% of the estimated maximum attainable amount of distinct HLA class II-presented proteins had been identified.

On the peptide level, 25,076 distinct HLA class II-presented peptides were eluted from medulloblastomas (n=28), obtaining 66% of the estimated maximum attainable coverage (Figure 29 B). Subsequent to comparative profiling, all antigens represented by at least one tumor-exclusive HLA class II-presented peptide were subjected to hotspot analysis (Figure 29 A). Medulloblastoma-associated HLA class II presentation hotspots were defined to have a minimum length of eight AA and to be covered by peptides identified in at least five patients, while not having matching sequences in benign samples. This identified a set of eleven antigens harboring regions uniquely presented on tumor tissue with peptide-specific frequencies reaching up to 43% of positive HLA peptidomes. Half of these hotspot targets were represented within the immunopeptidome of all medulloblastoma subgroups or of both adult und childhood tumors, respectively. One hotspot target each was exclusively presented by non-SHH/non-WNT and SHH-activated tumors each, whereas non-SHH/non-WNT and SHH-activated medulloblastomas had two proteins harboring HLA class II presentation hotspots in common. Furthermore, SHH- and WNT-activated or non-SHH/non-WNT and WNT-activated neoplasms shared one protein with hotspot-derived peptides each, respectively. Peptide sequences, a listing of positive patients, and the corresponding source protein are given in Supplementary Table 13.

**A**



**B**
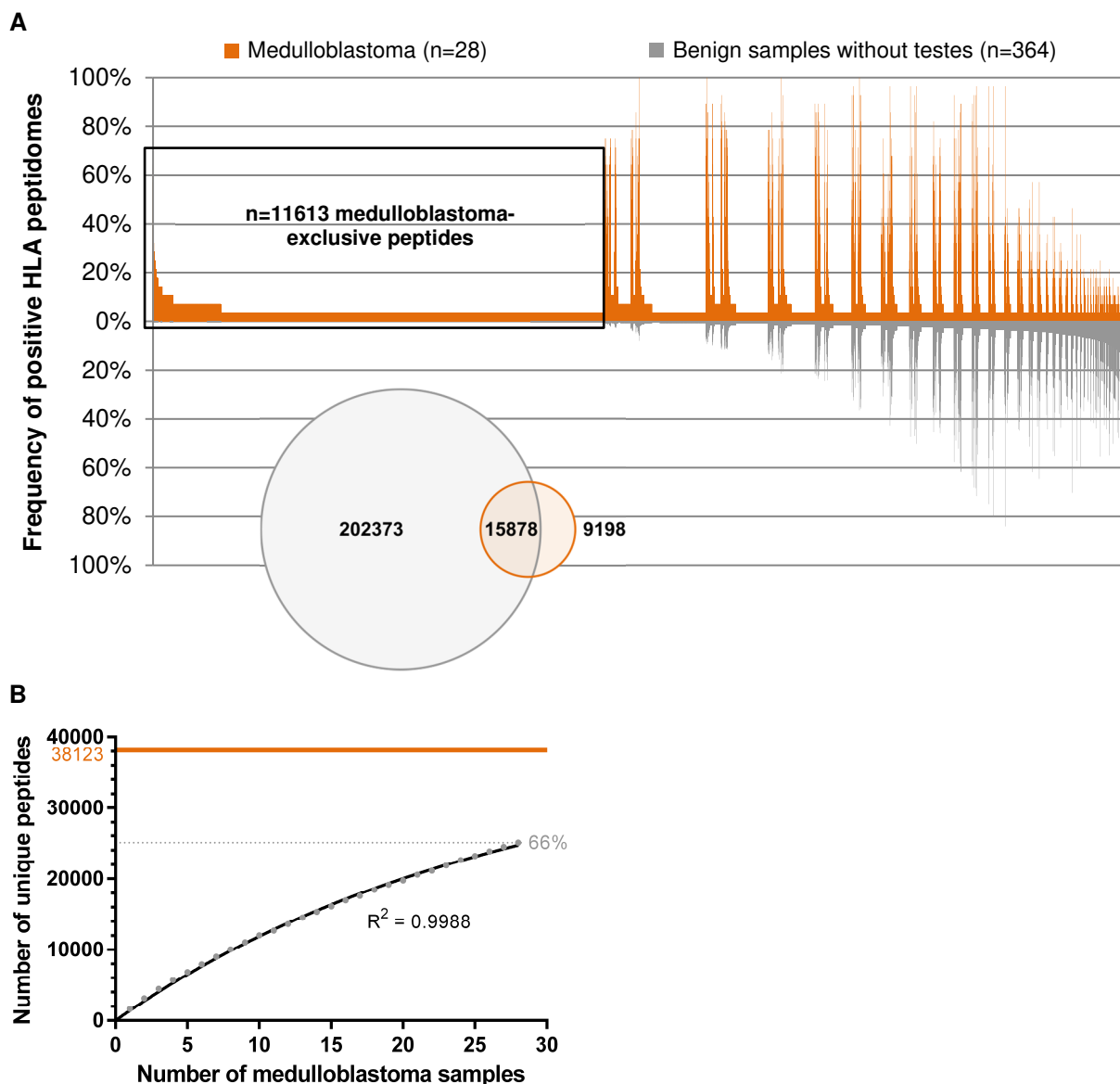


**Figure 29. HLA class II peptidomics to define medulloblastoma-associated peptides (A) Comparative profiling of HLA class II peptides presented on medulloblastoma *versus* an in-house benign database.** Every peptide evaluated for tumor association is represented by a bar in the waterfall plot (associated with the x-axis), whereas the y-axis shows the frequency of positive HLA peptidomes, separately for medulloblastoma (n=28) and benign samples without testes (n=364 covering 30 different human tissues). Being detected on a maximum of one non-CNS-related tissue, n=11,613 peptides were designated as medulloblastoma-exclusive. Corresponding source proteins were subjected to hotspot analysis. The number of distinct HLA class II-restricted peptides per group is illustrated by the Venn diagram on the left, whereby the overlap cannot map the permission of one positive non-CNS-related sample within the benign dataset. **(B) Saturation analysis for the identification of HLA class II-presented peptides in medulloblastoma tissue.** For each source count, the mean number of peptides was calculated by 1,000 random samplings. Using non-linear regression, an exponential function with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) was fitted. The goodness of fit was in the uppermost range ($R^2$ = 0.9988). Based on this curve, the maximum attainable number of distinct peptides was estimated (highlighted as solid line). With the available number of 28 malignant samples, 66% of the estimated maximum attainable amount of distinct HLA class II-presented peptides had been identified.

Comparing medulloblastoma-associated HLA class I- (n=15) and II-presented antigens (n=10) as well as medulloblastoma-associated HLA class I- (n=38 source proteins) and II-restricted peptides (n=11 source proteins), revealed a largely unique antigenic repertoire inherent to HLA class I and II peptidomes. However, IGFBPL1, INSM1, and INSM2 yielded both HLA class I- and II-presented target peptides.

### *Established CTAs and TAAs naturally presented on medulloblastoma*

Considering a total number of 366 established CTAs and TAAs (3.2.1) as well as 16 antigens reported to be associated with medulloblastoma (2.3.2; n=15 overlapped with the general list of CTAs and TAAs), the present HLA peptidome dataset acquired from medulloblastomas was screened for previously published tumor antigens. Of these, n=117 and n=84 were represented by HLA class I ligands and HLA class II-presented peptides, respectively. Although identification rates were comparatively high, CTAs and TAAs were basically presented at low frequency, especially those exclusively identified on medulloblastoma. While none of the HLA class I-presented TAAs and CTAs fulfilling the aforementioned criteria to be designated as tumor-exclusive antigen were identified on more than one specimen, 16 ones were represented by medulloblastoma-exclusive HLA class I ligands on at least two and a maximum of four tumors. On HLA class II, 13 antigens were exclusively identified in the peptidome of medulloblastomas, with RAD51 and GPC2 being the only ones reaching a total of two positive tumors. Eight further CTAs and TAAs were represented by tumor-exclusive HLA class II-presented peptides on 7-14% of neoplasms (Figure 30, Supplementary Table 14).

Frequency of positive
HLA class I peptidomes [%]

Frequency of positive
HLA class II peptidomes [%]

Legend:
- Medulloblastoma (n=28)
- Benign samples without testes (n=418 / n=364)
- ◆ Medulloblastoma-exclusive peptides
- ◆ Medulloblastoma-exclusive peptides identified on ≥ 2 tumors
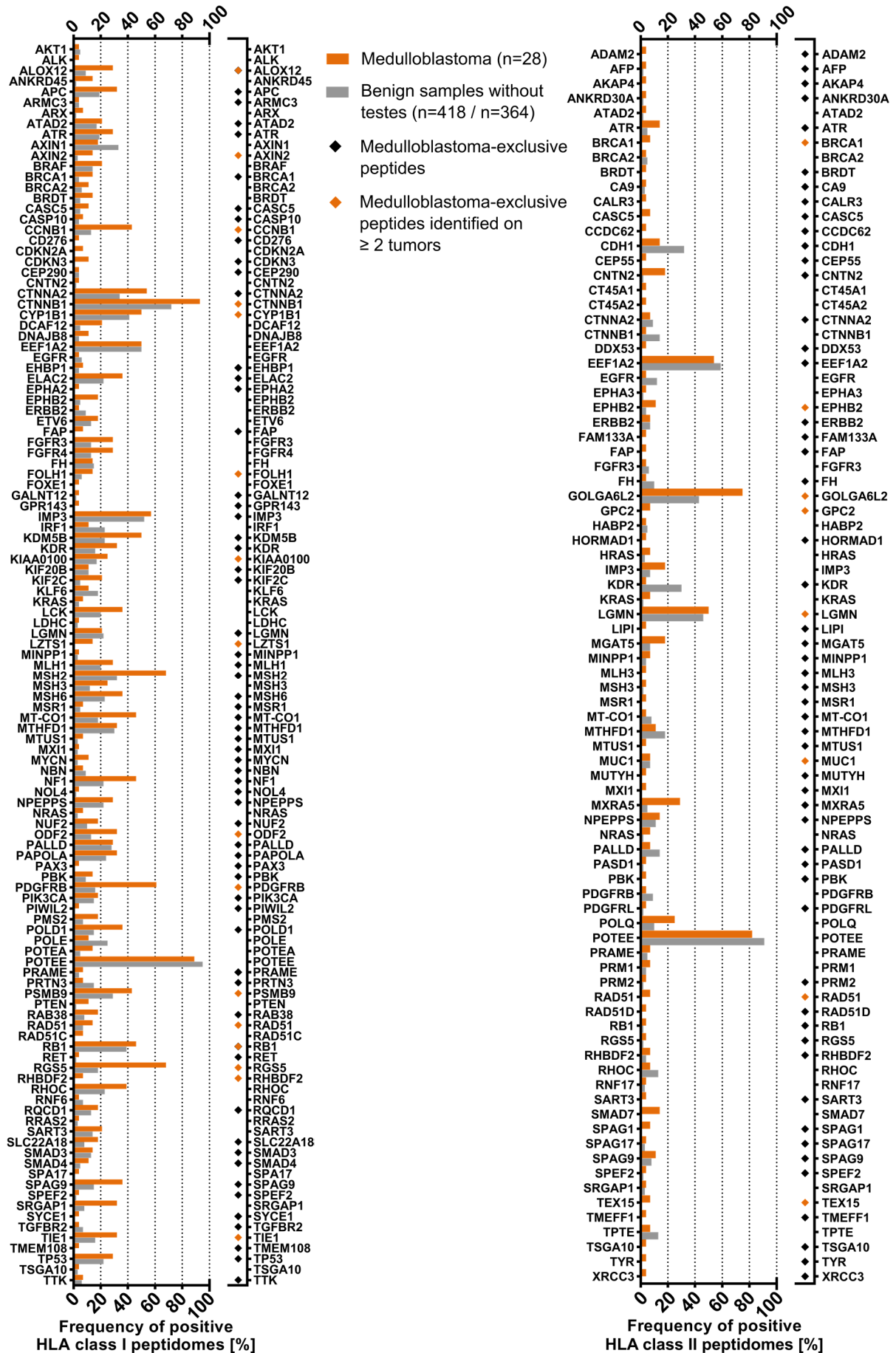
**Figure 30. Identification of established TAAs, CTAs, and medulloblastoma-associated antigens across the present HLA peptidome dataset.** The present immunopeptidomic dataset included HLA class I ligands derived from 116 TAAs and CTAs (left panel), with 16 being represented by medulloblastoma-exclusive peptides on at least two tumors (highlighted with orange diamonds). The frequency of positive HLA peptidomes was assessed based on HLA class I ligands for tumor samples, whereby benign hits were reported independent of HLA binding probabilities of the underlying peptide identifications. Concerning HLA class II (right panel), a total of 85 TAAs and CTAs were found to be naturally presented on medulloblastoma. Among these, eight yielded tumor-exclusive peptides attaining a presentation frequency of $\geq$ 7% (highlighted with orange diamonds). While peptides mapping to multiple source proteins were considered to calculate the frequency of positive HLA peptidomes, these were excluded to report the representation by medulloblastoma-exclusive peptides. CTAs and TAAs exclusively identified on benign samples were not listed.

# 5 Discussion

Medulloblastoma is the most common pediatric malignancy of the CNS, which represents the top-ranking localization of childhood tumors and the leading cause of death due to cancer in children.[1-3] Surgery, chemotherapy, and radiotherapy achieve cure rates of up to 75%, however, radiation of the developing brain causes severe long-term sequelae and recurrent medulloblastoma has remained largely incurable.[4-9] Cancer immunotherapy may replace radiotherapy in multimodal therapeutic regimens and represent an option to manage disease recurrence. Herein, we present the first investigation of the immunopeptidomic landscape of medulloblastoma to define HLA class I- and II-restricted candidate targets.

Both HLA class I- and II-presented peptides were eluted in considerable amounts from 28 primary medulloblastoma samples proving this cancer entity to be well suitable for studies of the HLA peptidome. Expression of HLA molecules and presentation of a manifold repertoire of peptides, which seems to apply for medulloblastoma, does also represent a prerequisite for immunotherapeutic intervention. This result was actually not expected, since several components of the antigen processing machinery (e.g. HLA-A, -B, and -C, calnexin, $\beta_2$m, LMP2, LMP7) have previously been reported to be down-regulated in medulloblastoma.[16] We could only observe the apparent down-modulation of HLA expression when analyzing the medulloblastoma cell lines MM8A, D341, D283 Med, and DAOY (800-2,200 µl cell pellet volumes) yielding 32-772 HLA class I and 98-354 HLA class II peptides when acquiring two technical replicates consuming 14% sample share each on an Orbitrap Fusion Lumos (data not shown). This again emphasizes that the use of cell lines represents an inferior substitute for primary human tumor samples.[17] Nevertheless, there were obvious hints of reduced HLA class I expression in specimen SA4. We ruled out poor quality of the tissue lysate or W6/32 antibody, failure during ZipTip$_{C18}$® purification, and technical issues during LC-MS/MS, as HLA class II peptide yields were fine, the identical W6/32 batch passed quality control and was employed for several samples with high numbers of HLA class I peptide identifications, direct injection was performed, and as the HLA class I peptide eluate was measured prior to that of HLA class II, while the LC-MS/MS system ran at good performance. This presumption will be validated, once analysis of RNA expression data has been completed. Relative HLA class I and II peptide yields obtained from fresh frozen tissue were significantly higher as compared with samples previously embedded in Bambanker medium or Tissue-Tek®O.C.T.™. This may, on the one hand, be reasoned by remnants of embedding material falsifying determined

sample weights and, on the other hand, by interferences to antibody binding efficiency during HLA-IP and/or also during MS. The latter appears very plausible as samples previously embedded in Tissue-Tek® O.C.T.™ showed a large number of interfering peaks in the total ion chromatogram (from minute 80 of the LC gradient) comparable in height to the typical CHAPS peak observed around minute 105 to 110. It turned out that these were series of peaks of the positively charged PEG with a distance of 44 m/z. PEG is – like CHAPS – known to cause ion suppression (and isobaric interference) in LC-MS/MS.[18-20] A different average purity of HLA class I peptide eluates was observed across the four different institutions, which can be reasoned by discrepancies in sample collection. In Zürich, sample collection for immunopeptidomics has been a well-established routine process since many years, whereas surgical staff in Hamburg, Sankt Augustin, and Würzburg has far less experience with sample requirements for HLA peptidomics. This problem could be addressed by transferring standard operating procedures of sample collection with the purpose to isolate HLA ligands from Zürich, providing samples with the best purities, to other institutions.

The present study population having a female-to-male ratio of 2:3 and a median age of onset of eight years constituted a representative cohort of medulloblastoma patients.[1] In total, n=4 WNT-activated (14%), n=7 SHH-activated (25%), n=2 Group 3, n=2 Group 4, n=8 non-WNT/non-SHH (not differentiated into Group 3 and Group 4), and n=5 without annotation to a subgroup were analyzed including two cases of adult medulloblastoma. The 60 distinct HLA class I allotypes cover 99.95% of the world population, whereby 92.08% of all individuals are expected to be positive for at least three allotypes. We found medulloblastoma to be a – albeit rather sparse – source of candidate targets for cancer immunotherapy. Antigens appearing tumor-exclusive in the analysis but showing a highly CNS-associated expression profile were excluded since we do not expect to achieve 100% saturation of HLA class I and II protein identifications with the currently available number of 23 benign brain and cerebellum samples. Thus, it is conceivable that brain-specific proteins appear as false-positive tumor-exclusive antigens in comparative profiling. In contrast to other tumors including meningioma, T cells targeting medulloblastoma cross the blood-brain barrier and may then cause on-target off-tumor toxicities in cells presenting CNS-specific antigens.[21-24] The search for frequently presented medulloblastoma-associated antigens uncovered 15 and ten for HLA class I and II, respectively. Among these, IGFBPL1 (36%), SPINK8 (29%), WNT5A (25%), CBX4 (21%), ESCO1 (21%), SHISA9 (18%), SYCP3 (18%), and CCDC59 (18%) were the most frequent ones. The neurotrophic insulin-like growth factor axis has a prominent role in regulating CNS development and promoting the growth of tumor cells, including medulloblastoma, ependymoma, meningioma, and glioma, *via* anti-apoptotic and mitogenic peptides.[25-28] Several members of the insulin-like growth factor binding protein family have been associated with ependymomas and medulloblastomas by being elevated in comparison with control cerebella and indicating poor prognosis when expressed at high levels.[29] SPINK8, spanning only 97 AA,[30] is not expressed above background levels (10 TPM) in benign human tissues and has been suggested to be epididymis-specific.[10,31,32] Other Kazal-type serine peptidase inhibitors such as SPINK1, also called secretory tumor-associated trypsin inhibitor, have been found to be over-expressed in various cancer entities, to promote tumor progression, and to correlate with adverse prognosis.[33-37] In colorectal carcinoma, SPINK1 has been suggested to act by inhibiting Metallothionein tumor suppressors.[34] A member of the oncogenic WNT protein

family was identified with WNT5A, which is renowned for tumor-associated elevated expression.[38,39] WNT5A was, however, not specific for tumors of the WNT-activated subgroup, but identified in SHH-activated and non-WNT/non-SHH medulloblastomas as well. Furthermore, HLA class I-presented peptides derived from WNT5A were identified in 51 malignant samples other than medulloblastoma. An oncogenic function exerted by activation of Notch1 signaling has also been attributed to CBX4.[40] Moreover, CBX4-mediated sumoylation activates the oncogenic DNA repair protein BMI-1, which has been implied for targeted therapy in pediatric diffuse intrinsic pontine glioma.[41-45] Besides promoting cell cycle progression as well as cancer cell migration and metastasis,[40,41,45] CBX4 is also angiogenic by promoting VEGF expression and governing the hypoxia-inducible factor 1-α.[46] The acetyltransferase ESCO1 accomplishes sister chromatid cohesion during the S phase of DNA replication facilitating and speeding the traverse of replication forks – a beneficial feature for malignant cells.[47-49] In turn, little is known about SHISA9 (seven PubMed entries[50]), which is not expressed with more than 7 TPM in any benign human tissue.[10] SHISA9, also named CKAMP44, is a subunit of synaptic glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors with regulatory functions in neurotransmission and synaptic plasticity.[51-53] SYCP3 did not only exhibit a CTA-like expression profile,[10] but is also described as testis-specific protein regulating sister chromosome segregation during meiosis with aberrant expression in malignant – including brain tumor – cells.[54-58] Since the related SYCP1 is listed in the CTDatabase,[15] it appears obvious that SYCP3 represents a novel CTA naturally presented on HLA class II molecules. CCDC59, referred to as thyroid transcription factor 1-associated protein 26, is a poorly studied protein (seven PubMed entries[59]) with reported function in the regulation of surfactant protein synthesis in the lung.[60-62]

Investigating acquired immunopeptidomic data for medulloblastoma-associated peptides unveiled a set of 34 HLA class I ligands derived from 38 antigens presented on 18-29% of tumors as well as of eleven antigens harboring medulloblastoma-associated HLA class II presentation hotspots giving rise to naturally presented peptides across at least five patients. It was conspicuous that the two most frequent medulloblastoma-associated HLA class I ligands were 10-mers, which is not the typical length of HLA class I-presented peptides, annotated to multiple HLA allotypes not perfectly matching the motif. Moreover, we observed a large number of HLA-A*25:01 and -A*26:01 ligands among both medulloblastoma-associated and CTA- or TAA-derived tumor-exclusive peptides. A total of 17 samples included in the benign dataset were positive for HLA-A*25:01 and/or -A*26:01 with none of them representing a CNS-related tissue. This implies an insufficient coverage of the tissue of tumor origin regarding these HLA allotypes and all tumor-exclusive peptides binding to HLA-A*25:01 or -A*26:01 were consequently excluded from candidate target lists. Conversely, the benign dataset of CNS-related samples covered twelve HLA-A allotypes (A*01:01, A*02:01, A*02:05, A*03:01, A*11:01, A*23:01, A*24:02, A*30:01, A*32:01, A*68:01, A*68:02, A*69:01), 16 HLA-B allotypes (B*07:02, B*08:01, B*13:02, B*14:02, B*15:01, B*27:05, B*35:01, B*35:03, B*35:08, B*37:01, B*40:02, B*44:02, B*45:01, B*49:01, B*50:01, B*58:01), and 10 HLA-C allotypes (C*02:02, C*03:03, C*03:04, C*04:01, C*06:02, C*07:01, C*07:02, C*07:04, C*08:02, C*16:01). Medulloblastoma-exclusive peptide hits were only pursued when the corresponding HLA class I allotype was contained in the CNS subset of the benign reference peptidome database. It was striking that overlapping medulloblastoma-associated HLA class I- and II-presented

antigens (n=10) as well as tumor-associated HLA class I- and II-restricted peptides, revealed a largely unique antigenic repertoire inherent to HLA class I and II peptidomes with only three antigens (IGFBPL1, INSM1, INSM2) yielding both HLA class I- and II-presented target peptides. This underlines the necessity to respect both HLA classes on both antigen and peptide level for comprehensive target discovery approaches. Besides that, we found a significant proportion of candidate targets to be pan-medulloblastoma peptides or antigens as being coincidently eluted from WNT-activated, non-WNT/non-SHH, and SHH-activated neoplasms (n=5 / n=13 HLA class I antigen / peptide targets, n=3 / n=5 HLA class II antigen / hotspot targets). Likewise, almost half of all medulloblastoma-associated antigens and peptides was shared by childhood and adult tumors (n=7 / n=12 HLA class I antigen / peptide targets, n=6 / n=6 HLA class II antigen / hotspot targets). In this context, it should be added that the number of targets presented independent of patient age is expected to be even higher, as only two cases of adult medulloblastoma were included in the present cohort. This allows the conclusion that medulloblastoma subgroups exhibit a common antigenic signature promising that defined targets are broadly applicable in medulloblastoma immunotherapy. Combining the list of medulloblastoma-associated antigens and peptides presented on HLA class I (n=66 candidate target peptides for cancer immunotherapy), these achieve a world population coverage of 98.64% whereby an average of 14 peptides are expected to match per patient. Established CTAs and TAAs failed to fulfil the requirements of frequent and tumor-exclusive presentation as being either anecdotally detected in very few tumors or being broadly identified on both malignant and benign tissues. Considering these turned out to be no valuable contribution to defining candidate targets for medulloblastoma immunotherapy. This strongly advises against basing target discovery approaches solely on RNA expression, protein, or immunohistochemistry data, as the HLA peptidome represents an autonomous layer molded by the antigen processing machinery.[17,63-66]

Once analysis of transcriptome and DNA methylation profiling data has been completed, we aim to investigate whether an imprint of the present subgroup manifests at these levels. Moreover, we selected a set of 15 HLA class I-restricted peptides covering 14 antigens and common HLA allotypes (HLA-A*01, -A*02, -A*03, -A*11, -A*68, -B*35) to undergo immunogenicity testing. This will include priming of naïve T cells from healthy donors and – if possible – medulloblastoma patients and will be performed by Dr. med. Julia Velz and Gioele Medici in the Laboratory for Molecular Neuro-Oncology at the University of Zürich.

In conclusion, the data presented demonstrate that medulloblastoma is eligible for immunotherapeutic intervention. We mapped the antigenic landscape of medulloblastoma in an unprecedented depth unveiling a novel set of canonical non-mutated tumor-associated peptides and antigens with natural and frequent presentation on HLA class I and II molecules. These may guide the development of peptide-specific immunotherapies such as vaccination with DCs and peptides or T cell-based strategies which may eventually represent an option to manage disease recurrence and replace radiotherapy in multimodal therapeutic regimens.

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# CHAPTER 4

## The immunopeptidome of meningioma reveals candidate targets for immunotherapies and delineates modulated presentation of HLA ligands in comparison with autologous dura

# 1   Abstract

Meningiomas are the most common neoplasms of the brain and CNS. Tumor localization and disease recurrence exacerbate the therapeutic situation with surgery and radiotherapy representing the only established therapy regimens. Cancer immunotherapy may meet the high demand for contemporary treatments, however, little is known about the antigens to be targeted. Herein, we present the first investigation of the immunopeptidomic landscape of 33 meningiomas for naturally presented target antigens for application in antigen-specific immunotherapeutic approaches. Meningeal neoplasias proved to be a rich source of novel tumor-associated peptides and antigens presented on HLA class I and II molecules. Remarkably, a large fraction were pan-meningioma targets presented across all WHO grades. Established cancer-testis and tumor-associated antigens, however, lacked frequent and/or meningioma-exclusive HLA presentation. In a subset of patients with autologous tumor-free dura being available, relative quantitation of HLA class I- and II-restricted peptide abundances delineated common patterns of modulated peptide presentation. In conclusion, the defined set of meningioma-associated antigens and peptides provides prime candidates to be pursued in the development of future immunotherapies as being naturally, exclusively, and frequently presented on native meningioma tissue. This may contribute to overcome the lack of therapeutic options for non-resectable and recurrent, especially high-grade, meningiomas.

# 2   Introduction

Meningeal tumors constitute the most frequent brain and CNS neoplasms accounting for an average annual incidence of 30,551 in the US. Despite being diagnosed as non-malignant in almost 99% of cases, these tumors nevertheless cause severe health issues including, among others, seizures and focal neurological deficits.[1-4] Complete surgical resection of meningiomas localized at the skull base, which is rich in neurovascular structures and where 38% of WHO grade I tumors arise, can be challenging and tumor recurrence of those meningiomas with a more malignant behavior despite multiple resections and radiotherapy is a common clinical problem. Poor progression-free and overall survival rates in particular affect those patients with subtotal resection.[5,6] So far, no chemotherapy with meningioma as indication has been approved by the FDA and no standard-of-care for the management of recurrent meningiomas has been established.[5,7] Consequently, there is a high demand for contemporary treatment strategies supplementing surgery and radiotherapy – a demand that could be elegantly serviced by cancer immunotherapy.

One additional reason for investigating T cell-based immunotherapy in meningioma is the fact that the tumor is supplied by vascular branches of the external carotid artery.[8,9] This is a major difference to the other brain tumor entities discussed previously (glioblastoma and medulloblastoma) which arise within the brain parenchyma itself. The latter (so-called intra-axial) lesions are supplied by branches of the internal carotid artery.[10-12] Within this vascular territory, the actively controlled and dynamic blood-brain barrier (neurovascular unit) creates a partially immunoprivileged niche within the brain parenchyma, but also within the tumor niche and thereby counteracts the entrance of T cells.[13-15]

Potenital T-cell targets are underinvestigated in meningiomas and no studies have been conducted to investigate the antigenic repertoire naturally presented on native tumor tissue. We employed an immunopeptidome-centric approach to define meningioma-associated peptides and antigens. For comparative profiling, the in-house benign HLA peptidome database was complemented with autologous tumor-free dura, which was available from nine patients and represents the tissue of tumor origin. Moreover, relative quantitation of HLA class I and II ligand abundances on tumor *versus* autologous non-neoplastic dura was performed delineating HLA ligands with either significant over- or under-representation in tumor HLA peptidomes. Candidate target antigens were queried against the GTEx database, in which RNA expression data acquired across a large set of benign human tissues are made publicly available, to identify such with a CTA-like expression profile as well as proteins with no confirmed expression in any tissue.[16]

# 3 Methods

## *Patient collective*

Written informed consent of the 33 patients included in the present study was obtained in accordance with the Declaration of Helsinki protocol and the local review board (Kantonale Ethikkommission Zürich; KEK-ZH-Nr. 2015-0163) before surgery. All patients underwent surgery at the Department of Neurosurgery of the University Hospital Zürich, whereby tissue samples were snap frozen in liquid nitrogen and stored at -80°C until use. Meningioma and autologous tumor-free dura specimens along with clinical metadata were kindly provided by Dr. med. Julia Velz, Dr. med. Sophie Shi-Yüng Wang, and PD Dr. med. Marian Christoph Neidert (University Hospital Zürich, Department of Neurosurgery).

All patients had histopathologically confirmed meningioma (n=22 WHO grade I, n=9 WHO grade II, and n=2 WHO grade III) with three samples obtained during surgery at disease recurrence. The present study population had a female-to-male ratio of 2:1 and a median age of onset of 59 [34-83] years. Autologous tumor-free dura supplementing the in-house benign HLA peptidome dataset with the tissue of tumor origin to define meningioma-associated antigens was available from nine patients. Further, this enabled LFQ of relative HLA class I and II ligand abundances on meningioma *versus* autologous dura for five or six patients, respectively. The median available amount of tissue for HLA-IP accounted to 1448 [249-3372] mg for meningioma and to 974 [300-2355] mg for dura samples. Individual patient and sample characteristics are listed in Table 8, whereby a closer look on HLA allotype frequencies in the study cohort is provided in CHAPTER 5.

**Table 8. Clinical and experimental metadata of the 33 meningioma patients included in the present study.** Age of onset was defined as the age at initial diagnosis. HLA class II allotypes were only available for those samples sequenced at HistoGenetics. Nine meningiomas with available autologous tumor-free dura are marked in grey.

| Internal sample ID | Gender Age of onset [years] | WHO grade | HLA typing | Sample mass HLA-IP [mg] | Relative quantitation HLA class I / II |
|---|---|---|---|---|---|
| MNG1 Tissue from 1st recurrence at 4.7 years after initial diagnosis | ♀ 68 | II | A*24:02;A*29:02;B*18:01;B*44:03;C*16:01;C*07:01 | 1075 | |
| MNG2 | ♀ 57 | I | A*30:01;A*03:01;B*13:02;B*07:02;C*07:02;C*06:02 | 684 | |
| MNG3 | ♀ 46 | I | A*01:01;A*68:01;B*40:01;B*57:01;C*03:04;C*06:02 | 1002 | |
| MNG4 | ♀ 81 | I | A*01:01;A*03:01;B*07:02;B*51:08;C*16:02;C*07:02 | 3683 | |
| MNG5 | ♀ 43 | I | A*01:01;A*24:02;B*08:01;B*13:02;C*06:02;C*07:01 | 1275 | |
| MNG6 | ♀ 67 | I | A*02:01;A*68:01;B*18:01;B*35:03;C*04:01;C*07:01 | 1536 | |
| MNG7 | ♀ 78 | I | A*30:02;A*01:01;B*08:01;B*07:02;C*07:01;C*07:01 | 1530 | |
| MNG499 | ♂ 69 | I | A*11:01;A*68:01;B*51:01;B*51:02;C*15:02;C*15:02 DRB1*04:04;DRB1*04:04;DRB4*01:01;DRB4*01:01; DQB1*03:02;DQB1*03:02;DQA1*03:01;DQA1*03:01 | 1827 | |
| MNG501 | ♀ 72 | II | A*02:01;A*02:01;B*15:01;B*51:01;C*03:03;C*15:02 DRB1*13:01;DRB1*15:01;DRB3*02:02;DRB5*01:01; DQB1*06:02;DQB1*06:03;DQA1*01:03;DQA1*01:02 | 1355 | |
| MNG612 Tissue from 2nd recurrence at 10.9 years after initial diagnosis | ♀ 34 | III | A*32:01;A*02:01;B*51:01;B*27:05;C*14:02;C*01:02 | 4858 | |
| MNG623 MNG623 Dura | ♀ 64 | I | A*29:02;A*31:01;B*18:01;B*44:03;C*07:01;C*16:01 DRB1*07:01;DRB1*11:04;DRB3*02:02;DRB4*01:01; DQB1*02:01;DQB1*03:01;DQA1*02:01;DQA1*05:01 | 522 300 | – – |
| MNG624 | ♀ 74 | I | A*01:01;A*01:01;B*08:01;B*35:01;C*04:01;C*07:01 DRB1*01:01;DRB1*03:01;DRB3*01:01;DQB1*02:01; DQB1*05:01;DQA1*01:01;DQA1*05:01 | 581 | |
| MNG628 MNG628 Dura | ♂ 65 | II | A*02:01;A*24:02;B*18:01;B*35:01;C*04:01;C*12:03 DRB1*11:04;DRB1*14:01;DRB3*02:01;DRB3*02:02; DQB1*03:01;DQB1*05:03;DQA1*01:01;DQA1*05:01 | 1167 473 | – – |
| MNG632 | ♂ 42 | I | A*30:02;A*68:01;B*35:01;B*35:01;C*04:01;C*04:01 | 1637 | |
| MNG634 Tissue from 1st recurrence at 7.3 years after initial diagnosis | ♂ 49 | I | A*24:02;A*31:01;B*13:02;B*55:01;C*06:02;C*01:02 | 915 | |
| MNG635 | ♀ 59 | II | A*24:02;A*31:01;B*13:02;B*55:01;C*06:02;C*01:02 | 249 | |
| MNG636 | ♀ 59 | I | A*03:01;A*33:01;B*38:01;B*44:03;C*02:02;C*12:03 | 1613 | |
| MNG637 | ♀ 37 | I | A*02:01;A*02:01;B*18:01;B*55:01;C*03:03;C*12:03 | 3021 | |
| MNG638 | ♀ 57 | II | A*24:02;A*02:01;B*18:01;B*14:02;C*08:02;C*07:01 | 1171 | |
| MNG641 | ♀ 59 | II | A*02:01;A*03:01;B*07:02;B*41:01;C*07:02;C*17:01 DRB1*11:04;DRB1*16:01;DRB3*02:02;DRB5*02:02; DQB1*03:01;DQB1*05:02;DQA1*01:02;DQA1*05:01 | 3372 | |
| MNG642 | ♂ 56 | II | A*11:01;A*34:01;B*15:35;B*51:01;C*04:01;C*07:02 DRB1*04:05;DRB1*15:02;DRB4*01:01;DRB5*01:01; DQB1*04:01;DQB1*05:02;DQA1*01:02;DQA1*03:01 | 1769 | |
| MNG646 | ♀ 63 | I | A*01:01;A*03:01;B*08:01;B*40:02;C*02:02;C*07:01 DRB1*03:01;DRB1*04:01;DRB3*01:01;DRB4*01:01; DQB1*02:01;DQB1*03:02;DQA1*03:01;DQA1*05:01 | 3025 | |
| MNG661 MNG661 Dura | ♂ 42 | I | A*24:02;A*30:02;B*18:01;B*49:01;C*03:03;C*12:03 DRB1*04:05;DRB1*11:04;DRB3*02:02;DRB4*01:01; DQB1*03:01;DQB1*03:02;DQA1*03:01;DQA1*05:01 | 1454 974 | – – |
| MNG666 | ♀ 67 | I | A*02:01;A*03:01;B*07:02;B*39:01;C*07:02;C*12:03 DRB1*11:01;DRB1*13:01;DRB3*02:02;DRB3*02:02; DQB1*03:01;DQB1*06:03;DQA1*01:03;DQA1*05:01 | 2339 | |
| MNG673 | ♀ 44 | I | A*02:01;A*29:02;B*44:03;B*57:01;C*03:04;C*16:01 DRB1*07:01;DRB1*13:01;DRB3*02:02;DRB4*01:01; DQB1*02:01;DQB1*06:03;DQA1*01:03;DQA1*02:01 | 2658 | |
| MNG679 MNG679 Dura | ♀ 49 | I | A*23:01;A*29:02;B*44:03;B*44:03;C*04:01;C*16:01 DRB1*07:01;DRB1*07:01;DRB4*01:01;DRB4*01:01; DQB1*02:01;DQB1*02:01;DQA1*02:01;DQA1*02:01 | 1135 605 | + + |
| MNG682 | ♀ 72 | I | A*26:01;A*29:02;B*15:01;B*35:01;C*04:01;C*04:01 DRB1*01:01;DRB1*14:01;DRB3*02:02;DQB1*05:03; DQB1*05:01;DQA1*01:01;DQA1*01:01 | 1346 | |

| | | | | | |
|---|---|---|---|---|---|
| MNG700<br>MNG700 Dura | ♂<br>44 | I | A*02:01;A*32:01;B*27:05;B*44:02;C*02:02;C*05:01<br>DRB1*04:01;DRB1*09:01;DRB4*01:01;DRB4*01:01;<br>DQB1*03:01;DQB1*03:03;DQA1*03:01;DQA1*03:01 | 874<br>380 | –<br>+ |
| MNG702<br>MNG702 Dura | ♂<br>41 | I | A*01:01;A*02:01;B*37:01;B*51:01;C*02:02;C*06:02<br>DRB1*11:01;DRB1*13:01;DRB3*01:01;DRB3*02:02;<br>DQB1*03:01;DQB1*06:03;DQA1*01:03;DQA1*05:01 | 1441<br>2355 | +<br>+ |
| MNG734 | ♂<br>54 | III | A*02:01;A*66:01;B*39:31;B*40:02;C*02:02;C*12:03<br>DRB1*12:01;DRB1*16:02;DRB3*02:02;DRB5*02:02;<br>DQB1*03:01;DQB1*05:02;DQA1*01:02;DQA1*05:01 | 1259 | |
| MNG814<br>MNG814 Dura | ♂<br>83 | II | A*03:01;A*68:01;B*07:02;B*07:02;C*07:02;C*07:02<br>DRB1*14:01;DRB1*04:04;DRB3*02:02;DRB4*01:01;<br>DQB1*03:02;DQB1*05:03;DQA1*01:01;DQA1*03:01 | 896<br>1324 | +<br>+ |
| MNG819<br>MNG819 Dura | ♀<br>70 | I | A*02:01;A*23:01;B*15:01;B*50:01;C*03:03;C*06:02<br>DRB1*03:01;DRB1*04:01;DRB3*02:02;DRB4*01:01;<br>DQB1*02:01;DQB1*03:02;DQA1*03:01;DQA1*05:01 | 1543<br>988 | +<br>+ |
| MNG833<br>MNG833 Dura | ♂<br>63 | II | A*24:02;A*24:02;B*35:01;B*51:01;C*01:02;C*04:01<br>DRB1*11:01;DRB1*13:01;DRB3*02:02;DRB3*02:02;<br>DQB1*03:01;DQB1*06:03;DQA1*01:03;DQA1*05:01 | 1735<br>1689 | +<br>+ |

### HLA typing

HLA class I and II allotypes of MNG499, MNG501, MNG623, MNG624, MNG628, MNG641, MNG642, MNG646, MNG661, MNG666, MNG673, MNG679, MNG682, MNG700, MNG702, MNG734, MNG814, MNG819, and MNG833 were determined on 4-digit level by next-generation sequencing of tumor tissue at HistoGenetics (New York, USA). For remaining patients, 4-digit HLA class I typing was kindly provided by Nagarajan Paramasivam (German Cancer Research Center, Division of Theoretical Bioinformatics and Heidelberg Center for Personalized Oncology) as retrieved from whole genome sequencing using the Polysolver[17] algorithm. A detailed description on whole genome sequencing of meningioma and matched blood as control was performed can be retrieved from Paramasivam *et al.*[18]

### HLA-IP and subsequent LC-MS/MS to identify HLA-presented peptides

HLA class I- and II-presented peptides were isolated from primary human tissue and analyzed by LC-MS/MS as described in 3.2. All peptide eluates were analyzed on an LTQ Orbitrap XL, whereby residual sample volume was subjected to measurement on an Orbitrap Fusion Lumos. For those samples acquired on both devices (Figure 31 A), peptide and protein lists were merged manually with peptide-specific scores being reported for every LC-MS/MS system.

# 4 Results

## 4.1 Peptide yields of HLA-IPs and HLA class I allotype coverage

To define candidate targets for meningioma immunotherapy naturally presented on HLA, we analyzed 33 primary tumor and nine autologous tumor-free dura specimens. The meningioma cohort included 58 distinct HLA class I allotypes covering 99.98% of the world population, whereby 94.60% of all individuals are expected to be positive for at least three allotypes (Supplementary Figure 8). The most frequent allotypes among meningioma patients were HLA-A*02:01 (20%), -A*03:01 (12%), -A*01:01 (11%), -A*24:02 (11%), -B*18:01 (11%), -B*07:02 (9%), -B*35:01 (9%), -B*51:01 (9%), -C*04:01 (14%), -C*07:01 (12%), and -C*06:02 (11%) (Supplementary Table 15).

A median of 2649 [505-4939] and 1038 [185-3891] HLA class I ligands were identified from meningioma or dura samples, respectively. All HLA class I peptide eluates except that of

MNG637 exhibited a purity of at least 70% and none of the samples were censored for low peptide yield or low percentage of HLA class I ligands. Concerning HLA class II, median peptide yields came up to 1882 [301-5720] and 852 [283-1371]. The total number of unique HLA class I and II peptides, HLA class I ligands as well as the purity of HLA class I peptide eluates are given in Figure 31 A for every specimen. The length distribution of HLA class I ligands clearly peaked at 9 AA length, whereas HLA class II-presented peptides were typically 13- to 18-mers. All investigated samples had comparable length distribution profiles independent of dignity or peptide yield (Figure 31 B). Taking tissue masses subjected to HLA-IP into account (Table 8), enabled us to investigate whether peptide yields correlate with sample quantities used. However, the amount of dura or meningioma tissue did neither correlate with the number of HLA class I nor with the number HLA class II peptide identifications per technical replicate (Figure 31 C). Considering the relative number of identified unique peptides per technical replicate and per one mg of tissue input revealed a significantly increased yield of HLA class I as compared with HLA class II peptides eluted from meningioma tissues (Figure 31 D).

**A**



* LC-MS/MS performed on both LTQ Orbitrap XL and Orbitrap Fusion Lumos

**B**

**C**



**D**
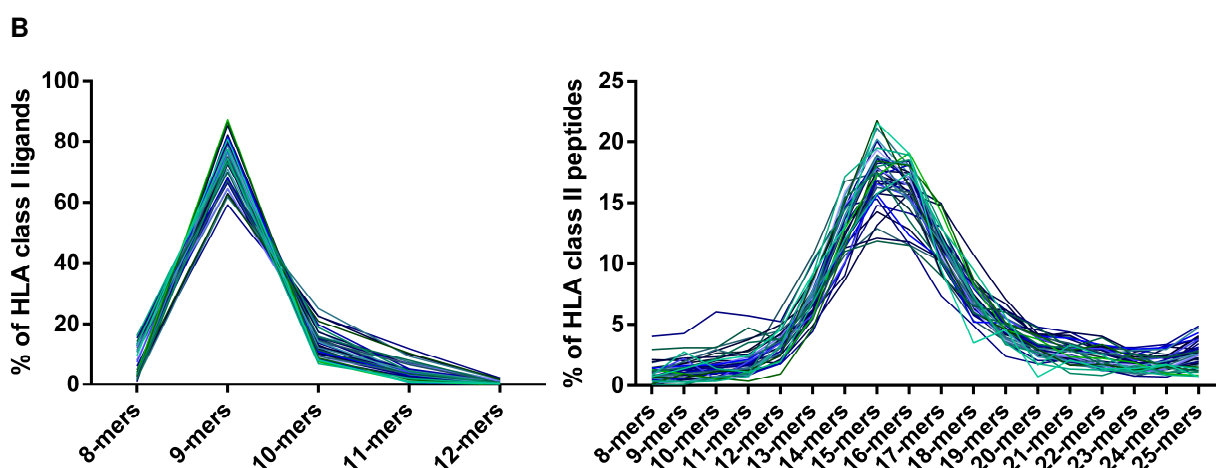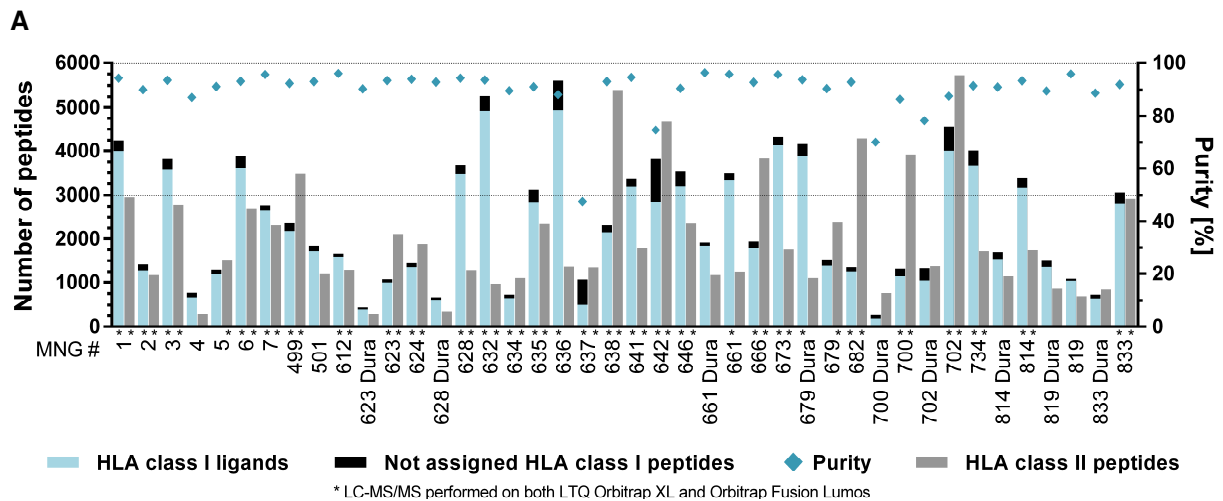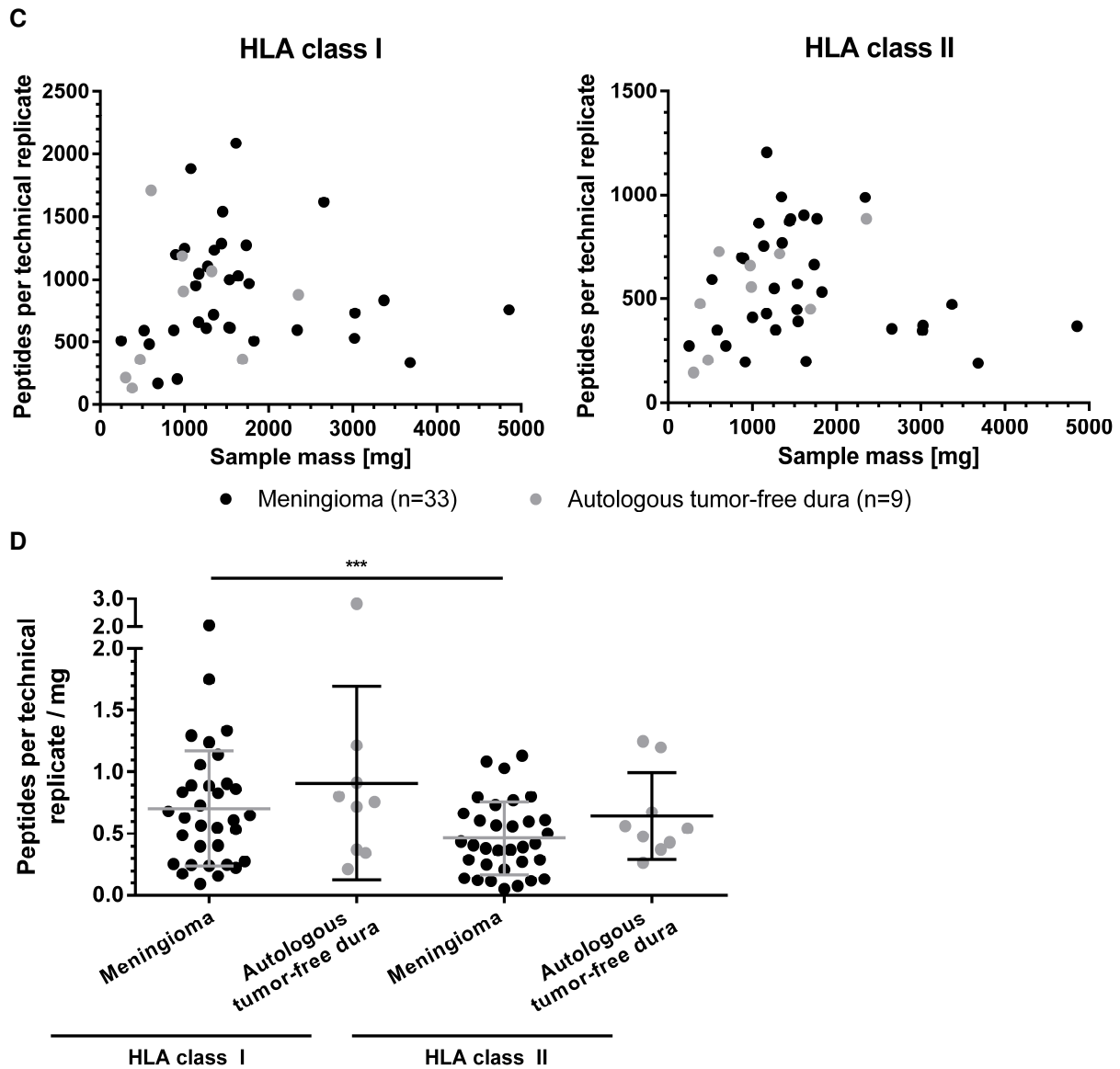


**Figure 31. Number and length distribution of identified HLA class I- and II-presented peptides.** **(A) HLA class I and II peptide yields of meningioma and autologous tumor-free dura tissue.** Calculated purities refer to the proportion of HLA class I peptides annotated to an HLA allotype of the respective patient. Asterisks indicate data acquisition on both LC-MS/MS systems. **(B) Length distribution of HLA class I ligands and HLA class II peptides.** Across the entire dataset, 9-mers constituted 72% of HLA class I ligands, whereas 71% of HLA class II-presented peptides had a length between 13 and 18 AA. Each line represents data of one sample with tumors being illustrated in blueish and dura samples in greenish shades. **(C) Unique peptides per sample and technical replicate** *versus* **amount of tissue subjected to HLA-IP.** To exclude device artefacts, only measurements acquired on an LTQ Orbitrap XL were evaluated. Likewise, only technical replicates consuming 20% sample share each were considered and such diluted for relative quantitation of peptide abundances in paired meningioma and tumor-free dura were excluded from this analysis. By non-linear regression (one-phase association) exponential functions with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) were fitted. However, the goodness of fit was poor for all these models: $R^2$ = 0.08 (meningioma HLA class I) / 0.20 (dura HLA class I) / 0.05 (meningioma HLA class II) / 0.50 (dura HLA class II). Similarly, a correlation analysis across these normally distributed data (according to D'Agostino & Pearson omnibus normality test) did not identify a significant correlation of sample quantities used and the number of peptides identified: two-tailed *p*-values = 0.8875 (meningioma HLA class I) / 0.6039 (dura HLA class I) / 0.2862 (meningioma

HLA class II) / 0.0542 (dura HLA class II). **(D) Peptide yields per technical replicate normalized to one mg of tissue input.** To investigate, whether the relative number of peptide identifications differs between meningioma and autologous tumor-free dura as well as between HLA class I- and II-IPs of the same sample, Wilcoxon matched-pairs signed rank tests were performed (normalized peptide yields did not have Gaussian distributions according to D'Agostino & Pearson omnibus normality test). The only significant difference was observed between the number of HLA class I and II peptides eluted from meningeal tumors (two-tailed $p$-value = 0.0001).

## 4.2 Definition of meningioma-associated antigens

By immunoaffinity chromatography and LC-MS/MS, the repertoire of HLA class I and II peptides and corresponding antigens naturally presented on 33 meningiomas was mapped. To define meningioma-associated antigens and peptides, an in-house benign database comprising 30 distinct primary human organs (n=418 HLA class I and n=364 HLA class II peptidome datasets) supplemented with nine autologous tumor-free dura samples representing the tissue of tumor origin was subtracted. The term meningioma-associated was assigned to peptides and antigens that were never identified on CNS-related tissues (brain, cerebellum, spinal cord, and dura) and for which a maximum of one non-CNS-related sample was positive. Moreover, the frequency of positive primary non-meningeal human malignancies (n=841 samples for HLA class I; n=593 samples for HLA class II) encompassing 36 cancer entities was evaluated. As additional criterium to select targets for cancer immunotherapies, RNA expression data acquired across a large set of benign human tissues and deposited in the GTEx database[16] was reported for every candidate antigen. Further, this allowed the identification of antigens not known to be expressed in any tissue (defined as less than ten TPM in any tissue) as well as such with a classical CTA-like expression profile (not exceeding ten TPM in other organs than testis).

***Meningioma-associated HLA class I-presented antigens and peptides***

HLA class I peptidome analyses of meningioma (n=33) and autologous tumor-free dura (n=9) allowed the identification of 10,431 and 5,242 distinct source proteins represented by HLA class I ligands on neoplastic or tumor-free meningeal tissue, respectively. These represent between 82% (dura) and 93% (meningioma) of the estimated maximum attainable amount of distinct source proteins (Figure 32 C). Despite the vast overlap of meningiomas with benign samples and/or autologous tumor-free dura, 98 antigens were exclusively presented by at least two meningeal tumors (Figure 32 A). Following manual curation of the underlying peptides for HLA motifs as well as multi-mapping to several source proteins, a set of 28 meningioma-associated antigens and corresponding peptides naturally presented on 9-30% of tumors was created. Among these, NMNA2, WNT5A, TBX15/18, and OSR1 were the most frequent ones (Figure 32 B). Despite only two WHO grade III tumors being included in the present dataset, seven of the meningioma-associated antigens were identified across all WHO grades (NMNA2, TBX15/18, XXLT1, SLC25A44, RHOD, CREBL2, PLCD4) and one each was shared by WHO grade I and III (NINJ2) or WHO grade II and III tumors (TMEM87A), respectively. WHO grade I and II meningiomas had 16 antigens in common and three antigens were exclusively part of the immunopeptidome of WHO grade I meningeal neoplasias (TC1D2, B4GALT4, MTU1). Peptide sequences and their HLA restriction, a listing of positive patients, and the GTEx profile of the corresponding source protein can be retrieved from Supplementary

Table 16. Of note, none of these meningioma-associated HLA class I antigens exhibited a CTA-like expression profile.

**A**
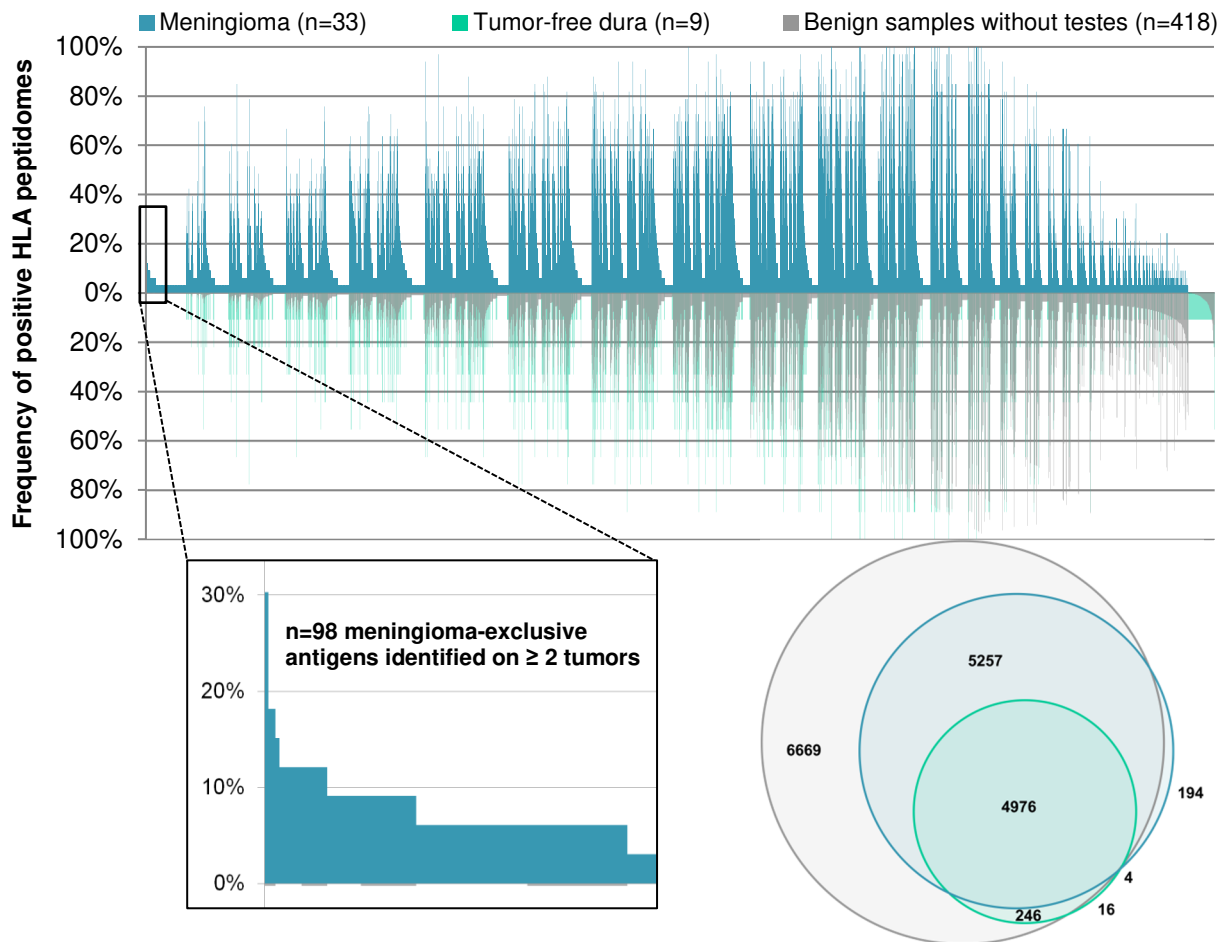


**B**



protein Wnt-5a (WNT5A)   forkhead box protein (FOX) E3, D4-like 1/2/3/5/6

insulin-like growth factor-binding protein 6 (IGFBP6)   folate receptor gamma (FOLR3)   sterol O-acyltransferase 2 (SOAT2)

Rho-related GTP-binding protein RhoD (RHOD)   protein odd-skipped-related 1 (OSR1)

solute carrier family 25 member 44 (SLC25A44)   cAMP-responsive element-binding protein-like 2 (CREBL2)

transmembrane protein 87A (TMEM87A)   NKG2-C type II integral membrane protein (NKG2C) / NKG2-E type II integral membrane protein (NKG2E)

protein AF-9 (AF-9)   nicotinamide mononucleotide protein SSX5/9 (SSX5/9)

cadherin-3 (CDH3)

ninjurin-2 (NINJ2)   adenylyltransferase 2 (NMNA2) Frizzled-7 (FZD7)

globoside alpha-1,3-N-acetylgalactosaminyltransferase 1 (GBGT1)   mitochondrial tRNA-specific 2-thiouridylase 1 (MTU1)

1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase delta-4 (PLCD4)   beta-1,4-galactosyltransferase 4 (B4GALT4)

E3 ubiquitin-protein ligase ZNRF2 (ZNRF2)   paraneoplastic antigen Ma2 (PNMA2)   xyloside xylosyltransferase 1 (XXLT1)

transmembrane protein 255B (TMEM255B)   Tctex1 domain-containing protein 2 (TC1D2)

uncharacterized protein C8orf34 (C8orf34) T-box transcription factor TBX15/18 (TBX15/18)

**C**
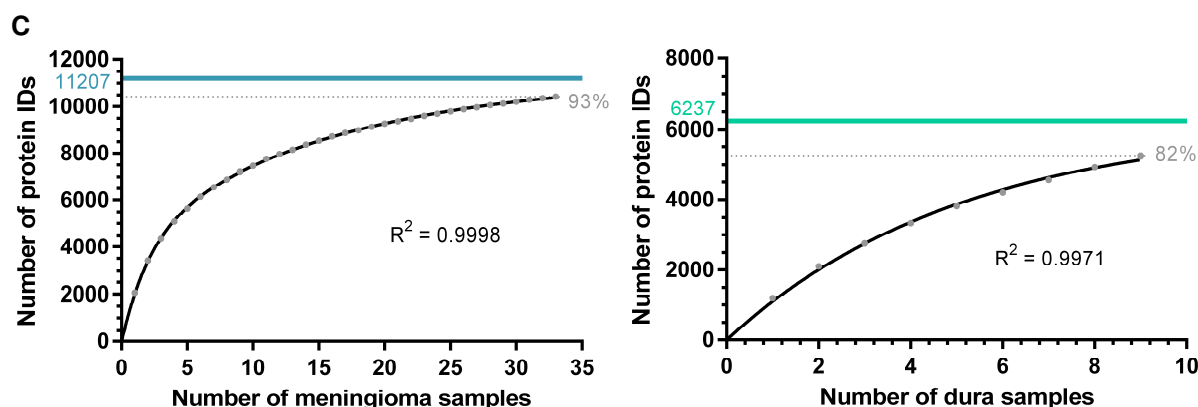


**Figure 32. Definition of meningioma-associated antigens based on class I immunopeptidome analyses. (A) Comparative profiling of the HLA class I peptidome of meningioma *versus* an in-house benign database complemented by tumor-free dura.** Each bar in this waterfall plot (associated with the x-axis) represents a single source protein, whereas the frequency of positive HLA peptidomes is shown on the y-axis, separately for meningioma (n=33), tumor-free dura (n=9), and benign samples without testes (n=418 covering 29 different human tissues). Those source proteins detected on a maximum of one non-CNS-related tissue were designated as meningioma-exclusive, whereby n=98 were identified on at least two meningeal tumors (highlighted as enlarged view on the left). The Venn diagram on the right illustrates the number of distinct HLA class I-presented antigens per group, however, the overlaps cannot map the permission of one positive non-CNS-related sample within the benign dataset. **(B) Word cloud of meningioma-associated antigens.** Based on comparative profiling and subsequent quality control of underlying peptides (HLA motifs as well as multi-mapping to several source proteins), a set of 28 meningioma-associated antigens naturally presented on 9-30% of meningeal tumors was defined. The font size in the word cloud is proportional to the frequency of positive meningeal tumors. **(C) Saturation analysis for the identification of antigens represented by HLA class I ligands on meningioma or tumor-free dura tissue.** For each source count, the mean number of antigens was calculated by 1,000 random samplings. Using non-linear regression, exponential functions with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) were fitted. For both models, the goodness of fit was in the uppermost range ($R^2$ = 0.9998 and $R^2$ = 0.9971). Based on these curves, the maximum attainable number of distinct source proteins was estimated (highlighted as solid lines). With the available number of 33 meningioma and nine tumor-free dura samples, 93% or 82% of the estimated maximum attainable amount of distinct HLA class I-presented proteins had been identified, respectively.

On the peptide level, 38,038 and 9,170 distinct HLA class I ligands were eluted from meningeal neoplasms (n=33) and tumor-free dura (n=9), obtaining 77% (meningioma) and 38% (dura) of the estimated maximum attainable coverage (Figure 33 B). Although the HLA class I peptidome of meningioma showed a pronounced high overlap with that of benign samples and/or autologous tumor-free dura, 2,515 meningioma-exclusive peptides presented on at least two tumors were identified (Figure 33 A). Subsequent to manual curation, a set of 74 peptides derived from 68 antigens and presented on 15-30% of tumors was defined. Among these, HLA ligands derived from OGN, FOXC2, and IFI44L were the most frequent ones. Overall, 31 peptides were presented across all WHO grades, 36 were shared by WHO grade I and II tumors, and two peptides were eluted from both WHO grade I and III meningiomas. One and four peptides were exclusively identified from WHO grade III or I meningeal neoplasms, respectively. Peptide sequences and their HLA restriction, a listing of positive patients, and the corresponding source protein are given in Supplementary Table 17.

**A**



**B**
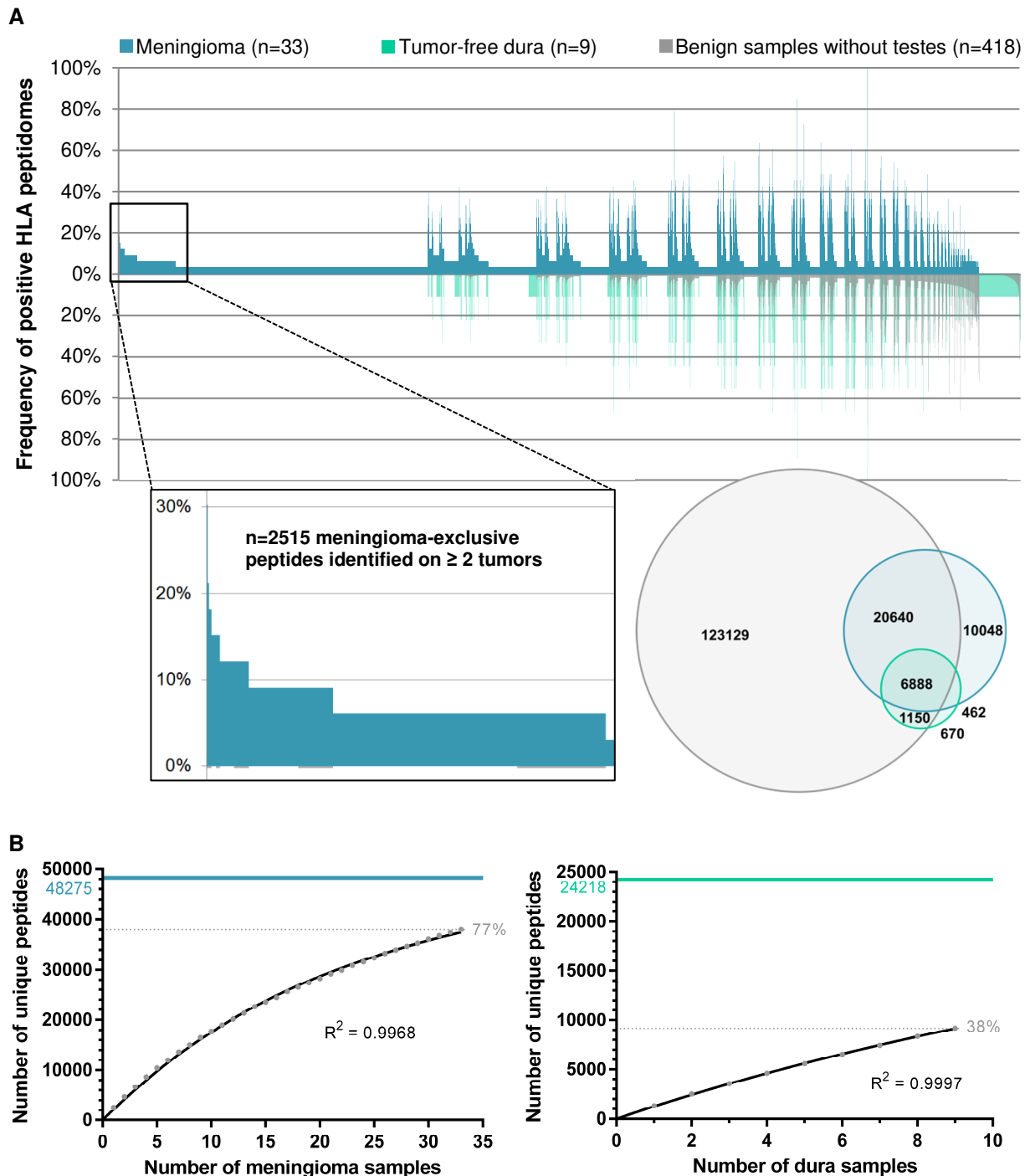


**Figure 33. HLA class I peptidomics to define meningioma-associated peptides. (A) Comparative profiling of HLA class I ligands presented on meningioma *versus* an in-house benign database supplemented with tumor-free dura.** Every peptide evaluated for tumor association is represented by a bar in the waterfall plot (associated with the x-axis), whereas the y-axis shows the frequency of positive HLA peptidomes, separately for meningioma (n=33), tumor-free dura (n=9), and benign samples without testes (n=418 covering 29 different human tissues). Peptides were designated as meningioma-exclusive, when detected on a maximum of one non-CNS-related tissue, whereby n=2,515 were identified on at least two meningeal tumors (highlighted as enlarged view on the left). The number of distinct HLA class I ligands per group is illustrated by the Venn diagram on the right, whereby the overlaps cannot map the permission of one positive non-CNS-related sample within the benign dataset. **(B) Saturation analysis for the identification of HLA class I ligands in meningioma or tumor-free dura tissue.** For each source count, the mean number of peptides was calculated by 1,000 random samplings. Using non-linear regression, exponential functions with a forced y-intercept of 0 (internal

data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) were fitted. For both models, the goodness of fit was in the uppermost range ($R^2$ = 0.9968 and $R^2$ = 0.9997). Based on these curves, the maximum attainable number of distinct peptides was estimated (highlighted as solid lines). With the available number of 33 meningioma and nine tumor-free dura samples, 77% or 38% of the estimated maximum attainable amount of distinct HLA class I ligands had been identified, respectively.

Combining the list of peptides derived from HLA class I-presented meningioma-associated antigens (Supplementary Table 16) with that of HLA class I ligands designated as meningioma-associated (Supplementary Table 17) gave n=141 candidate target peptides for cancer immunotherapy. These cover 99.57% of the world population (Supplementary Figure 9), whereby an average of 21 peptides are expected to match per patient. The population coverage on a per-country basis is shown in Figure 34.
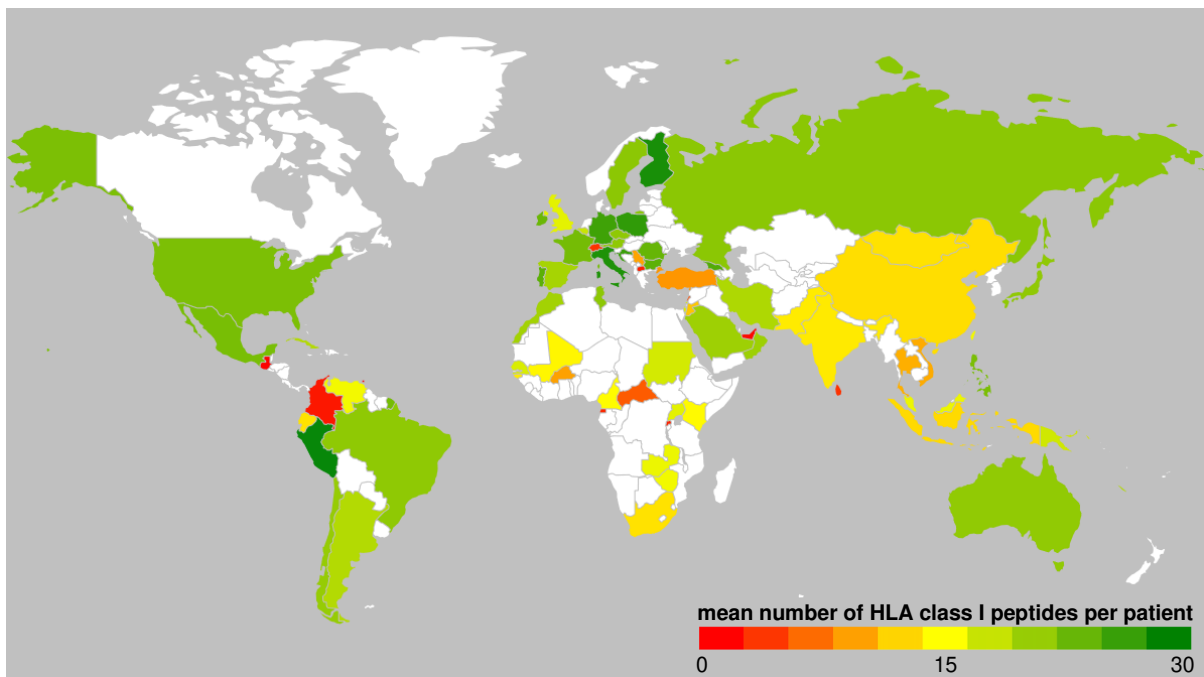


**Figure 34. Population coverage of meningioma-associated HLA class I peptides.** Using the population coverage tool provided by the IEDB Analysis Resource[19], the population coverage of the n=141 candidate target peptides for meningioma immunotherapy was calculated on a per-country basis. On average, 21 HLA class I peptides match per patient worldwide. For visualization of the United Kingdom, the individual values of England, Northern Ireland, Scotland, and Wales were multiplied with the relative area portion. Countries not included in the IEDB tool or not covered by the geographic heat map add-on of Microsoft Excel are colorless.

### *Meningioma-associated HLA class II antigens*

HLA class II peptidome analyses of meningioma (n=33) and autologous tumor-free dura (n=9) allowed the identification of 7,535 and 2,180 distinct source proteins giving rise to HLA class II-restricted peptides on neoplastic or tumor-free meningeal tissue, respectively. These represent between 70% (dura) and 72% (meningioma) of the estimated maximum attainable amount of distinct source proteins (Figure 35 C). Despite the vast overlap of meningiomas with benign samples and/or autologous tumor-free dura, 167 antigens were exclusively presented by at least two meningeal tumors (Figure 35 A). Following manual curation of the underlying peptides for peptide length and the presence of length variants as well as multi-mapping to several source proteins, a set of 37 meningioma-associated antigens and corresponding peptides naturally presented on 9-30% of tumors was created. Among these, PRSS35, A4GALT, FBN2, and SNED1 were the most frequent ones (Figure 35 B). Six of the meningioma-associated antigens were identified across all WHO grades (A4GALT, FBN2, SNED1, MPP6, PCED1B, ANGEL2) and 24 ones were shared by WHO grade I and II tumors. Six antigens were exclusively part of the immunopeptidome of WHO grade I meningeal neoplasias (CAPN14, KIR2DS4, TIMM44, ECD, CD86, RFWD3), whereas WHO grade I and III meningiomas had one antigen in common (KLC2). Peptide sequences, a listing of positive patients, and the GTEx profile of the corresponding source protein can be retrieved from Supplementary Table 16. Of note, three of these meningioma-associated HLA class II antigens (C1orf112, SIRPD, TTLL6) exhibited a CTA-like expression profile and were not listed in the CTDatabase[20].

**A**
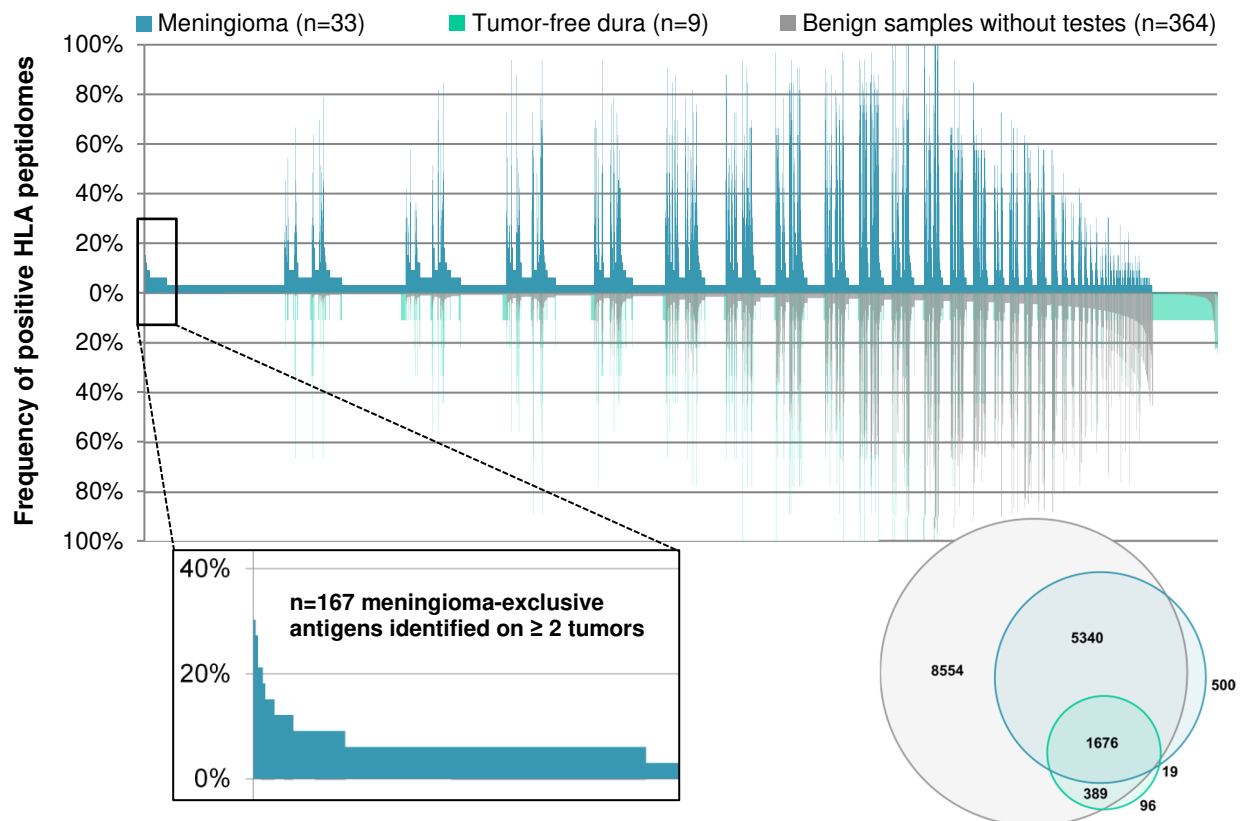
**B**

serine/threonine-protein kinase Sgk3 (SGK3)    MAGUK p55 subfamily member 6 (MPP6)

lactosylceramide 4-alpha-uncharacterized protein KIAA1586 (KIAA1586)

galactosyltransferase (A4GALT) highly divergent homeobox (HDX)   calpain-14 (CAPN14)

probable allantoicase (ALLC)    nucleoporin GLE1 (GLE1)

bone morphogenetic protein 5 (BMP5) nuclear factor of activated T-cells, cytoplasmic 2 (NFATC2)

fibrillin-2 (FBN2)    methylenetetrahydrofolate reductase (MTHFR)    transcription factor SOX-6 (SOX6)

T-lymphocyte activation antigen CD86 (CD86)    uncharacterized protein C1orf112 (C1orf112)    PC-esterase domain-containing protein 1B (PCED1B)

protein SGT1 (ECD)

mucin-4 (MUC4) inactive serine protease 35 (PRSS35)

melanoma-associated antigen 10 (MAGEA10)   mitochondrial import inner membrane translocase subunit TIM44 (TIMM44)

N-acetyltransferase ESCO1 (ESCO1)    zinc finger E-box-binding homeobox 2 (ZEB2)    tubulin polyglutamylase TTLL6 (TTLL6)

ZAR1-like protein (ZAR1L)    killer cell immunoglobulin-like receptor 2DS4 (KIR2DS4)    calcium-binding protein 39-like (CAB39L)

protein angel homolog 2 (ANGEL2) dorsal root ganglia homeobox protein (DRGX) signal-regulatory protein delta (SIRPD)

G protein-regulated inducer of neurite outgrowth 2 (GPRIN2)   meteorin-like protein (METRNL)    kinesin light chain 2 (KLC2)

signal transducer and activator of transcription 5B (STAT5B)    E3 ubiquitin-protein ligase RFWD3 (RFWD3)    ubiquitin conjugation factor E4 B (UBE4B)

sushi, nidogen and EGF-like domain-containing protein 1 (SNED1)

**C**
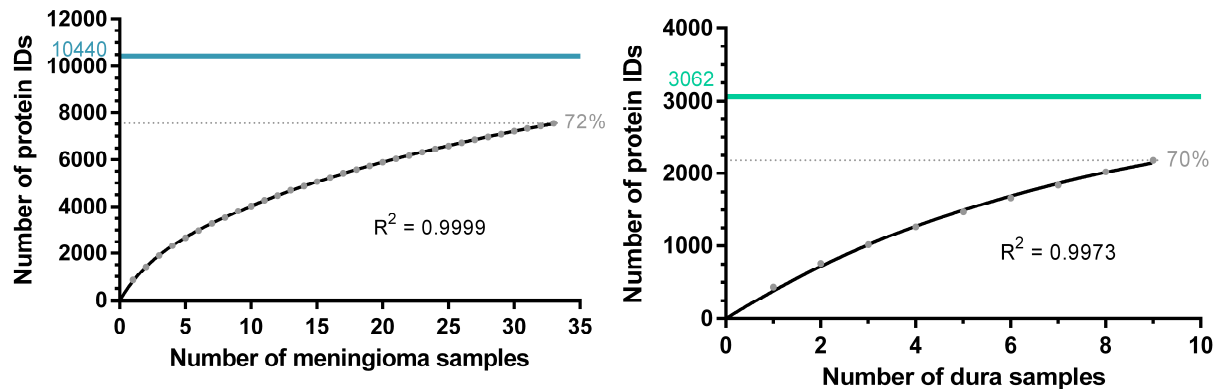


**Figure 35. Definition of meningioma-associated antigens based on class II immunopeptidome analyses. (A) Comparative profiling of the HLA class II peptidome of meningioma *versus* an in-house benign database supplemented with tumor-free dura.** Each bar in this waterfall plot (associated with the x-axis) represents a single source protein, whereas the frequency of positive HLA peptidomes is shown on the y-axis, separately for meningioma (n=33), tumor-free dura (n=9), and benign samples without testes (n=364 covering 30 different human tissues). Those source proteins detected on a maximum of one non-CNS-related tissue were designated as meningioma-exclusive, whereby n=167 were identified on at least two meningeal tumors (highlighted as enlarged view on the left). The Venn diagram on the right illustrates the number of distinct HLA class II-presented antigens per group, however, the overlaps cannot map the permission of one positive non-CNS-related sample within the benign dataset. **(B) Word cloud of meningioma-associated antigens.** Based on comparative profiling and subsequent quality control of underlying peptides (peptide length and/or presence of length variants as well as multi-mapping to several source proteins), a set of 37 meningioma-associated antigens naturally presented on 9-30% of meningeal tumors was defined. The font size in the word cloud is proportional to the frequency of positive meningeal tumors. **(C) Saturation analysis for the identification of antigens represented by HLA class II peptides on meningioma or tumor-free dura tissue.** For each source count, the mean number of antigens was calculated by 1,000 random samplings. Using non-linear regression, exponential functions with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) were fitted. For both models, the goodness of fit was in the uppermost range ($R^2$ = 0.9999 and $R^2$ = 0.9973). Based on these curves, the maximum attainable number of distinct source proteins was estimated (highlighted as solid lines). With the available number of 33 meningioma and nine tumor-free dura

samples, 72% or 70% of the estimated maximum attainable amount of distinct HLA class II-presented proteins had been identified, respectively.

On the peptide level, 36,979 and 9,170 distinct HLA class II-presented peptides were eluted from meningeal neoplasms (n=33) and tumor-free dura (n=9), obtaining 54% (meningioma) and 50% (dura) of the estimated maximum attainable coverage (Figure 36 B). Subsequent to comparative profiling, all antigens represented by at least one meningioma-exclusive HLA class II-presented peptide were subjected to hotspot analysis (Figure 36 A). Meningioma-associated HLA class II presentation hotspots were defined to have a minimum length of eight AA and to be covered by peptides identified in at least five patients, while not having matching sequences in benign samples. This identified a set of 44 antigens harboring regions uniquely presented on tumor tissue with peptide-specific frequencies reaching up to 45% of positive HLA peptidomes. A total of 15 proteins were represented with hotspot-derived peptides across all WHO grades, whereas presentation hotspots within 26 proteins were shared by WHO grade I and II tumors. Further, one meningioma-associated HLA class II presentation hotspot was exclusively detected in WHO grade I meningeal neoplasms, whereas WHO grade I and III tumors had two hotspot targets in common. Peptide sequences, a listing of positive patients, and the corresponding source protein are given in Supplementary Table 18.

Comparing meningioma-associated HLA class I- (n=36) and II-presented antigens (n=37) as well as meningioma-associated HLA class I- (n=68 source proteins) and II-restricted peptides (n=44 source proteins), revealed a unique antigenic repertoire inherent to HLA class I and II peptidomes. STAB1 represented the only shared antigen yielding both meningioma-associated HLA class I and II ligands.

**A**

**B**
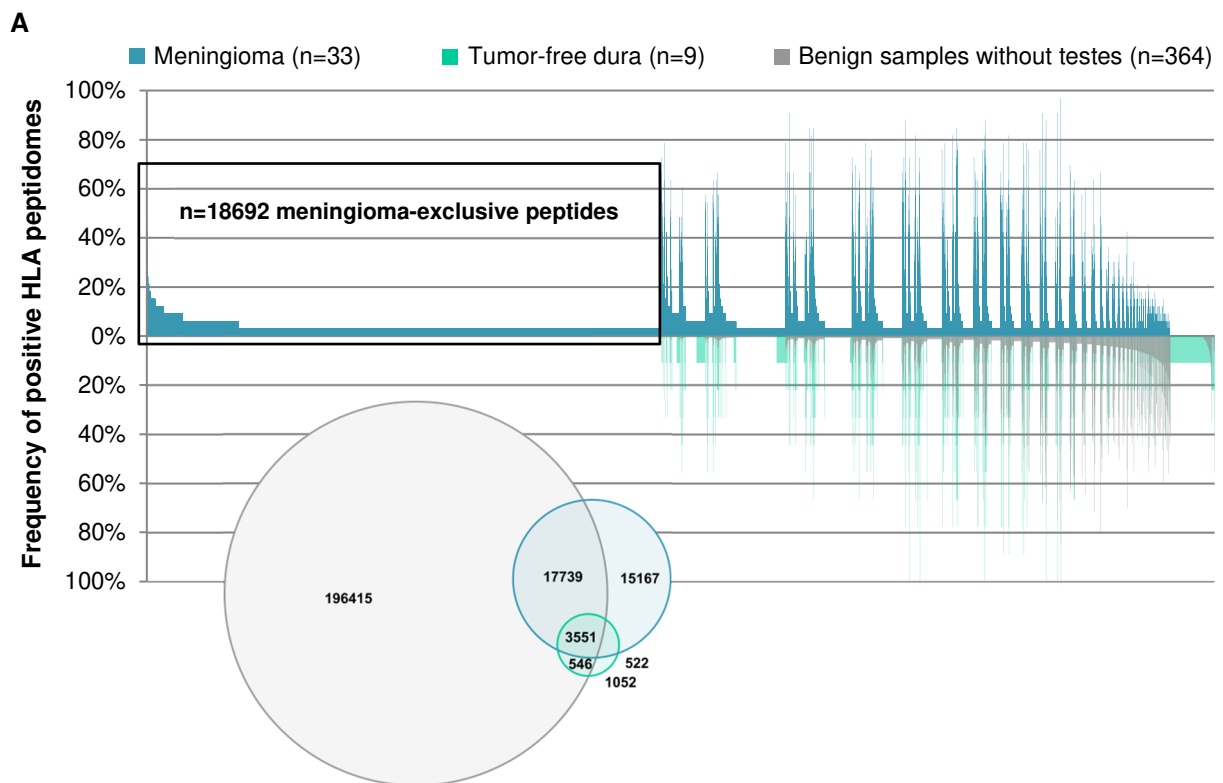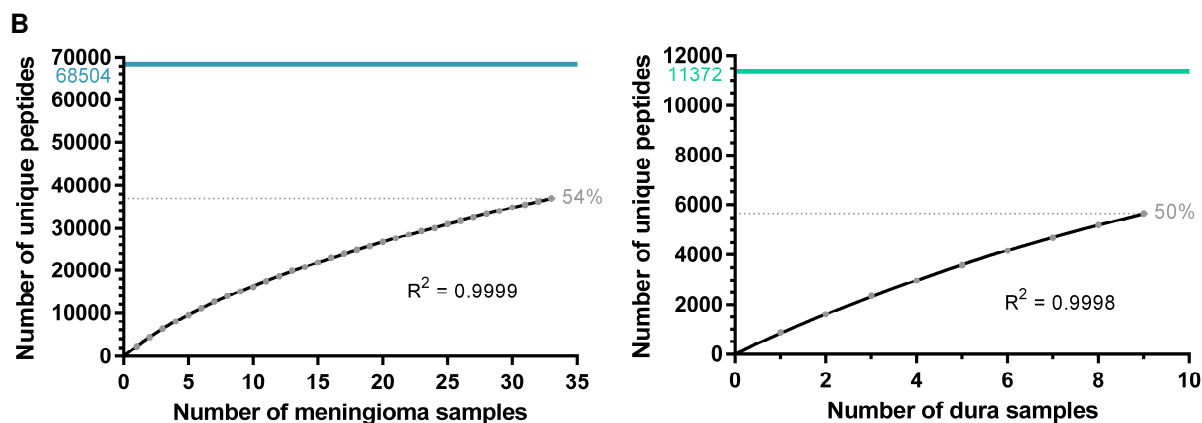


**Figure 36. HLA class II peptidomics to define meningioma-associated peptides (A) Comparative profiling of HLA class II peptides presented on meningioma *versus* an in-house benign database supplemented with tumor-free dura.** Every peptide evaluated for tumor association is represented by a bar in the waterfall plot (associated with the x-axis), whereas the y-axis shows the frequency of positive HLA peptidomes, separately for meningioma (n=33), tumor-free dura (n=9), and benign samples without testes (n=364 covering 30 different human tissues). Being detected on a maximum of one non-CNS-related tissue, n=18,692 peptides were designated as meningioma-exclusive. Corresponding source proteins were subjected to hotspot analysis. The number of distinct HLA class II-restricted peptides per group is illustrated by the Venn diagram on the left, whereby the overlaps cannot map the permission of one positive non-CNS-related sample within the benign dataset. **(B) Saturation analysis for the identification of HLA class II-presented peptides in meningioma or tumor-free dura tissue.** For each source count, the mean number of peptides was calculated by 1,000 random samplings. Using non-linear regression, exponential functions with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) were fitted. For both models, the goodness of fit was in the uppermost range ($R^2$ = 0.9999 and $R^2$ = 0.9998). Based on these curves, the maximum attainable number of distinct peptides was estimated (highlighted as solid lines). With the available number of 33 meningioma and nine tumor-free dura samples, 54% or 50% of the estimated maximum attainable amount of distinct HLA class II-presented peptides had been identified, respectively.

### *Natural HLA presentation of established CTAs and TAAs*

Considering a total number of 366 established CTAs and TAAs (3.2.1) as well as 15 antigens reported to be associated with meningeal neoplasias (2.3.2; n=10 overlapped with the general list of CTAs and TAAs), the present HLA peptidome dataset acquired from meningiomas was screened for previously published tumor antigens. Of these, n=145 and n=126 were represented by HLA class I ligands and HLA class II-presented peptides, respectively. Despite these high identification rates, presentation frequencies of CTAs and TAAs were in general low, especially of those exclusively identified on meningeal tumors. Among 23 HLA class I-presented TAAs and CTAs fulfilling the aforementioned criteria to be designated as meningioma-exclusive antigen, SSX5, SSX9, DDX43, and DDX53 were the most frequent ones (6-12% positive tumors). 18 additional TAAs and CTAs were represented by meningioma-exclusive HLA class I ligands on at least two and a maximum of four meningiomas (Figure 37 A, Supplementary Table 19). On HLA class II, 26 antigens were exclusively identified in the peptidome of meningiomas, with MAGEA10, MUTYH, and CABYR being the most frequent ones (6-15% positive tumors). 20 further CTAs and TAAs were represented by meningioma-exclusive HLA class II-presented peptides on at least two and a maximum of five neoplasms (Figure 37 B, Supplementary Table 19).

**A**



**Frequency of positive HLA class I peptidomes [%]**

**Frequency of positive HLA class I peptidomes [%]**

■ Meningioma (n=33)　　■ Tumor-free dura (n=9)　　■ Benign samples without testes (n=418)

◆ Meningioma-exclusive peptides　　◆ Meningioma-exclusive peptides identified on ≥ 2 tumors
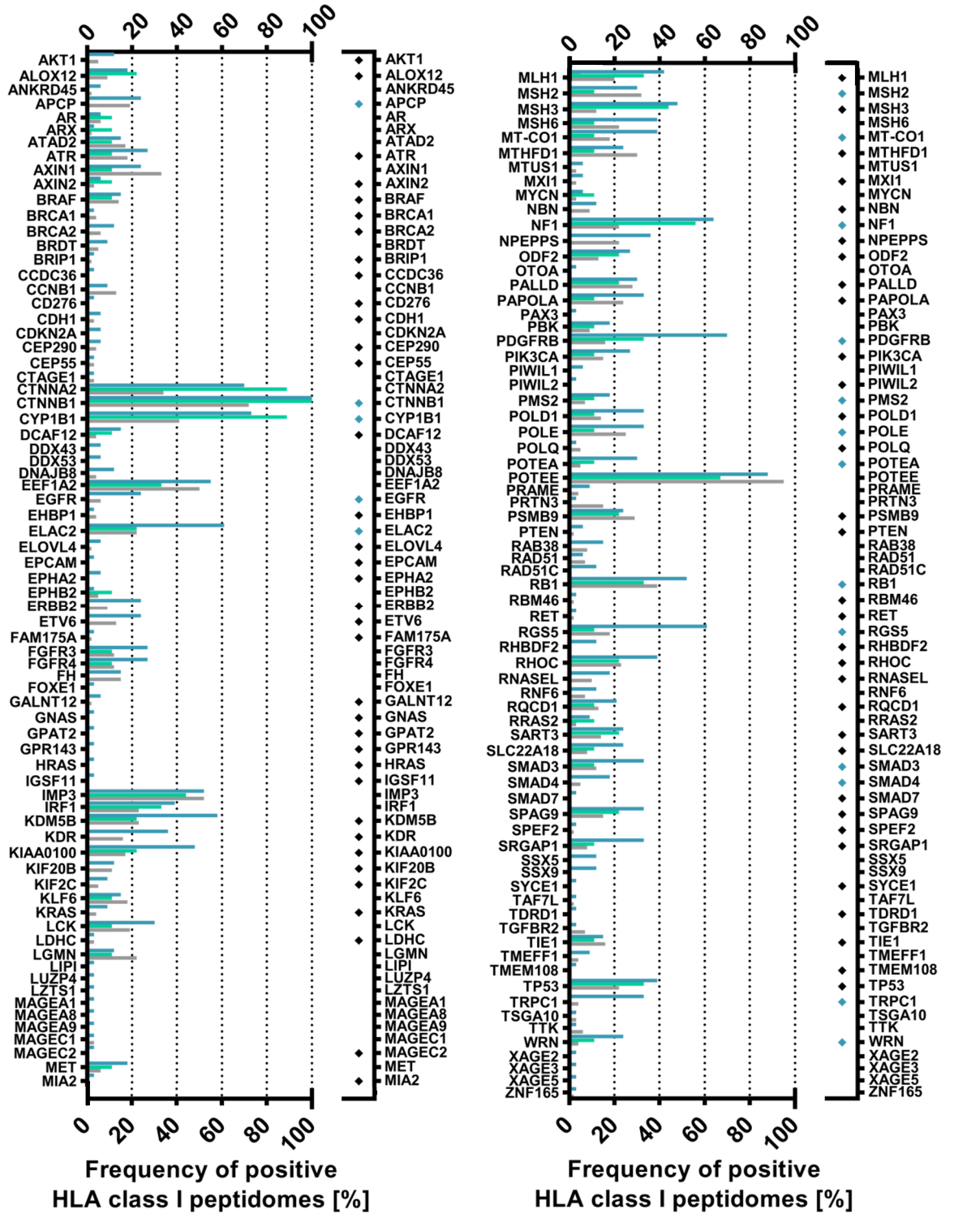
**B**
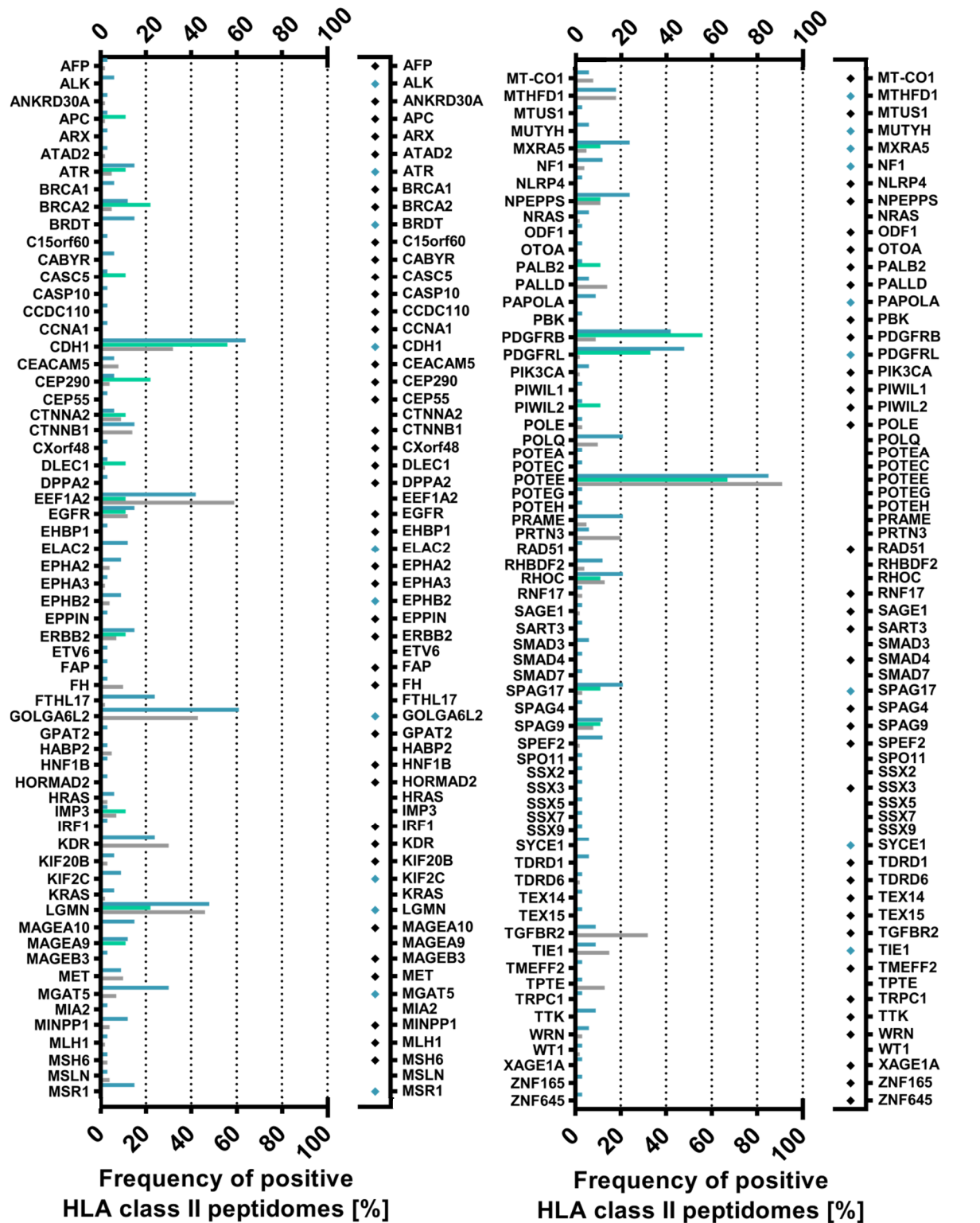


**Figure 37. Identification of established TAAs, CTAs, and meningioma-associated antigens across the present HLA peptidome dataset.** While peptides mapping to multiple source proteins were considered to calculate the frequency of positive HLA peptidomes, these were excluded to report the representation by meningioma-exclusive peptides. CTAs and TAAs exclusively identified on benign samples were not listed. **(A) CTAs and TAAs naturally presented on HLA class I molecules of**

**human meningioma and/or tumor-free dura tissue.** The present immunopeptidomic dataset included HLA class I ligands derived from 145 TAAs and CTAs, with 18 being represented by meningioma-exclusive peptides on at least two tumors (highlighted with blue diamonds). The frequency of positive HLA peptidomes was assessed based on HLA class I ligands for meningioma and dura samples, whereby benign hits were reported independent of HLA binding probabilities of the underlying peptide identifications. **(B) TAAs and CTAs represented in the HLA class II peptidomes of human meningioma and/or tumor-free dura tissue.** Among 126 naturally presented CTAs and TAAs, 20 were represented by meningioma-exclusive peptides on at least two tumors (highlighted with blue diamonds).

## 4.3 Relative HLA ligand abundances on meningioma *versus* dura

From nine meningioma patients not only tumor tissue, but also autologous tumor-free dura was available enabling relative quantitation of HLA ligand abundances. Significant modulation was defined by a corrected $p$-value $\leq 0.01$ and a fold change of mean AUC in (meningioma / dura) $\geq 4$ or $\leq 0.25$ regarding up- or down-modulated peptides, respectively. To obtain a deeper insight into general patterns of modulated HLA presentation on meningioma *versus* non-neoplastic dura, significantly modulated peptides were assigned to the source proteins they originated from. These protein lists were combined for all patients and subjected to comparative profiling unveiling HLA class I and II antigens recurrently represented by up- or down-modulated peptides, respectively. Moreover, functional annotation clustering identified biological processes in which antigens underlying modulated HLA presentation across meningioma patients were involved.

### *Significantly modulated HLA class I ligands*

LFQ-MS to identify significantly up- and down-modulated HLA class I ligands was possible for five meningioma patients and based on a mean number of $1,563 \pm 462$ HLA class I ligands evaluated for relative abundance per patient. On average, $12.00 \pm 5.85\%$ and $15.65 \pm 10.43\%$ of the patients' HLA class I peptidomes were subject to significant up- and down-modulation, respectively (Figure 38 A). Since these five patients shared a maximum of two HLA-A, -B, or -C allotypes, comparative profiling to identify common patterns of modulated HLA presentation was performed on source protein level. In total, 263 and 433 antigens were exclusively represented by up- or down-modulated peptides, respectively (Figure 38 B). Functional annotation clustering identified the up-modulated ones to be mainly involved in organelle organization, cellular protein localization, ribosome biogenesis, DNA-templated transcription, and histone modification, whereas the down-modulated ones play a role in antigen processing and presentation, intracellular signal transduction, negative regulation of signal transduction, membrane protein localization, and viral processes (Figure 38 C, Supplementary Table 21). Following manual curation of the underlying peptides for HLA motifs as well as multi-mapping to several source proteins, a set of 13 antigens represented by up-modulated peptides on meningioma *versus* autologous tumor-free dura in 40-60% of patients was defined. Among these, RLA2 was the most frequent one. Conversely, 18 proteins were under-represented in the HLA class I peptidome of meningioma as compared with autologous non-neoplastic dura (40-60% of patients). PRC2C was represented by down-modulated HLA-A*23:01 and -A*24:02 ligands on three out of five meningiomas (Figure 38 B, Supplementary Table 20).

**A**



Log₂ fold change (meningioma / tumor-free dura)

○ down-modulated    ○ not modulated    ○ up-modulated

**B**
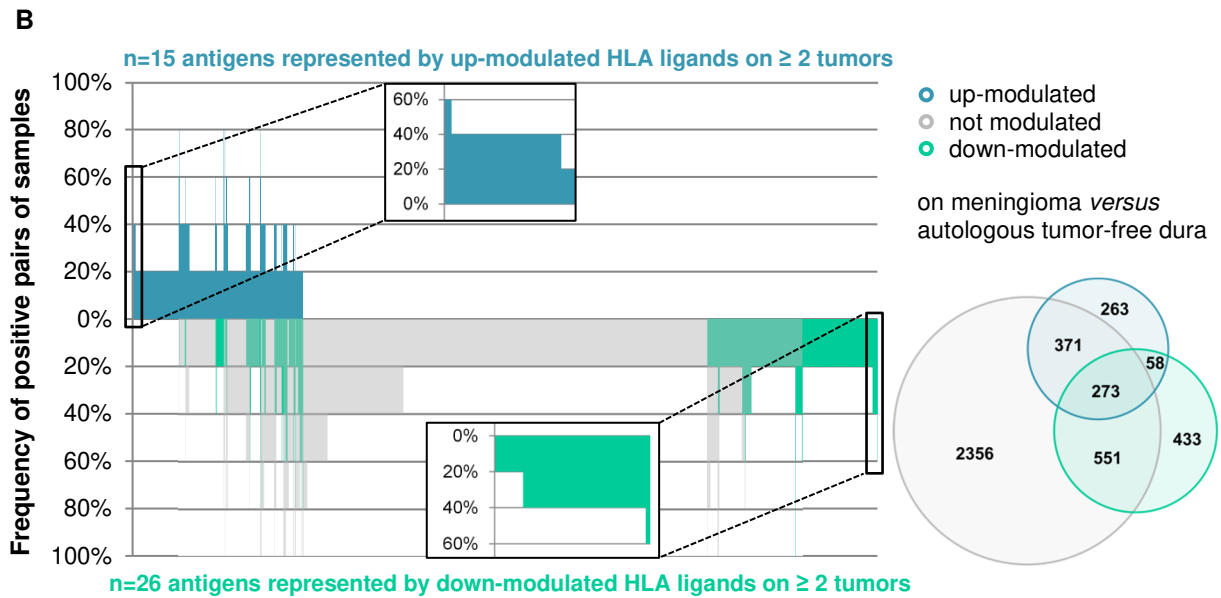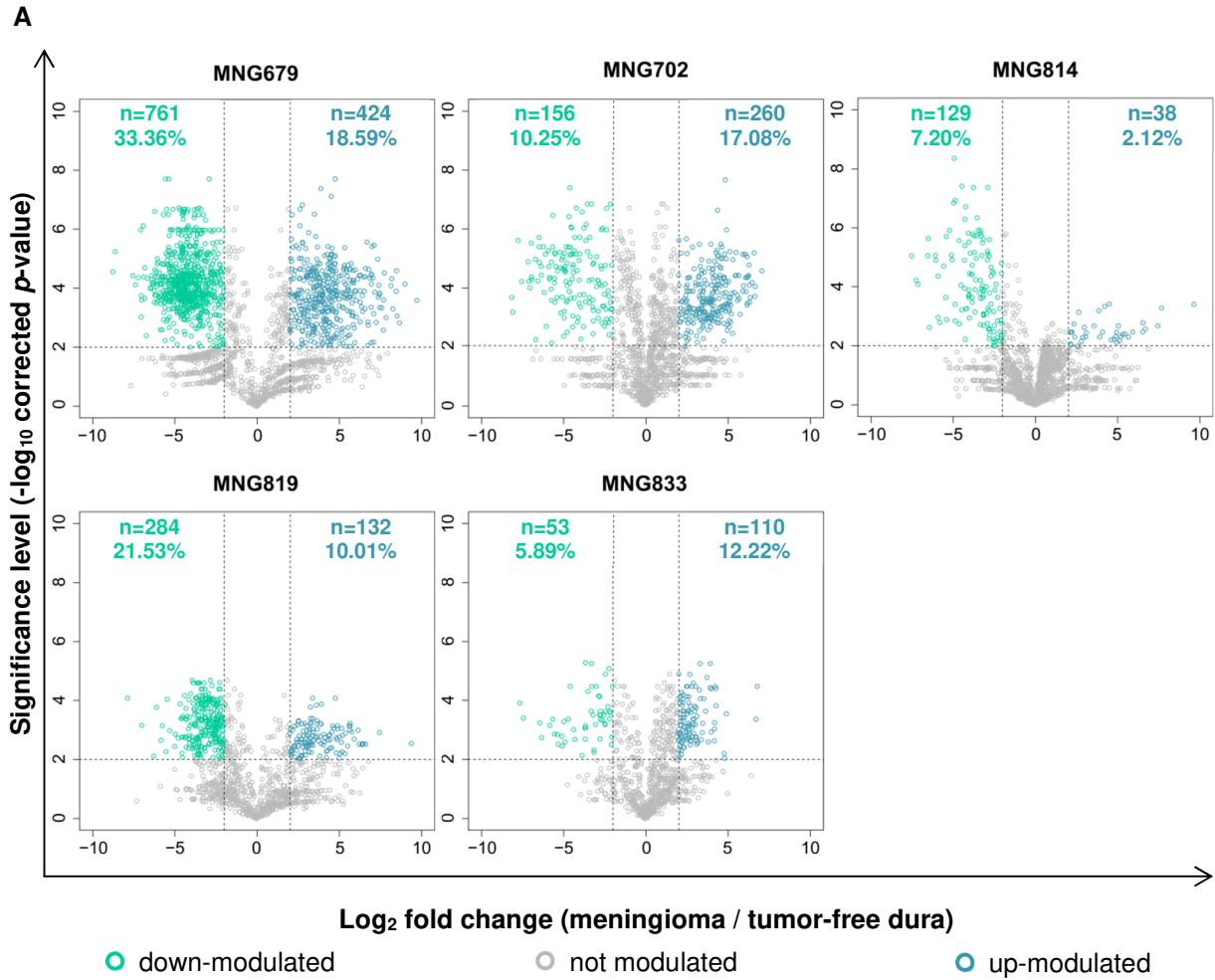
**C**
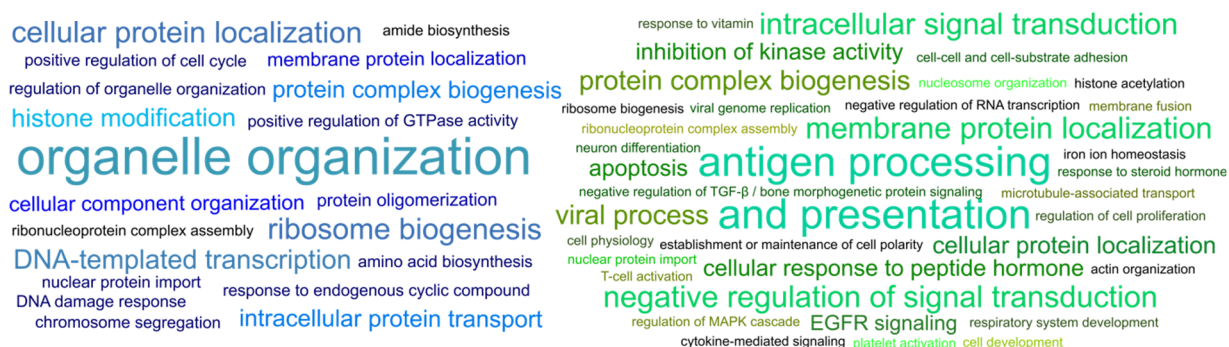


**Figure 38. Patterns of modulated HLA class I presentation in meningioma across five patients. (A) Volcano plots of relative HLA ligand abundances on meningioma *versus* autologous tumor-free dura.** By LFQ-MS, relative abundances of HLA class I ligands, each of which is represented by a dot, were compared. The x-axis indicates changes of abundance as $\log_2$ fold change and corresponding significance levels (after BH correction for multiple testing) are associated with the y-axis. Significant modulation was defined by a corrected *p*-value ≤ 0.01 and a fold change of mean AUC in (meningioma / dura) ≥ 4 or ≤ 0.25 regarding up- (highlighted in blue) or down-modulated (highlighted in green) peptides, respectively. The total number of up- and down-modulated peptides as well as their proportion in the patient's HLA class I peptidome are indicated in quadrants of each Volcano plot. Only four technical replicates were available from both dura and tumor tissue of patient MNG819. **(B) Comparative profiling of antigens corresponding to peptides displayed in Volcano plots.** Each bar in this waterfall plot (associated with the x-axis) represents a single protein, whereas the frequency of positive pairs of samples is shown on the y-axis. Comparing the source proteins of peptides underlying significant up- or down-modulation as well as of those not being modulated allowed the identification of exclusively and recurrently over- (n=15) or under-represented (n=26) antigens across five LFQ datasets acquired from meningioma and autologous tumor-free dura. **(C) Functional annotation of antigens exclusively represented by up- or down-modulated peptides.** Modulation-exclusive source proteins (n=263 over-represented antigens shown in the left and n=433 under-represented antigens shown in the right panel) were clustered for functional annotation with enrichment scores being proportional to the font size in the word clouds.

### *HLA class II-presented peptides with significant modulation*

LFQ-MS to identify significantly up- and down-modulated HLA class II-restricted peptides was possible for six meningioma patients and based on a mean number of 1,242 ± 330 HLA class II-presented peptides evaluated for relative abundance per patient. On average, 12.05 ± 6.13% and 12.53 ± 5.13% of the patients' HLA class II peptidomes were subject to significant up- and down-modulation, respectively (Figure 39 A). Comparative profiling to identify common patterns of modulated HLA presentation was performed on source protein level, since length variants with common core sequences cannot be adequately addressed across patients. In total, n=88 and n=67 antigens were exclusively represented by up- or down-modulated peptides, respectively (Figure 39 B). Annotating these to biological functions did only identify three clusters for over-represented antigens (cell-cell adhesion, ribonucleoside metabolism, actin organization) and two clusters for under-represented antigens (response to oxidative stress, protein catabolism; Figure 39 C, Supplementary Table 21). No common signature of modulated HLA class II presentation was identified across the six patients, with only two (VQA1, QCR8) and four (CATF, ACH10, BROMI, NSF) antigens being represented by up- or down-modulated peptides in two patients, respectively (Figure 39 B, Supplementary Table 20).

**A**



**B**
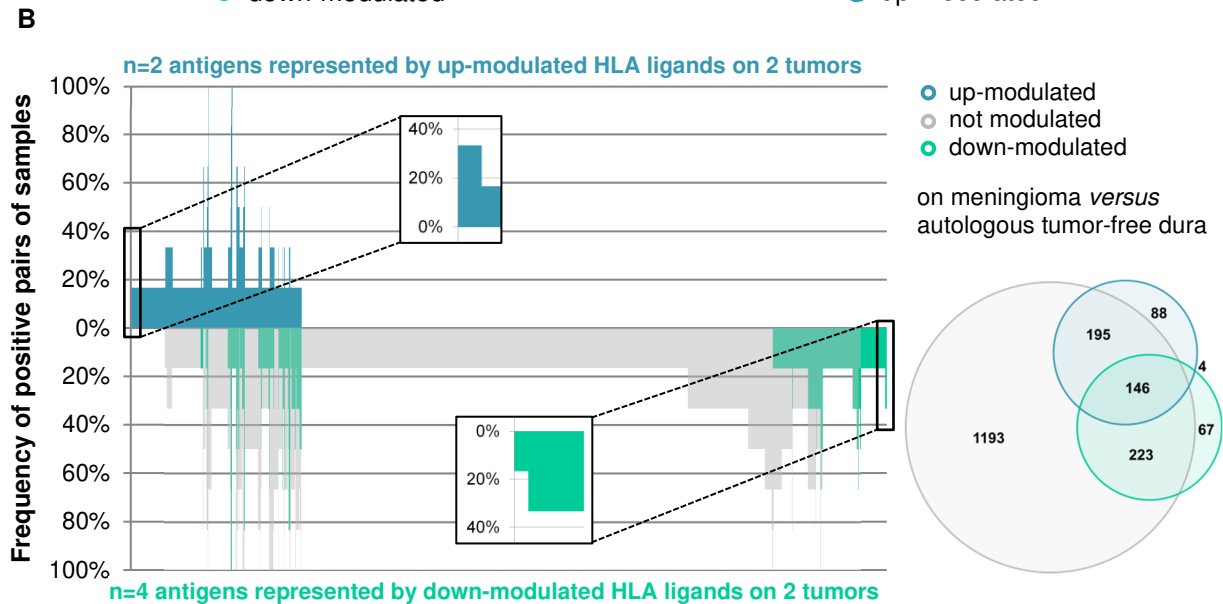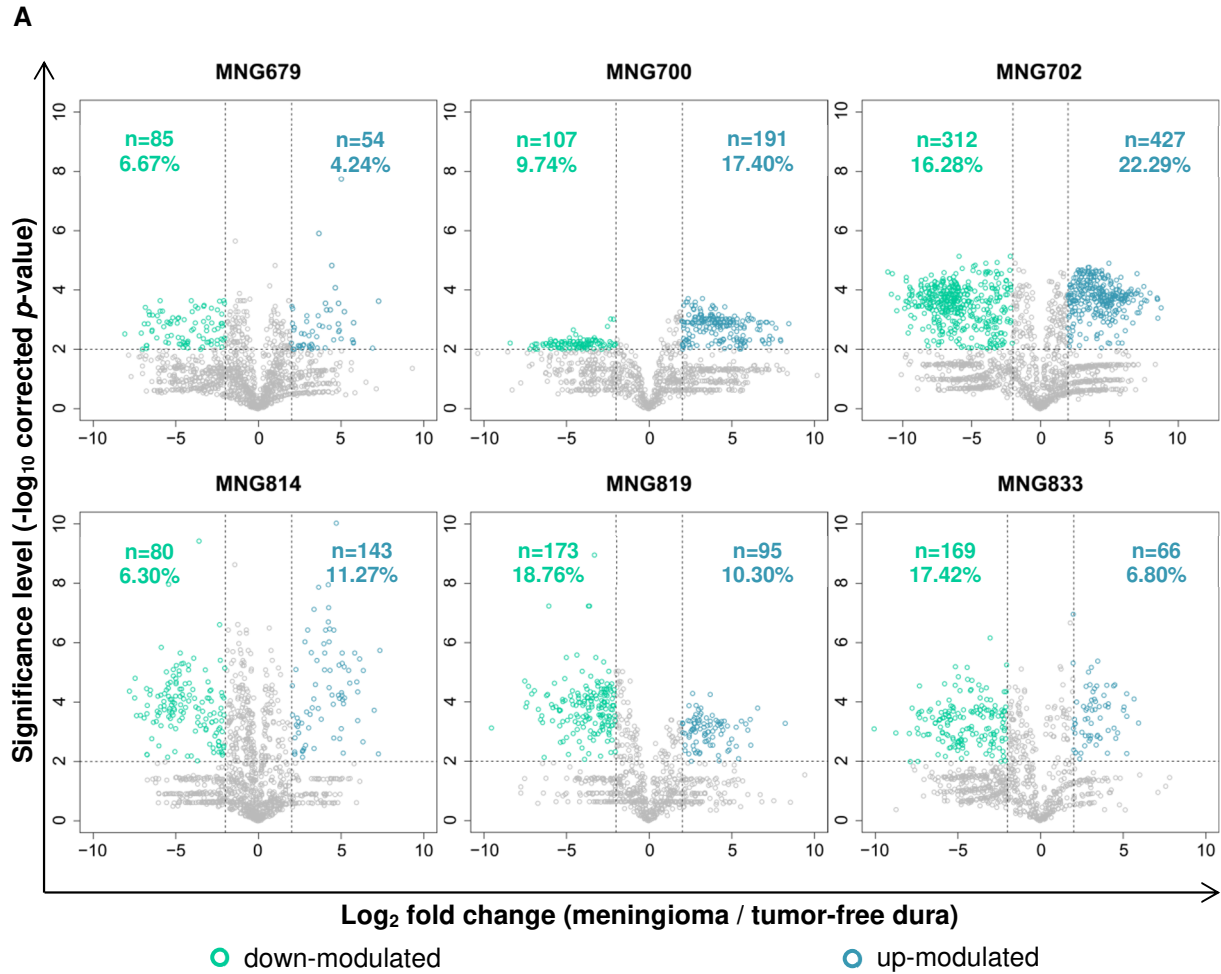


**C**

actin organization

cell-cell adhesion

ribonucleoside metabolism

protein catabolism

response to oxidative stress

**Figure 39. Patterns of modulated HLA class II presentation in meningioma across six patients. (A) Volcano plots of relative HLA class II-presented peptide abundances on meningioma *versus* autologous tumor-free dura.** By LFQ-MS, relative abundances of HLA class II-restricted peptides,

each of which is represented by a dot, were compared. The x-axis indicates changes of abundance as log$_2$ fold change and corresponding significance levels (after BH correction for multiple testing) are associated with the y-axis. Significant modulation was defined by a corrected *p*-value ≤ 0.01 and a fold change of mean AUC in (meningioma / dura) ≥ 4 or ≤ 0.25 regarding up- (highlighted in blue) or down-modulated (highlighted in green) peptides, respectively. The total number of up- and down-modulated peptides as well as their proportion in the patient's HLA class II peptidome are indicated in quadrants of each Volcano plot. **(B) Comparative profiling of antigens corresponding to peptides displayed in Volcano plots.** Each bar in this waterfall plot (associated with the x-axis) represents a single protein, whereas the frequency of positive pairs of samples is shown on the y-axis. Comparing the source proteins of peptides underlying significant up- or down-modulation as well as of those not being modulated allowed the identification of exclusively and recurrently over- (n=2) or under-represented (n=4) antigens across six LFQ datasets acquired from meningioma and autologous tumor-free dura. **(C) Functional annotation of antigens exclusively represented by up- or down-modulated peptides.** Modulation-exclusive source proteins (n=88 over-represented antigens shown in the left and n=67 under-represented antigens shown in the right panel) were clustered for functional annotation with enrichment scores being proportional to the font size in the word clouds.

# 5 Discussion

Neoplasms of the meninges constitute more than one third of all brain and CNS tumors. Despite being largely non-malignant, meningiomas cause severe health issues and impossibility of complete surgical resection owing to localization and tumor recurrence despite surgery and radiation apply to a significant fraction.[1-6] Cancer immunotherapy may complement surgery and radiotherapy as well as meet the high demand for therapeutic regimens to manage disease recurrence.[5,7] The natural presentation of candidate target antigens on HLA molecules has so far not been investigated. Herein, we present a large-scale immunopeptidomic study defining meningioma-associated HLA class I- and II-restricted peptides.

Meningioma proved to be suitable for HLA peptidomic efforts, yielding considerable numbers of HLA class I- and II-presented peptides from a total of 33 primary tumor samples. Furthermore, HLA expression and presentation of a manifold repertoire of peptides is a prerequisite for immunotherapeutic approaches. Likewise, several thousands of distinct HLA class I- and II-restricted peptides were eluted from autologous tumor-free dura being available from nine patients. Non-neoplastic dura was analyzed to supplement an in-house benign database already covering 30 distinct human tissues with that of tumor origin to define meningioma-associated antigens and peptides. There was no indication for decreased HLA expression on meningioma *versus* tumor-free dura yielding comparable amounts of HLA class I and II peptides per mg of tissue input. However, this comparison cannot delineate peptide identifications on tumor cells and such on stromal or infiltrating immune cells, which are part of bulk tumor tissue.[21] Moreover, it is conceivable that cellular density varies between neoplastic and tumor-free meningeal tissue. A significant difference was observed between the relative number of HLA class I ligands and HLA class II-presented peptides identified per technical replicate and mg of meningioma tissue. The fact that the two compared groups had overlapping error bars, however, weakens the confidence of the underlying statistical calculation.

The present study population having a female-to-male ratio of 2:1 and a median age of onset of 59 constituted a representative cohort of meningioma patients.[1] In total, n=22 WHO grade I, n=9 WHO grade II, and n=2 WHO grade III tumors were analyzed. The 58 distinct HLA class I allotypes cover 99.98% of the world population, whereby 94.60% of all individuals are expected to be positive for at least three allotypes. The search for frequently presented meningioma-associated antigens revealed 28 and 37 for HLA class I and II, respectively. Among these, NMNA2 (30%), PRSS35 (30%), A4GALT (27%), FBN2 (21%), SNED1 (21%) WNT5A (18%), TBX15/18 (18%), and OSR1 (15%) were the most frequent ones. NMNA2 catalyzes nicotinamide adenine dinucleotide biosynthesis reflecting high energy consumption, accelerating glycolysis, and promoting cancer cell survival.[22-25] WNT5A is a member of the oncogenic WNT protein family, which is known to be expressed in meningioma and many other tumors.[26,27] In turn, little is known about the inactive serine protease PRSS35 (twelve PubMed entries[28]), which has been reported to be expressed in ovaries[29] as well as in the context of renal fibrosis,[30] oocyte maturation,[31] and epithelial-to-mesenchymal transition (EMT) of ovarian cancer cells.[32] The latter acts *via* activation of the SMAD pathway and can be induced by WNT proteins,[32,33] which is in line with the detection of up-modulated SMAD2-derived HLA class I ligands on two out of five meningiomas, 16 unique (non-multi-mapping) HLA class I ligands originating from WNT1, WNT2B, WNT5A, WNT6, or wntless homolog in 19 out of 33 tumors, 30 HLA class I ligands derived from FZD1, FZD2, FZD4, FZD6, FZD7, FZD8, and secreted frizzled related protein 2 across 25 out of 33 meningiomas as well as 42 unique (non-multi-mapping) CTNNB1-derived HLA class I ligands presented on every of the 33 analyzed tumors. These findings may indicate that activation of WNT signaling pathways in meningeal neoplasms is reflected by the immunopeptidome. With A4GALT, a meningioma-associated antigen acting as key regulator of both epithelial-to-mesenchymal and mesenchymal-to-epithelial transition of cancer cells was identified.[34] EMT can not only be induced by WNT, but also upon TGF-β stimulation.[33] Extracellular TGF-β is sequestered in microfibrils by FBN1 and FBN2, whereby the TGF-β capacity of FBN1 is higher. FBN1 has been reported to be down-regulated in tumor endothelial cells shifting the FBN1/FBN2 ratio in microfibrils towards FBN2. This causes a locally increased concentration of active TGF-β in the tumor microenvironment promoting not only EMT of tumor cells, but also angiogenesis.[33,35] SNED1 is described to be up-regulated in several tumor entities including meningioma[36], to promote tumor invasiveness and metastasis formation, and to correlate with poor outcomes of estrogen- and progesterone-receptor-negative breast cancer patients when expressed at high levels.[37] T-box transcription factors such as TBX15 and TBX18 play a pivotal role in embryonic development and are hijacked by tumor cells.[38-41] OSR1, a serine/threonine protein kinase of the WNK pathway, participates in increasing the metastatic potential and proliferation rate of tumor cells as well as in promoting angiogenesis by interfering with PI3K-AKT, TGF-β, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling networks.[42,43]

Besides meningioma-associated antigens, meningioma-associated peptides potentially arising from differential antigen processing in tumor cells were defined. The applied threshold of five positive samples for reported meningioma-exclusive HLA class I ligands is for certain biased towards the most frequent HLA class I allotypes within the cohort. However, this also increases the confidence of meningioma exclusivity by ensuring sufficient coverage by HLA peptidomes included in the benign dataset supplemented with tumor-free dura specimens. In

addition, meningioma-exclusive targets from frequent allotypes have the advantage of being relevant for a large proportion of the potential patient population. Searching acquired HLA class I and II peptidome data for meningioma-associated peptides unveiled a set of 74 HLA class I ligands derived from 68 antigens presented on 15-30% of tumors as well as 44 antigens harboring meningioma-associated HLA class II presentation hotspots giving rise to naturally presented peptides across at least five patients. Remarkably, comparison of meningioma-associated HLA class I- and II-presented antigens as well as meningioma-associated HLA class I- and II-restricted peptides revealed a unique antigenic repertoire inherent to HLA class I and II peptidomes. Thus, it is inevitable to consider both HLA classes on both antigen and peptide level for comprehensive target discovery approaches. It is noteworthy that among the defined meningioma-associated peptides and proteins, seven HLA class I-presented antigens, 31 HLA class I ligands, six HLA class II-presented antigens, and 15 source proteins harboring hotspot regions proved to be pan-meningioma antigens or peptides presented across all WHO grades. Those tumor-associated antigens and peptides uniquely identified in tumors of the same WHO grade represented a clear minority within each of the four candidate target groups. This indicates that there are common antigenic signatures across differently graded meningiomas and promises broad application of defined targets for the immunotherapy of meningioma. Taking together meningioma-associated antigens and peptides presented on HLA class I molecules (n=141 candidate target peptides), these achieve a world population coverage of 99.57% with an average number of 21 peptides matching per patient. Assuming that half of these candidates will be excluded during the course of immunogenicity testing, which appears overestimated according to our previous experience with immunopeptidomics-based target definition approaches,[44] the number of peptides would still be enough to achieve sufficient coverage of the world population.

The HLA class II dataset contained three meningioma-associated CTAs (C1orf112, SIRPD, TTLL6), which have so far not been listed in the CTDatabase[20], whereas none of the HLA class I-presented meningioma-associated antigens exhibited a CTA-like RNA expression profile. Screening the present HLA peptidomes for 369 established CTAs, TAAs, or antigens reported to be meningioma-associated resulted in high overall identification rates. However, most antigens were also presented on tumor-free dura or benign tissues. SSX5/SSX9 and MAGEA10 had already been identified as meningioma-associated antigens *via* comparative profiling. Remaining meningioma-exclusive antigens and peptides derived from established TAAs and CTAs were characterized by infrequent HLA presentation refuting these as prime targets for cancer immunotherapies. This again emphasizes that the immunopeptidome represents an autonomous layer strongly influenced by the antigen processing machinery and cannot be expected to mirror the proteome or even the transcriptome.[45-49] Thus, target definition for immunotherapeutic approaches is not recommended to be solely based on immunohistochemistry, protein, or RNA expression data, but calls for investigating the antigenic repertoire naturally presented on HLA molecules instead.

Moreover, relative quantitation of HLA-presented peptide abundances on meningioma *versus* autologous tumor-free dura was performed. 13 antigens were recurrently and exclusively represented by up-modulated HLA class I ligands with the ribosomal protein RLA2[36] being the most frequent one (60% of patients). This is in line with the finding that source proteins of up-

modulated HLA class I ligands functionally clustered for involvement in anabolic processes, especially RNA transcription and protein biosynthesis. Likewise, 18 proteins were frequently under-represented in tumor HLA class I peptidomes. Among these, PRC2C, which is involved in hematopoietic cell differentiation,[36] was the most frequent one (60% of tumors). Antigen processing and presentation, the functional annotation of under-represented source proteins with the highest enrichment score, can, however, be designated as an artefact arising from few peptides mapping to a large number of protein accessions annotated to HLA class II molecules. Besides that, many antigens represented by down-modulated HLA class I ligands were associated with cellular signal transduction and protein localization. While proteins promoting cell cycle progression were over-represented, such involved in apoptosis or the regulation of cell proliferation were under-represented in the HLA class I peptidome of meningiomas. This may indicate that increased proliferative activity and concomitant inhibition of apoptosis are reflected by quantitative changes in the immunopeptidome. In turn, modulation of HLA class II peptide presentation did not show a common signature across patients, with only six antigens underlying the same modulation of presentation in two patients. Likewise, only few clusters of functional annotation were identified among over- and under-represented antigens.

HLA-A*02:01 was not only the most frequent HLA class I allotype in the meningioma cohort, but also has a pronounced high allele frequency across the world (meningioma cohort: 23%; China: 13-19%; Germany: 12-27%; Japan 12%; Russia: 30%; USA: 4-28%).[50] Thus, all HLA-A*02:01-restricted peptides derived from meningioma-associated antigens as well as meningioma-associated HLA-A*02:01 ligands presented on at least 18% of tumors were selected for synthesis. Immunogenicity testing including priming of naïve T cells from healthy donors and – if possible – meningioma patients will be performed by Dr. med. Julia Velz and Gioele Medici in the Laboratory for Molecular Neuro-Oncology at the University of Zürich.

Taken together, our study demonstrates that meningioma is suited for immunotherapeutic intervention. The unprecedented investigation of the antigenic repertoire of meningeal neoplasms delineated a novel set of non-mutated meningioma-associated antigens and peptides naturally presented on HLA class I and II molecules. These enable developing peptide-specific immunotherapies such as peptide and DC vaccination or T cell-based approaches which may ultimately contribute to overcome the lack of therapeutic options for non-resectable and recurrent meningiomas.

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# CHAPTER 5

## A meta-analysis comparing the immunopeptidomic landscape of intracranial neoplasms

# 1 Abstract

Intracranial neoplasias are among the most common and most severe tumor diseases in both children and adults. We performed a large meta-analysis including 123 HLA class I and II peptidome datasets acquired from n=40 glioblastoma, n=28 medulloblastoma, and n=33 meningioma patients. Each of these neoplasms is characterized by a unique underlying cellular background suggesting that there might be – besides similarities – also profound differences in the antigenic landscape of these brain tumors. Specific HLA genotypes are associated with increased or decreased susceptibility to autoimmune and infectious diseases and have recently been found to coin response to cancer immunotherapy. This encouraged us to screen the three cohorts for altered HLA allotype frequencies. We found six HLA class I allotypes to be significantly enriched among patients suffering from intracranial neoplasias. Additionally, we identified WNT5A and ESCO1 as pan-brain tumor antigens, while other candidate targets for cancer immunotherapy exhibited largely entity-specific HLA presentation. Taken together, we demonstrated that immunopeptidomics-based target definition inevitably has to be performed for every tumor entity individually and that intracranial neoplasias are associated with cumulative occurrence of particular HLA allomorphs.

# 2 Introduction

The intracranial neoplasms glioblastoma, medulloblastoma, and meningioma originate from different cell types, namely glial, embryonal neuroepithelial / cerebellar granule neural precursor, and meningeal cells.[1-5] To investigate, whether this difference in cellular origin is reflected by the antigenic landscape of these three tumors, the repertoires of HLA class I- and II-presented peptides were searched for similarities and differences. Linus Backert, a former colleague, tried to identify antigens shared between four hematological malignancies based on HLA peptidome data. However, multiple myeloma, acute myeloid, chronic myeloid, and chronic lymphocytic leukemias essentially had entity-specific tumor-associated antigens.[6] The immunopeptidomic datasets acquired during the course of this thesis were searched for pan-brain tumor antigens shared between glioblastoma, medulloblastoma, and meningioma.

The development and course of various autoimmune and infectious diseases has been associated with specific HLA class I and II alleles.[7-10] A cohort of 22 ependymoma patients has recently been found to have a significantly increased allotype frequency of HLA-A*26:01 as compared with the German population (internal data created by Lena Mühlenbruch at the Department of Immunology, University of Tübingen). Hence, the HLA class I allotype frequencies within the glioblastoma, medulloblastoma, and meningioma cohort were compared with the distribution in the German population.

# 3 Methods

***Acquisition of HLA peptidome data and definition of candidate target antigens***

Peptides presented on HLA class I and II molecules were isolated and analyzed as described previously (CHAPTER 1 / 3.2; CHAPTER 2-4 / 3). Definition of candidate targets for cancer immunotherapy was performed as reported in CHAPTER 2-4.

***HLA class I allele frequencies***

Tumor samples were collected during surgical resections performed in Switzerland and Germany and HLA typing was conducted as reported previously (CHAPTER 2-4 / 3). For each of the three patient cohorts, HLA class I allele frequencies were calculated (Supplementary Table 1, Supplementary Table 10, Supplementary Table 15). Owing to the poor data availability from Switzerland, allele frequencies calculated for each patient cohort were only set in relation to a large German reference population as retrieved from the Allele Frequency Net Database (Germany pop 8; n=39,689).[11] One-hit wonders (n=1 positive patient) were not considered for statistical evaluation employing Yates's continuity corrected $\chi^2$ tests.

# 4 Results

## 4.1 HLA class I allotypes associated with intracranial neoplasia

We investigated the HLA class I allele frequencies observed among glioblastoma, medulloblastoma, or meningioma patients in comparison with a large German reference population. HLA-A*02:01, -A*02:05, and -B*44:02 were found to be significantly enriched in glioblastoma, whereas increased allele frequencies of HLA-C*07:04 or HLA-A*29:02 and -A*30:02 were identified among medulloblastoma and meningioma patients, respectively (Figure 40).

**HLA-A**, **HLA-B**, and **HLA-C** allele frequencies [%]

- Glioblastoma (n=40)
- Medulloblastoma (n=28)
- Meningioma (n=33)
- Germany (n=39,689)
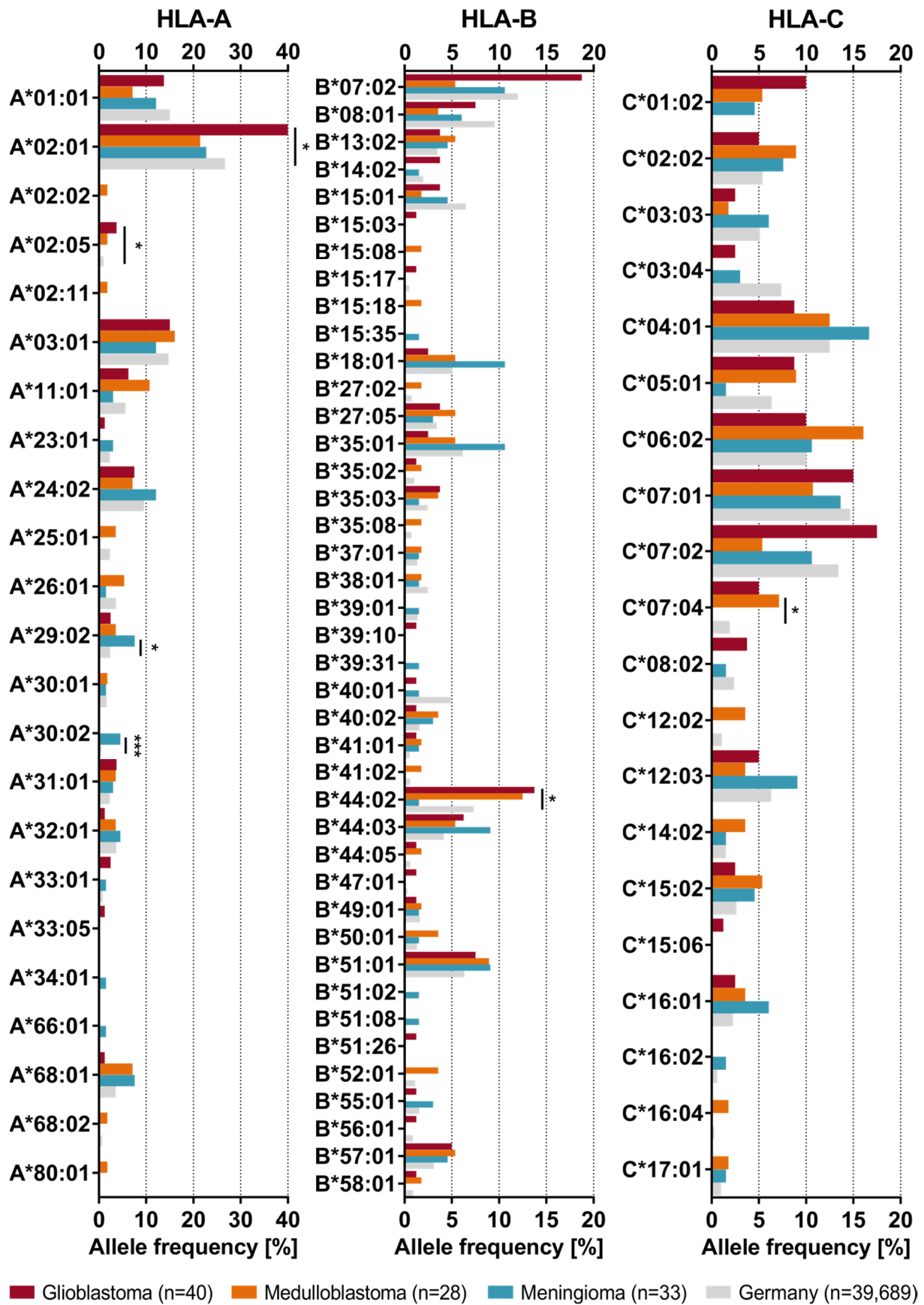
| Tumor entity | HLA allotype | n alleles (allele frequency) in patient / reference cohort | Two-tailed *p*-value | χ2 | OR (95% CI) |
|---|---|---|---|---|---|
| Glioblastoma | A*02:01 A*02:05 B*44:02 | 32 (40.0%) / 21,170 (26.7%) 3 (3.8%) / 738 (0.9%) 11 (13.8%) / 5,810 (7.3%) | 0.0102 0.0412 0.0464 | 6.594 4.166 3.967 | 1.8 (1.2-2.9) 4.2 (1.3-13.2) 2.0 (1.1-3.8) |
| Medulloblastoma | C*07:04 | 4 (7.1%) / 1,524 (1.9%) | 0.0184 | 5.560 | 3.9 (1.4-10.9) |
| Meningioma | A*29:02 A*30:02 | 3 (4.6%) / 437 (0.6%) 5 (7.6%) / 1,897 (2.4%) | 0.0187 0.0004 | 5.532 12.54 | 3.3 (1.3-8.3) 8.6 (2.7-27.5) |

**Figure 40. Association of HLA class I allotypes with intracranial neoplasias.** Using the Yates's continuity corrected $\chi^2$ test HLA class I allele frequencies among glioblastoma, medulloblastoma, and meningioma patients were compared with that of a large German population (upper panel). This identified a significantly increased allele frequency of three, one, and two HLA class I allotypes among glioblastoma, medulloblastoma, or meningioma patients, respectively (lower panel). Reference data for HLA-A*02:02, -A*66:02, and -C*01:02 were not available. Abbreviations not introduced in the text above: odds ratio (OR), confidence interval (CI).

## 4.2 The immunopeptidome of intracranial neoplasia in comparison

Considerable numbers of both HLA class I- and II-restricted peptides were eluted from glioblastomas (n=62), medulloblastomas (n=28), and meningiomas (n=33). Simple overlap analysis delineated only a small fraction of antigens to be entity-specific neither being identified in benign samples nor in any of the respective two other tumor types. The proportion of entity-specific proteins came up to 0.51-1.99% for HLA class I (Figure 41 A) and to 2.09-5.40% for HLA class II peptidomes (Figure 41 B) being highest for glioblastoma and lowest for medulloblastoma in each case. From a total of 12,792 HLA class I- and 9,678 HLA class II-presented antigens 260 ones were glioblastoma-associated on the peptide or protein level, respectively. For medulloblastoma, these numbers came up to 9,821 HLA class I- and 4,922 HLA class II- presented antigens comprising 71 tumor-associated ones. Immunopeptidome analysis of meningioma uncovered 10,431 and 7,535 proteins to be naturally presented on HLA class I and II molecules, whereby 184 yielded tumor-associated HLA ligands.

Comparing candidate targets of cancer immunotherapy defined for glioblastoma, medulloblastoma, and meningioma delineated an entity-specific repertoire of TAAs naturally presented on HLA molecules. However, WNT5A and ESCO1 proved to be pan-tumor antigens frequently and tumor-exclusively presented on all three intracranial neoplasms. Another 13 antigens each were shared by medulloblastomas and glioblastomas (OLIG3, NDUFA4L2, SHISA9, DCX, BTBD17, EFS, HES4, GFAP, PTPRZ1, KIF1A, CRMP1, AKAP9, NCAM1) or by meningiomas and glioblastomas (TMEM87A, TBX15, TBX18, SESN3, LRP1, FMNL2, STAB1, GRAMD3, OSGIN2, SERPINC1, FN1, COL5A2, SCG2), respectively (Figure 41 C, Supplementary Table 2-4, Supplementary Table 11-13, Supplementary Table 16-18).
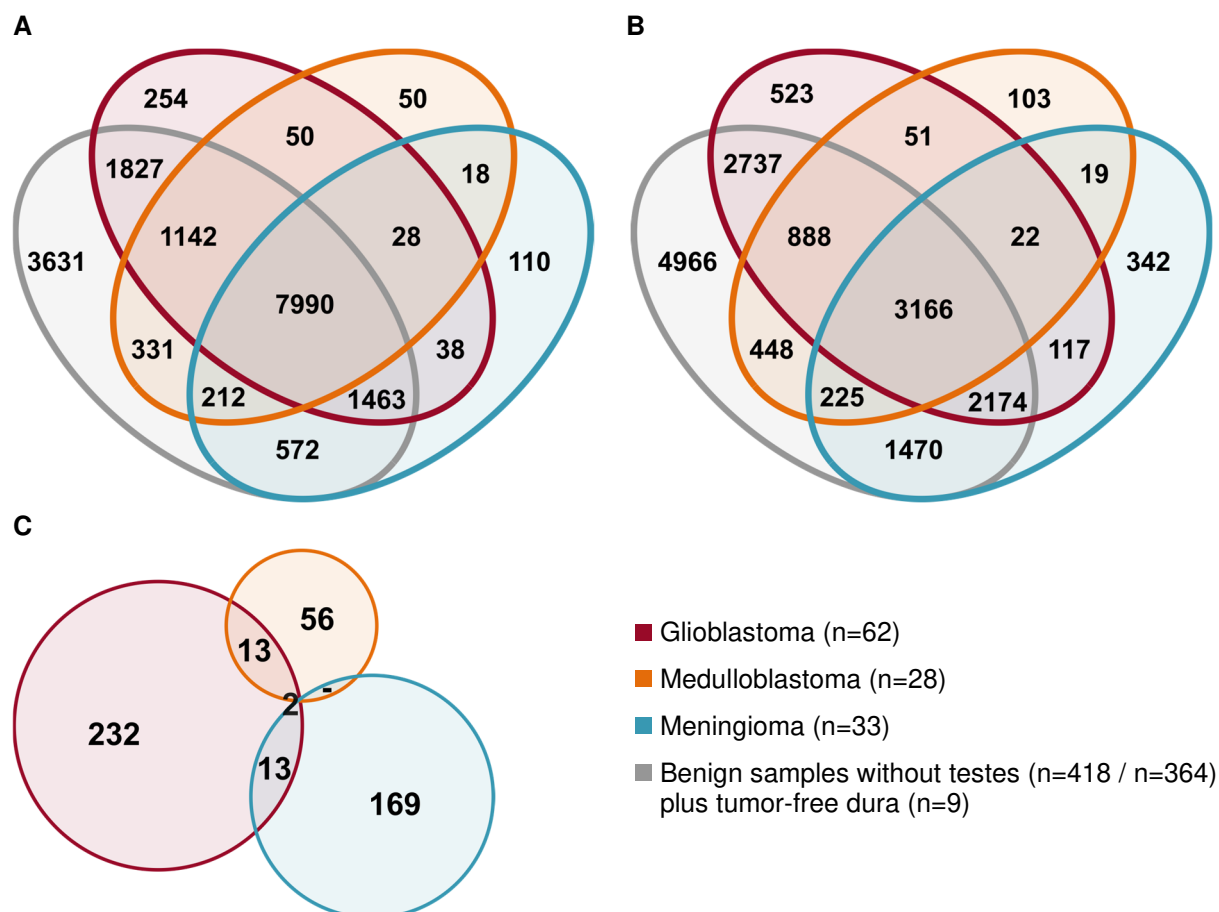
**Figure 41. Similarities and differences of the immunopeptidomic landscape of glioblastoma, medulloblastoma, and meningioma. Overlap of (A) HLA class I- and (B) HLA class II-presented antigens.** It should be noted that Venn diagrams cannot illustrate that tumor-exclusive proteins were permitted to be identified in one non-CNS-related sample within the benign dataset. **(C) Identification of shared and unique brain tumor antigens.** Combining the lists of HLA class I- and II-presented candidate targets for each tumor uncovered a small but notable overlap between the three entities. While 26 source proteins were tumor-exclusive or yielded tumor-associated peptides in two tumor types each, all investigated intracranial neoplasias had two tumor-associated antigens in common: WNT5A and ESCO1.

# 5 Discussion

Glial, embryonal neuroepithelial / cerebellar granule neural precursor, and meningeal cells represent the cellular origin of glioblastomas, medulloblastomas, and meningiomas.[1-5] We aimed at investigating whether the immunopeptidome and, most importantly, the repertoire of candidate targets for immunotherapy is imprinted by the cell lineage from which neoplastic transformation once started. Besides that, we evaluated the three patient cohorts presented herein for an association of intracranial neoplasias with specific HLA class I allomorphs.

Genetic predisposition represents a common element of multifactorial disease development. This encloses, among many others, the individual composition of HLA class I and II alleles. Autoimmune disorders, for instance, have a long history of HLA association including, for instance, ankylosing spondylitis (HLA-B*27), type 1 diabetes (HLA-DRB1*04:01–DQB1*03:02), narcolepsy (HLA-DQB1*06:02), and celiac disease (HLA-DQA1*05:01–DQB1*02:01 or HLA-DQA1*03:01–DQB1*03:02).[7] The individual HLA genotype does not only

influence the observed immune phenotype, the susceptibility for, and the course of autoimmune and infectious diseases, but also the response to cancer immunotherapy.[7-10,12] Maximal heterozygosity of HLA-A, -B, and -C alleles has been found to favor response to checkpoint inhibition thereby improving overall survival. In turn, HLA-B*15:01 has been suggested to impair neo-antigen-directed CD8+ T-cell responses.[12] Moreover, a cohort of 22 ependymoma patients has recently been found to have a significantly increased allotype frequency of HLA-A*26:01 as compared with the German population (internal data created by Lena Mühlenbruch at the Department of Immunology, University of Tübingen). Hence, the HLA class I allotype frequencies within the glioblastoma, medulloblastoma, and meningioma cohort were compared with the distribution in the German population. A significant fraction of tumor samples originated from surgeries performed in Switzerland, however, hardly any data on HLA allele frequencies had been available from this country. Assuming that the ethnic background and composition of the Swiss population is comparable to that of the German population, we used solely data from Germany for the comparison of HLA class I allotype frequencies. We found HLA-A*02:01, -A*02:05, and -B*44:02 to be significantly enriched among glioblastoma patients. The heavily elevated allele frequency of HLA-A*02:01 (40.0% *versus* 26.7%) might reason why previous immunotherapeutic efforts have focused on this allotype[13-15] representing not only an overall common HLA allomorph, but being naturally enriched in glioblastoma patient collectives. Conversely, increased allele frequencies of HLA-C*07:04 or HLA-A*29:02 and -A*30:02 were identified within the cohort of medulloblastoma or meningioma patients, respectively. Once HLA class II allotypes have been successfully imputed from whole exome and RNA sequencing data, we aim to investigate whether there are also HLA class II alleles associated with intracranial neoplasias.

The immunopeptidomic datasets acquired during the course of this thesis were compared with each other and searched for pan-brain tumor antigens shared between glioblastoma, medulloblastoma, and meningioma. Despite high peptide yields per sample as well as a large total amount of unique peptide and protein identifications, the number of candidate targets defined for medulloblastoma (n=71) was considerably smaller as compared with the other two tumor types (glioblastoma: n=260 / meningioma: n=184). This may indicate that the antigenic landscape of medulloblastomas resembles that of benign tissues more than meningeal tumors and glioblastomas do. We found the repertoire of HLA-presented antigens, especially of those with tumor association, to be shaped by the cellular origin of neoplastic cells. Apart from WNT5A and ESCO1 proven to be pan-brain tumor antigens, candidate targets exhibited entity-specific HLA presentation. This is in line with previous findings in hematological malignancies, namely multiple myeloma, acute myeloid, chronic myeloid, and chronic lymphocytic leukemia, strongly encouraging and requiring immunopeptidomic studies to be performed for every tumor entity individually.[6]

The yield of HLA class I and II peptides per mg of tissue is representative of the cellular density, HLA expression, and to some extent also of the degree of immune infiltration.[16-19] For both glioblastoma and medulloblastoma RNA sequencing was performed, which permits comparing the expression of all components of the antigen processing machinery including HLA class I and II molecules between these two tumor types. Subsequent to completion of transcriptome analysis, we aim to compare the number of peptide identifications relative to the amount of

tissue input and HLA expression between these two intracranial neoplasias. Including the meningioma dataset in this analysis is, however, not possible, as RNA sequencing data is lacking and data acquisition on another less sensitive LC-MS/MS system would introduce a technical bias.

In conclusion, we found six HLA class I allotypes to be associated with intracranial neoplasias as being significantly enriched among glioblastoma, medulloblastoma, or meningioma patients. Comparison of naturally presented candidate targets for cancer immunotherapy unveiled a unique repertoire of HLA-presented peptides inherent to every tumor entity as well as two pan-brain tumor antigens: WNT5A and ESCO1.

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# CHAPTER 6

## Implementation of a mix of ten synthetic heavy isotope-labeled peptides as retention time standard

# 1  Abstract

Synthetic peptide spike-ins have been established as part of good proteomic practice, but have so far not been implemented for immunopeptidomics. These offer tremendous potential not only for constant monitoring of chromatographic and mass spectrometric performance, but also for contemporary data acquisition strategies and quantitative approaches with enhanced identification and reproducibility rate, which depend on retention time (RT) alignment between measurements to be compared with each other. We implemented a set of ten heavy isotope-labeled RT peptides that are compatible with immunopeptidomic requirements. Following titration to determine the optimal concentration for each peptide, these served as base for performance monitoring of two liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS) systems over half a year. During the course of a procurement measure, the mix of ten RT peptides was used to compare five LC-MS/MS systems – especially regarding sensitivity – thus guiding device selection. Moreover, these RT peptides will enable deeper exploration of immunopeptidomes by data-independent acquisition and more accurate quantification strategies. Further, they will represent an essential tool in method development including optimization of collision energies for new LC-MS/MS systems used for immunopeptidomic research.

# 2  Introduction

Observed peptide retention times (RT) vary not only across different chromatographic systems, but also within the same system over time. Main influencing factors are solvent composition and solvent exchanges (mobile phase), flow rate, gradient settings, column material and column exchanges (stationary phase), and the volume comprised by the LC system. A standardized set of synthetic spike-in peptides enables indexing of RTs, monitoring of chromatographic performance, as well as comparison and calibration of collision energies between two mass spectrometers.[1] In relation to spike-in peptide RTs, peptide-specific RT indexes are calculated, represented by the dimensionless variable iRT, to align multiple LC-MS/MS runs.[2] RT alignment of LC-MS/MS measurements is of utmost importance when performing data-independent acquisition (DIA). Here, not only the most intense precursor ions are selected for fragmentation (as in case of DDA), but comprehensive sampling and fragmentation of peptide features is performed. This increases the identification rate as well as the reproducibility and quantifiability by eliminating the semi-stochastic process of peptide selection inherent to DDA. However, this results in chimeric fragment spectra that are difficult to demultiplex and impede assigning the correct chromatographic peak, which can be facilitated by spike-in RT standard peptides.[1,3-6]

Previous attempts employing iRT peptides (Biognosys)[2] had failed, as these proteomic RT standards were not compatible with immunopeptidomic LC-MS/MS methods (internal data created at the Department of Immunology, University of Tübingen). ProteomeTools Calibration Standard (PROCAL) was developed by JPT Peptide Technologies for proteomic purposes with tryptic digests as analyte as well. However, several of the contained peptides lie as doubly or triply positive precursor ions within the m/z window applied for HLA peptidome analyses.[1] After a pilot experiment using the entire mix of 40 peptides, the ten best peptides were synthesized

in-house. The PROCAL sequences are arbitrary and do not occur in any proteome according to present knowledge. However, when facing the emerging field of cryptic and neo-antigenic peptides with many sequences having remained unknown so far, we decided to use heavy isotope labels nevertheless. These ten RT peptides were titrated for optimal concentration in both LC-MS/MS systems currently operated at the Department of Immunology, University of Tübingen for immunopeptidomic studies.

# 3  Methods

### *LC-MS/MS to detect PROCAL spike-in peptides*

The commercially available PROCAL kit containing 40 RT peptides (JPT Peptide Technologies; Table 9) was dissolved in 100 µl $A_{Load}$ followed by vortexing and sonicating for 60 and 30 s, respectively. This yielded a stock solution containing 100 fmol/peptide/µl that was subsequently serially diluted with $A_{Load}$ (1:2 / 1:4 / 1:8) to a concentration of 50 / 25 / 12.5 fmol/peptide/µl. Synthetic peptides were diluted 1:5 with a standardized HLA class I peptide eluate prepared from JY cells (JY17#3) so that spike-in peptides had a concentration of 20 / 10 / 5 / 2.5 fmol/peptide/µl (equivalent to 100 / 50 / 25 / 12.5 fmol/peptide/measurement). Data were acquired on an Orbitrap Fusion Lumos with the standard method for HLA class I peptides (3.2.2) For data base search, a concatenated FASTA-formatted file consisting of the Swiss-Prot release from September 27th 2013 (20,279 reviewed protein sequences) and 40 PROCAL sequences was used.

**Table 9. PROCAL peptides with annotation of molecular weight and m/z values of doubly and triply positively charged precursor ions.** Precursor ions not detectable with the standard HLA class I LC-MS/MS method covering a mass range of 400-650 m/z are marked in red. Information were retrieved from Zolg *et al.*[1]

| Accession | Sequence | M [Da = g/mol] | [M+2H]++ [M+3H]+++ | Accession | Sequence | M [Da = g/mol] | [M+2H]++ [M+3H]+++ |
|---|---|---|---|---|---|---|---|
| RT0001 | YSAHEEHHYDK | 1414.5902 | 708.30 472.54 | RT0021 | HFALFSTDVTK | 1264.6452 | 633.33 422.56 |
| RT0002 | HEHISSDYAGK | 1242.5629 | 622.29 415.19 | RT0022 | TFTGTTDSFFK | 1250.5819 | 626.30 417.87 |
| RT0003 | TFAHTESHISK | 1256.6149 | 629.31 419.88 | RT0023 | VSGFSDISIYK | 1214.6183 | 608.32 405.88 |
| RT0004 | ISLGEHEGGGK | 1082.5356 | 542.28 361.85 | RT0024 | TFGTETFDTFK | 1292.5925 | 647.30 431.87 |
| RT0005 | LSSGYDGTSYK | 1176.5299 | 589.27 393.18 | RT0025 | TSIDSFIDSYK | 1274.6030 | 638.31 425.87 |
| RT0006 | FGTGTYAGGEK | 1086.4982 | 544.26 363.17 | RT0026 | ASDLLSGYYIK | 1228.6339 | 615.32 410.55 |
| RT0007 | VGASTGYSGLK | 1038.5346 | 520.27 347.19 | RT0027 | FLFTGYDTSVK | 1276.6339 | 639.32 426.55 |
| RT0008 | TASGVGGFSTK | 1010.5033 | 506.26 337.84 | RT0028 | GIFGAFTDDYK | 1232.5713 | 617.29 411.86 |
| RT0009 | SYASDFGSSAK | 1118.4880 | 560.25 373.84 | RT0029 | VYAETLSGFIK | 1226.6547 | 614.33 409.89 |
| RT0010 | LYSYYSSTESK | 1326.5980 | 664.31 443.21 | RT0030 | GFVIDDGLITK | 1176.6390 | 589.33 393.22 |
| RT0011 | LYTGAGYDEVK | 1214.5819 | 608.30 405.87 | RT0031 | GASDFLSFAVK | 1140.5815 | 571.30 381.20 |

| ID | Sequence | Mass | m/z | | ID | Sequence | Mass | m/z |
|---|---|---|---|---|---|---|---|---|
| RT0012 | TLIAYDDSSTK | 1212.5874 | 607.30 405.20 | | RT0032 | FFLTGTSIFVK | 1258.6961 | 630.36 420.57 |
| RT0013 | HLTGLTFDTYK | 1294.6557 | 648.34 432.56 | | RT0033 | VSSIFFDTFDK | 1304.6289 | 653.32 435.88 |
| RT0014 | FLASSEGGFTK | 1142.5608 | 572.29 381.86 | | RT0034 | GDFTFFIDTFK | 1336.6339 | 669.32 446.55 |
| RT0015 | GFLDYESTGAK | 1186.5506 | 594.28 396.52 | | RT0035 | LFISALVDFFK | 1298.7274 | 650.37 433.92 |
| RT0016 | ALFSSITDSEK | 1196.5925 | 599.30 399.87 | | RT0036 | SLFFIIDGFVK | 1284.7118 | 643.36 429.24 |
| RT0017 | FVGTEYDGLAK | 1198.5870 | 600.30 400.54 | | RT0037 | IDVYILALLLK | 1272.8057 | 637.41 425.28 |
| RT0018 | YALDSYSLSSK | 1232.5925 | 617.30 411.87 | | RT0038 | SILAFLYLYFK | 1376.7744 | 689.39 459.93 |
| RT0019 | HDTVFGSYLYK | 1328.6401 | 665.33 443.89 | | RT0039 | SLIFFLSTLLK | 1280.7744 | 641.39 427.93 |
| RT0020 | YFGYTSDTFGK | 1284.5663 | 643.29 429.20 | | RT0040 | FLISLLEEYFK | 1400.7591 | 701.39 467.93 |

### Selection of RT peptides for in-house synthesis

Mean RTs from up to four LC-MS/MS runs (2.5-20 fmol/peptide/µl) were calculated for each PROCAL peptide. The 90 min LC gradient was divided up into bins of 10 min, whereby between one and two RT peptides were chosen per bin. Criteria for RT peptide selection were (1) detection at 2.5-5 fmol/µl and (2) one labelable amino acid that is shared with all other RT peptides. The latter is important to reduce the number of dynamic modifications in raw data processing thus preventing an increase in FDR. In total, a set of twelve peptides were subjected to solid-phase peptide synthesis (Wirkstoffpeptidlabor, Department of Immunology, University of Tübingen), whereby heavy isotope-labeled leucine [L($^{13}C_6$; $^{15}N$)] was incorporated at one position each. The synthesis of RT005 and RT0030 failed for three times, thus reducing the set of RT peptides to ten.

### Titration of RT peptide mixes for two LC-MS/MS systems

To avoid overdosing of spike-in peptides which may interfere with detection of (low abundant) native peptides and to establish a sensitive performance monitoring method, RT peptides were titrated for each LC-MS/MS system. Synthetic peptides were weighed in and dissolved at 1 mg/ml. For this purpose, peptides were first completely dissolved in 100% DMSO, facilitated by vortexing, with subsequent addition of MS grade $H_2O$. Peptides were diluted with $A_{Load}$ to a concentration of 10 pmol/µl. The dilution factor (Table 10) was calculated from the peptide-specific molecular weight, the peptide purity as determined by analytical high-performance liquid chromatography (HPLC), and a peptide content of 75%, which is a reference value obtained by nitrogen determination (20% TFA and 5-10% $H_2O$ content in peptide powders produced in-house; internal data of the Wirkstoffpeptidlabor, Department of Immunology, University of Tübingen). By serial dilution with $A_{Load}$, peptides were adjusted to 50 / 10 / 5 / 2.5 / 1 / 0.5 / 0.1 fmol/peptide/µl followed by 1:10 dilution with JY-derived HLA class I peptides (Orbitrap Fusion Lumos JY17#3; LTQ Orbitrap XL: JY17#4). Data acquisition was performed with standard LC-MS/MS methods for HLA class I peptides (400-650 m/z), whereby the highest (5 fmol/peptide/µl) and lowest (0.01 fmol/peptide/µl) concentration was only measured on the LTQ Orbitrap XL or the Orbitrap Fusion Lumos, respectively. For data analysis, heavy isotope

labels (+7.017 Da) were permitted as additional dynamic modification for leucine residues and the Swiss-Prot release from September 27th 2013 (20,279 reviewed protein sequences) concatenated with ten RT peptide sequences was used as reference database.

**Table 10. Ten heavy isotope-labeled peptides synthesized as RT standard.** M/z values of doubly and triply positively charged precursor ions were retrieved from Skyline. Precursors not detectable with the standard HLA class I LC-MS/MS method covering a mass range of 400-650 m/z are marked in red. The penultimate column gives molarities of peptide solutions obtained by dissolving 1 mg peptide powder in 1 ml solvent; calculated from molecular weight, synthetic peptide purity, and a peptide content of 75%. Dilution factors to adjust peptide solutions to 10 pmol/µl are given in the last column.

| Accession | Sequence | M [Da = g/mol] | $[M+2H]^{++}$ $[M+3H]^{+++}$ | Peptide purity [%] | 1 mg/ml = X nmol/µl | Dilution factor > 10 pmol/µl |
|---|---|---|---|---|---|---|
| RT0004 | IS[L($^{13}C_6$; $^{15}N$)]GEHEGGGK | 1089.5357 | 545.7837 364.1915 | 87.18 | 0.6001 | 60.01 |
| RT0007 | VGASTGYSG[L($^{13}C_6$; $^{15}N$)]K | 1045.5346 | 523.7831 349.5245 | 86.83 | 0.6229 | 62.29 |
| RT0012 | T[L($^{13}C_6$; $^{15}N$)]IAYDDSSTK | 1219.5874 | 610.8096 407.5421 | 65.67 | 0.4038 | 40.38 |
| RT0014 | F[L($^{13}C_6$; $^{15}N$)]ASSEGGFTK | 1149.5608 | 575.7963 384.1999 | 83.18 | 0.5427 | 54.27 |
| RT0015 | GF[L($^{13}C_6$; $^{15}N$)]DYESTGAK | 1193.5507 | 597.7912 398.8632 | 88.7 | 0.5574 | 55.74 |
| RT0016 | A[L($^{13}C_6$; $^{15}N$)]FSSITDSEK | 1203.5925 | 602.8121 402.2105 | 88.78 | 0.5532 | 55.32 |
| RT0021 | HFA[L($^{13}C_6$; $^{15}N$)]FSTDVTK | 1271.6452 | 636.8385 424.8947 | 82.68 | 0.4876 | 48.76 |
| RT0029 | VYAET[L($^{13}C_6$; $^{15}N$)]SGFIK | 1233.6547 | 617.8432 412.2310 | 81.54 | 0.4957 | 49.57 |
| RT0031 | GASDF[L($^{13}C_6$; $^{15}N$)]SFAVK | 1147.5816 | 574.8066 383.5402 | 67.51 | 0.4412 | 44.12 |
| RT0032 | FF[L($^{13}C_6$; $^{15}N$)]TGTSIFVK | 1265.6962 | 633.8639 422.9117 | 71.08 | 0.4212 | 42.12 |

### *Comparison of five LC-MS/MS systems*

During the course of a procurement measure for a new LC-MS/MS system, standardized samples of JY-derived HLA class I peptides and ten heavy isotope-labeled RT peptides were measured on five devices coming into question. For this purpose, equimolar concentrations of ten heavy isotope-labeled RT peptides were spiked into a complex matrix of HLA class I peptides eluted from JY cells. Following serial 1:2 dilution from 0.2 fmol/peptide/µl to 0.1 / 0.05 / 0.025 / 0.0125 fmol/peptide/µl, samples were acquired on an Orbitrap Fusion Lumos (Thermo Fisher Scientific; Department of Immunology, University of Tübingen; 90 min gradient), an Orbitrap Fusion (Thermo Fisher Scientific; Immatics Biotechnologies GmbH; 70 min gradient), a Q Exactive HF (Thermo Fisher Scientific; Proteome Center Tübingen, University of Tübingen; 90 min gradient), a TimsTOF Pro with Parallel Accumulation Serial Fragmentation acquisition (Bruker Daltonics; 30 / 60 / 100 min gradient), and on a TripleTOF 6600 (AB SCIEX; 90 min gradient). M/z windows and gradient designs were attuned to immunopeptidomic demands as far as possible. Data was analyzed by Bruker Daltonics (TimsTOF Pro) and by Ana Marcu (Department of Immunology, University of Tübingen) employing the PEAKS 8.5 software (Bioinformatics Solutions Inc.), as this was compatible with all raw file formats. In brief, the fragmentation type was set to CID and database search against the Swiss-Prot release from September 27th 2013 (20,279 reviewed protein sequences)

concatenated with the sequences of ten RT peptides was performed at a precursor mass tolerance of 5 (Thermo Fisher Scientific instruments) or 10 ppm (TripleTOF 6600), a fragment mass tolerance of 0.02 (Thermo Fisher Scientific instruments) or 0.5 Da (TripleTOF 6600), and a FDR of 5% estimated by target-decoy database search ('decoy-fusion'). A maximum number of three variable modifications, comprising oxidation of methionine (+15.995 Da) and heavy isotope-labeled leucine (+7.017 Da), were permitted per peptide.

# 4 Results

## 4.1 Compatibility of PROCAL peptides with immunopeptidomics

PROCAL peptides were spiked at 20 / 10 / 5 / 2.5 fmol/peptide/µl into a matrix of HLA class I peptides eluted from JY cells and acquired on an Orbitrap Fusion Lumos. 34 out of 40 peptides were detectable in full length in at least one measurement, whereby the limit of detection was not reached with 2.5 fmol/µl for 29 peptides (Figure 42). These were distributed across the entire 90 min gradient (Figure 42 A). Of note, spike-in PROCAL peptides did not severely impede the detection of JY-derived peptides as visible by a constant number of unique peptide identifications across the titration series (Supplementary Table 22).

**A**
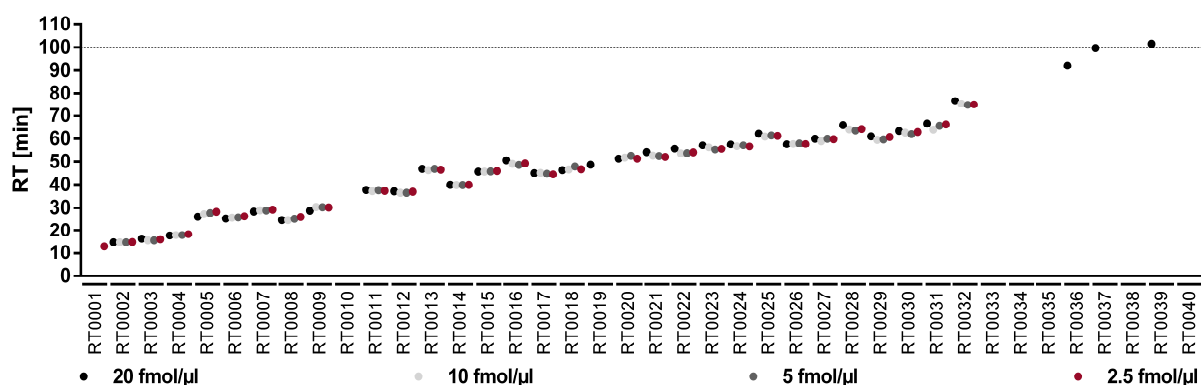


**B**
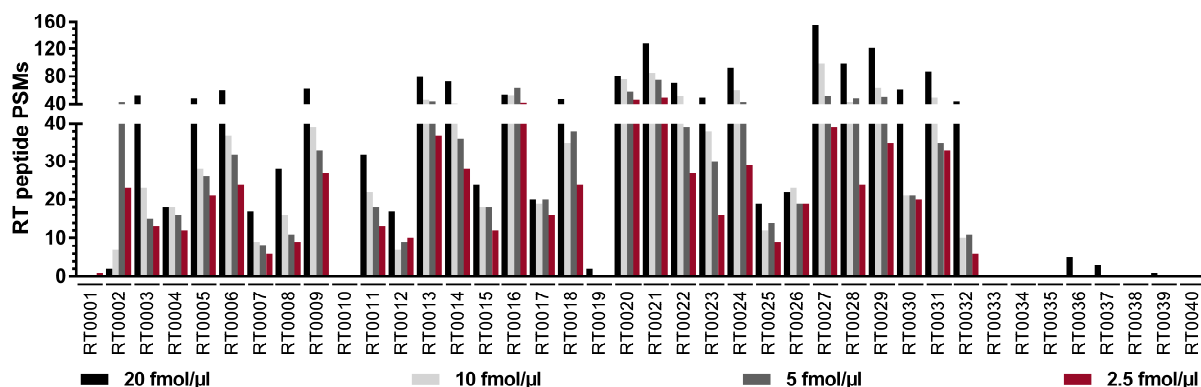
**C**
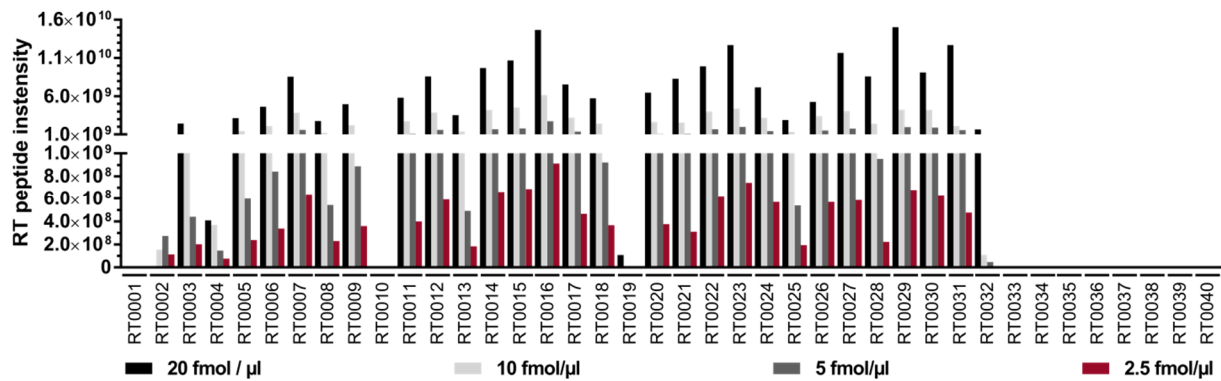


**Figure 42. Identification of PROCAL peptides titrated in a complex matrix of HLA class I peptides [2.5-20 fmol/peptide/µl].** RT peptide intensities (AUC), PSMs, and RTs were reported for the best scoring PSM detected in full length. Of note, the number of break variants increased with mounting peptide concentration. **(A) Observed RTs of RT0001-RT0040.** The dashed line indicates the end of the 90 min gradient. **(B) Number of PSMs identified per RT peptide** and **(C) RT peptide intensities.** RT0036, RT0037, and RT0039 eluted at the end of or after the 90 min gradient and had very low intensities $< 8 \times 10^5$ at 20 fmol/µl. RT0010, RT0033, RT0034, RT0035, RT0038, and RT0040 were not detected at all.

## 4.2 Titration of in-house heavy isotope-labeled RT peptides

Heavy isotope-labeled RT peptides were spiked at 0.01-1 fmol/peptide/µl or at 0.025-5 fmol/peptide/µl in a complex matrix of HLA class I ligands for the Orbitrap Fusion Lumos (Figure 43, Figure 44) and the LTQ Orbitrap XL (Figure 45, Figure 46), respectively. As already observed for PROCAL peptides, high concentrations of spike-in RT peptides did not affect the number of unique peptide identifications (Supplementary Table 22). Criteria to determine optimal concentrations were ≥ 2 PSMs (Figure 43, Figure 45) and an AUC comparable to JY-derived precursors of medium intensity (Figure 44, Figure 46).

**A**
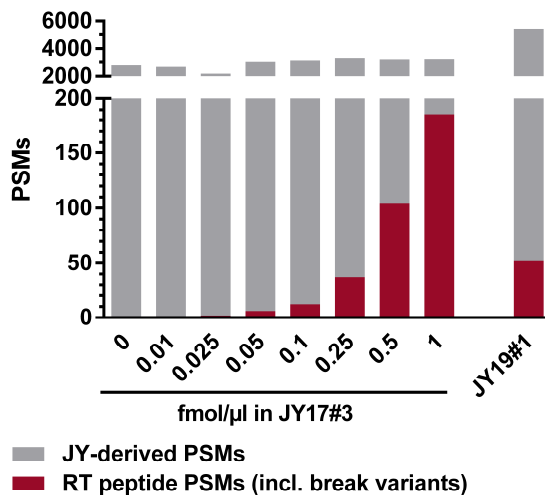


**C**
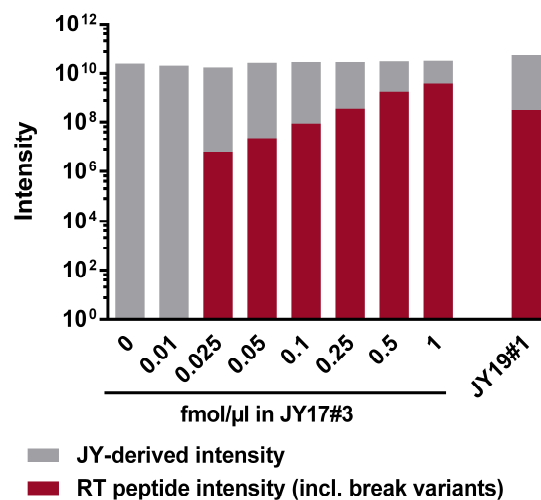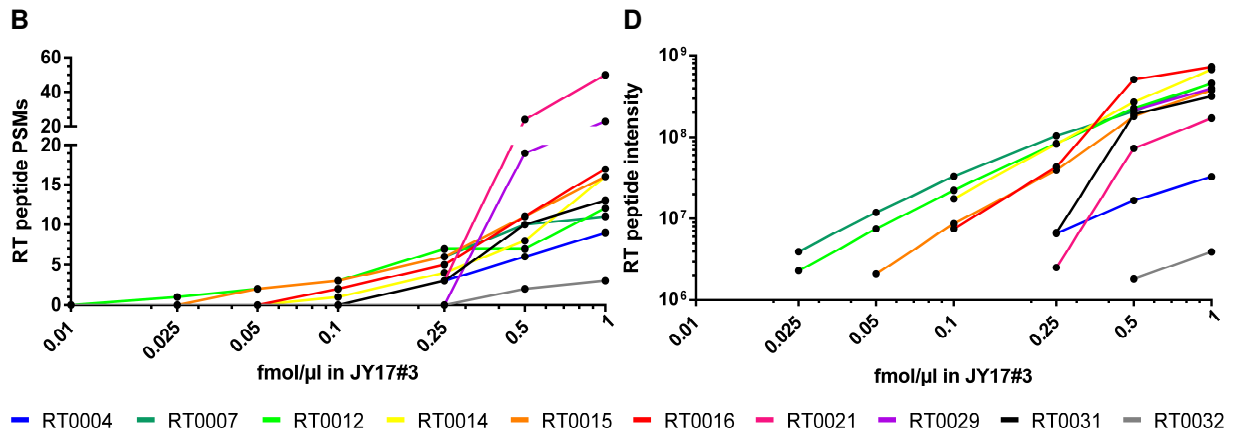
**Figure 43. Titration of RT peptides in JY17#3 for the Orbitrap Fusion Lumos.** Panel **(A)** and **(C)** include PSMs and intensities of both full-length and break variants, whereas **(B)** and **(D)** report dose-dependent PSM numbers and intensities for full-length peptides only.
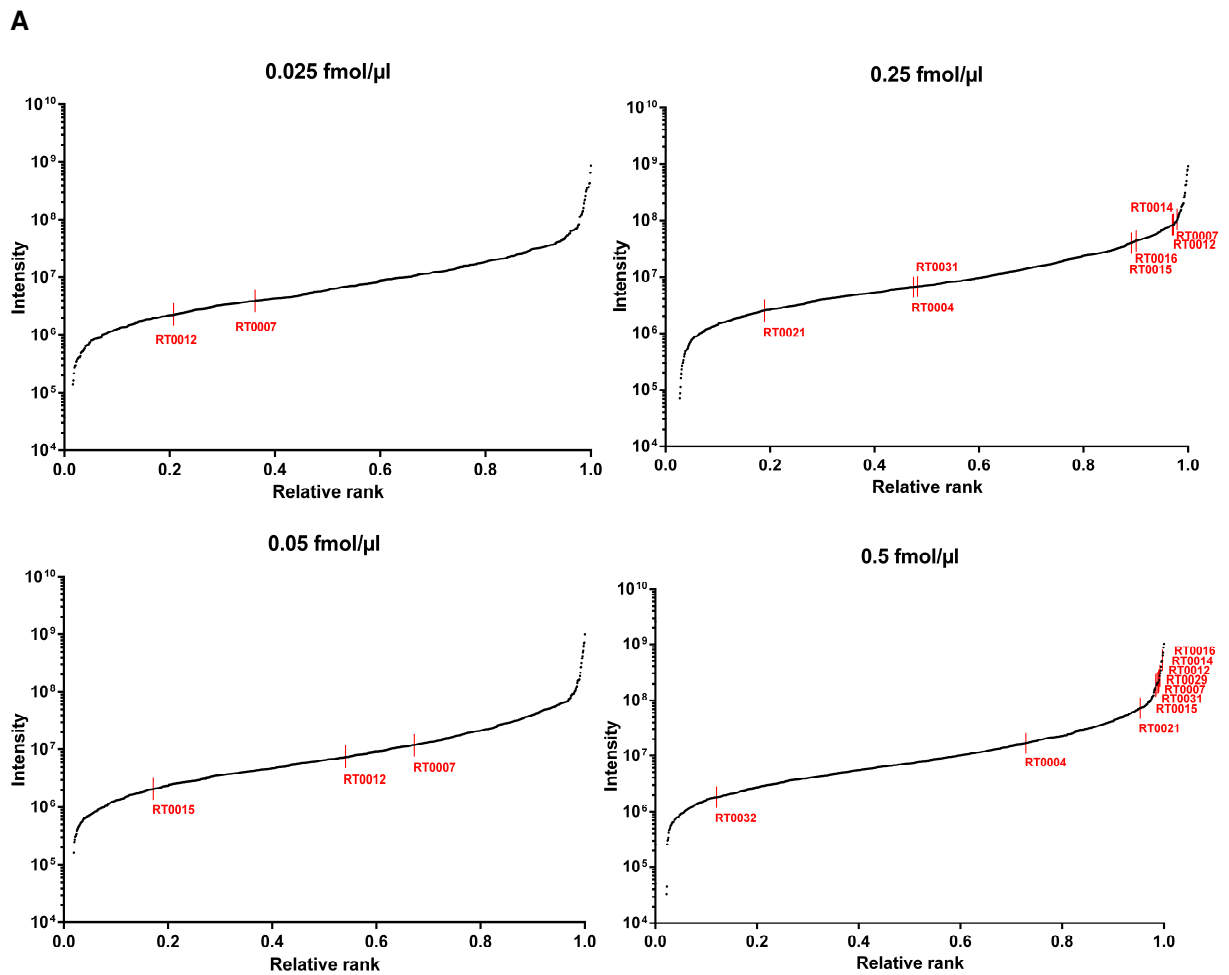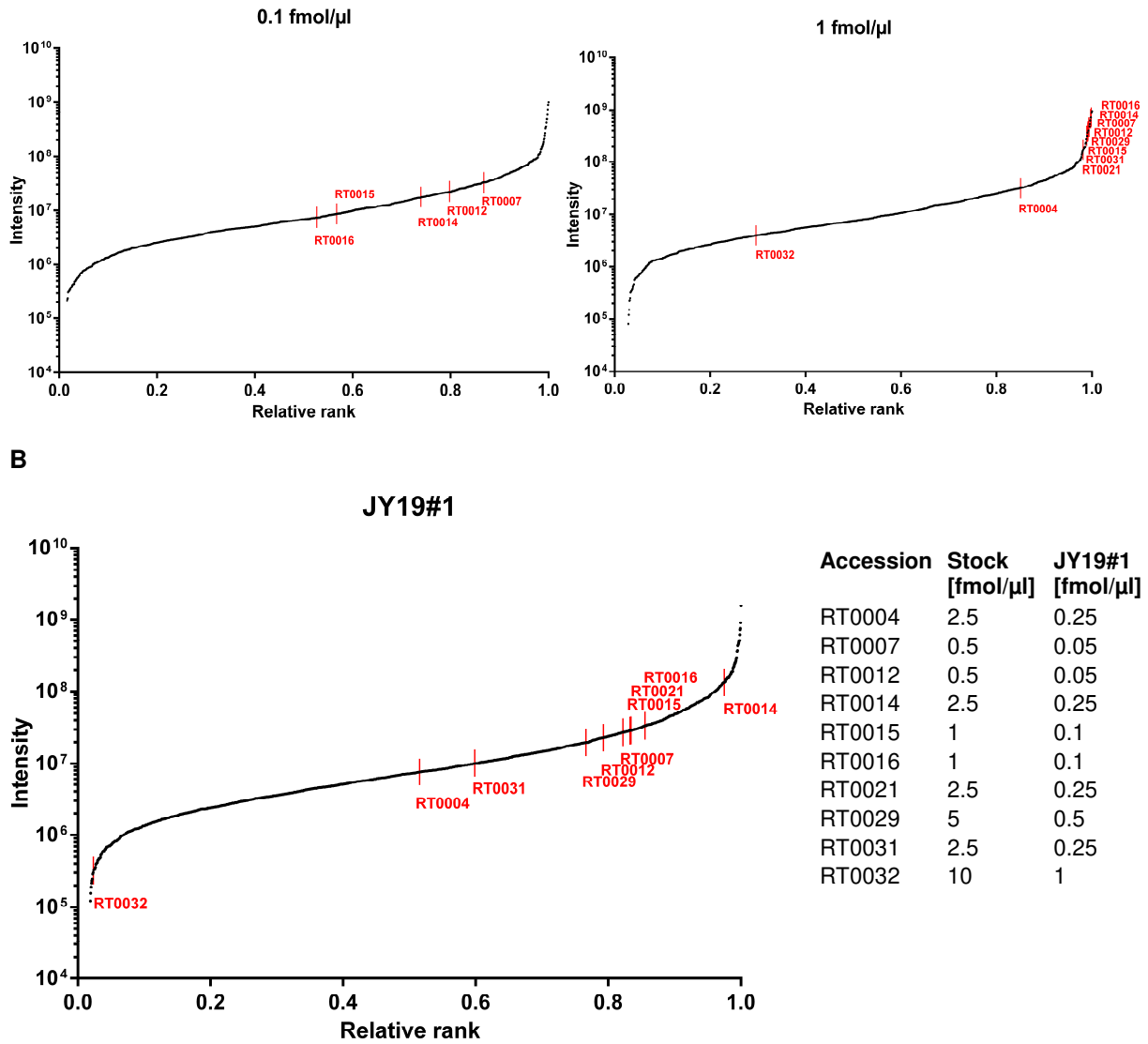
**Figure 44. Relative rank of full-length RT peptides in comparison with JY17#3-derived peptides (Orbitrap Fusion Lumos).** Best scoring PSMs were sorted by intensities and ranked according to their AUCs. RT peptides are marked in red for **(A) titration series** and **(B) titrated RT peptide mix spiked into the newly synthesized LC-MS/MS standard JY19#1.** RT peptide concentrations of ready-to-use stocks (to be diluted 1:10 with sample of interest) and in JY19#1 are listed on the right.
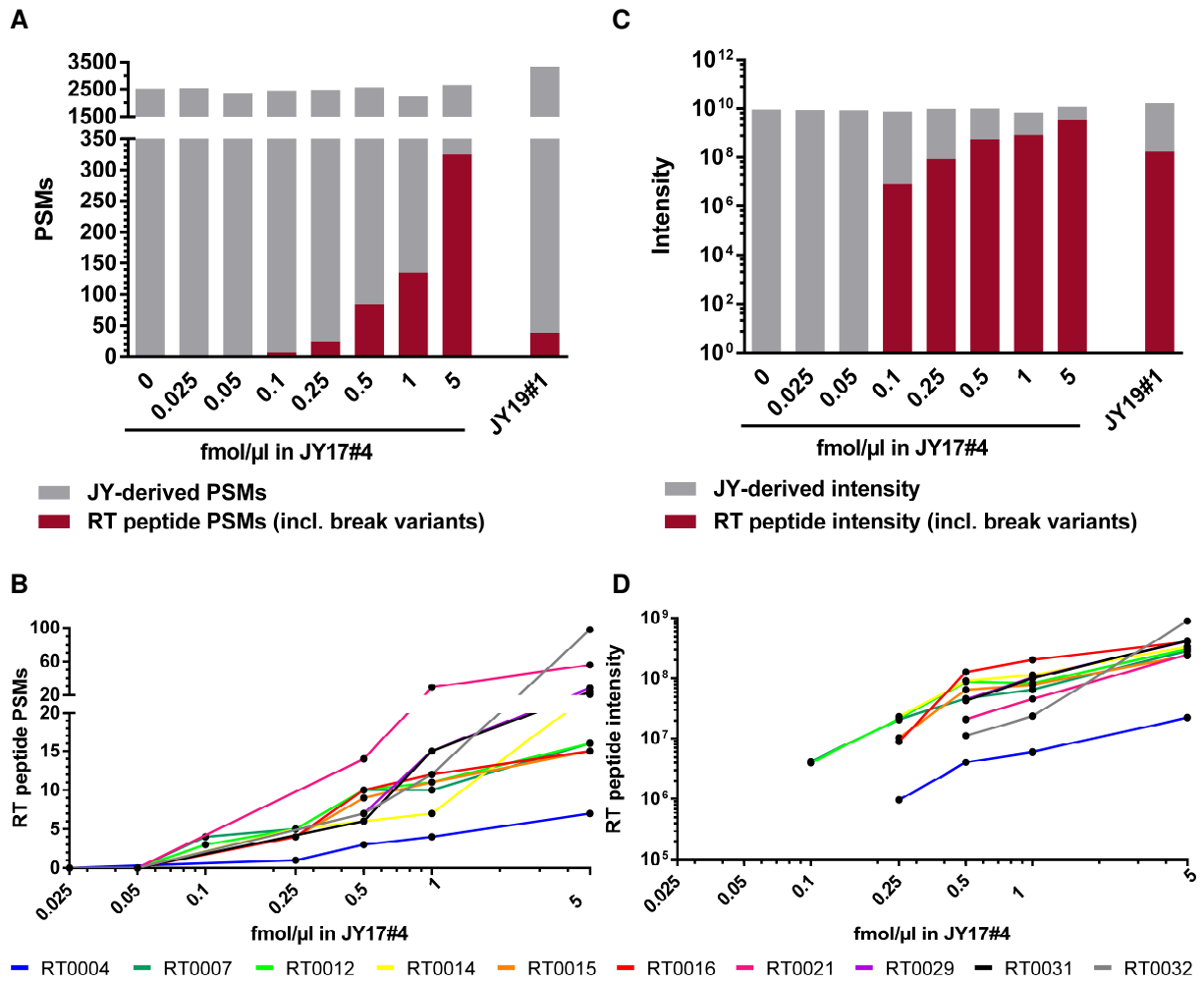
Figure 45. **Titration of RT peptides in JY17#4 for the LTQ Orbitrap XL.** Panel **(A)** and **(C)** include PSMs and intensities of both full length and break variants, whereas **(B)** and **(D)** report dose-dependent PSM numbers and intensities for full length peptides only.

**A**

**B**



Figure with title "JY19#1" showing Intensity (y-axis, $10^4$ to $10^9$) versus Relative rank (x-axis, 0.0 to 1.0). RT peptides marked in red: RT0032, RT0004, RT0007, RT0012, RT0015, RT0031, RT0029, RT0014, RT0016, RT0021.

| Accession | Stock [fmol/µl] | JY19#1 [fmol/µl] |
|---|---|---|
| RT0004 | 5 | 0.5 |
| RT0007 | 1 | 0.1 |
| RT0012 | 1 | 0.1 |
| RT0014 | 2.5 | 0.25 |
| RT0015 | 2.5 | 0.25 |
| RT0016 | 2.5 | 0.25 |
| RT0021 | 5 | 0.5 |
| RT0029 | 5 | 0.5 |
| RT0031 | 5 | 0.5 |
| RT0032 | 5 | 0.5 |
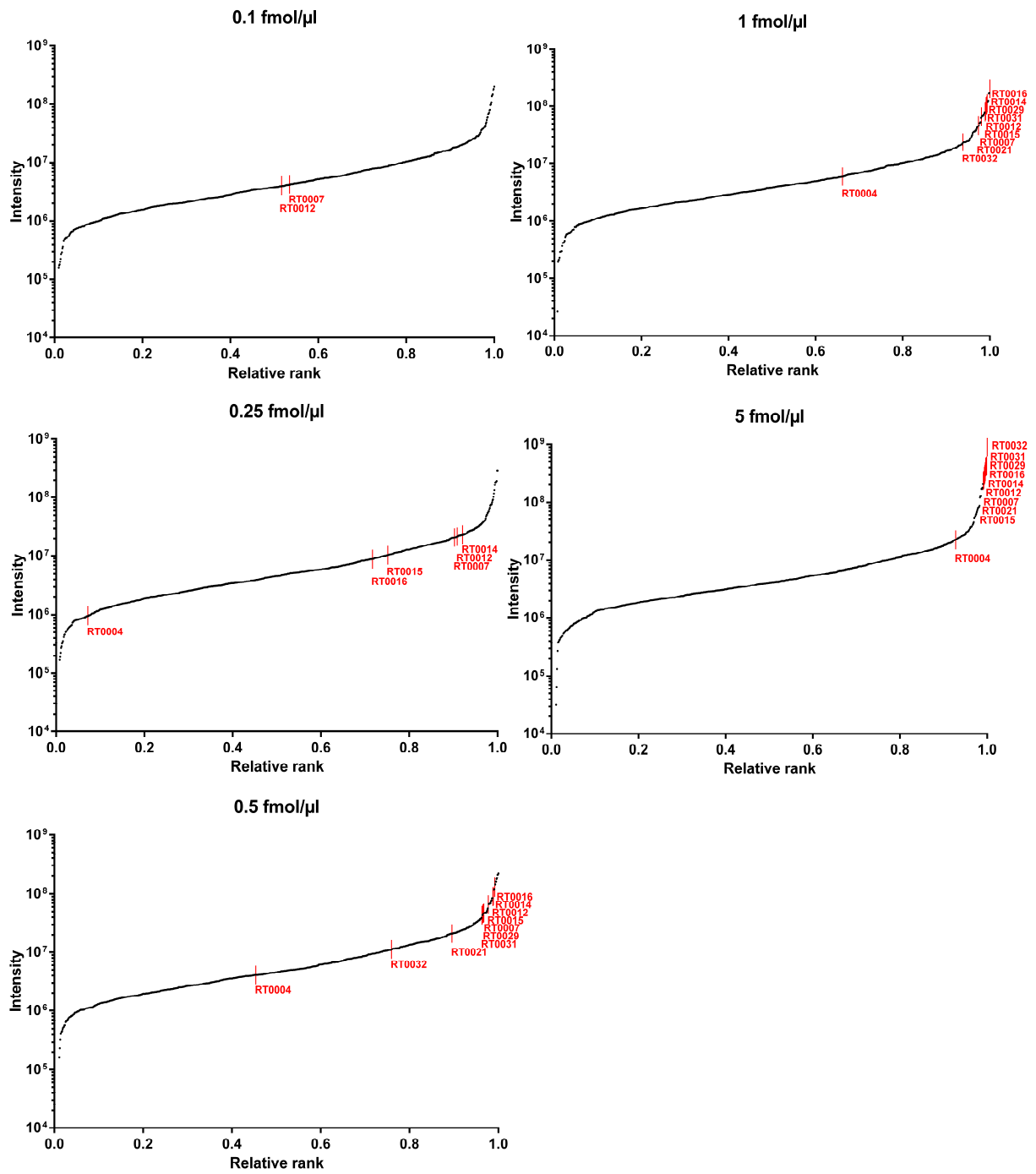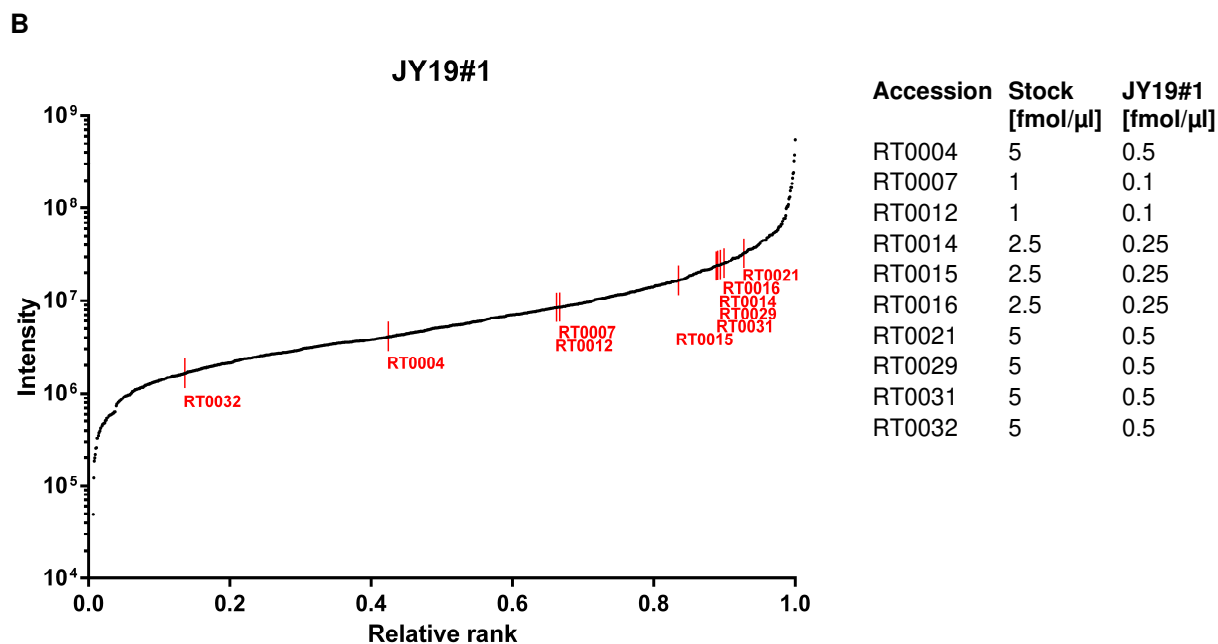
**Figure 46. Relative rank of full-length RT peptides in comparison with JY17#4-derived peptides (LTQ Orbitrap XL).** Best scoring PSMs were sorted by intensities and ranked according to their AUCs. RT peptides are marked in red for **(A) titration series** and **(B) titrated RT peptide mix spiked into the newly synthesized LC-MS/MS standard JY19#1.** RT peptide concentrations of ready-to-use stocks (to be diluted 1:10 with sample of interest) and in JY19#1 are listed on the right.

The LC-MS/MS standard JY19#1 composed of HLA class I ligands eluted from JY cells was prepared by Jens Bauer (Department of Immunology, University of Tübingen). Upon spike-in of ten RT peptides almost 500 vials of the lower concentrated and 300 vials of the higher concentrated standard each containing 25 µl sample (5 technical replicates) were filled for the Orbitrap Fusion Lumos and the LTQ Orbitrap XL, respectively. Moreover, almost 200 ready-to-use aliquots of 1000 µl RT peptide mix to be diluted 1:10 with sample were frozen for each LC-MS/MS system. Highly concentrated stocks of 1 mg/ml are available for every single RT peptide as well.

## 4.3  Performance monitoring of LC-MS/MS systems by RT peptides

The performance of the two LC-MS/MS systems at the Department of Immunology, University of Tübingen is constantly monitored by means of standardized HLA class I peptide eluates. Since implementation of RT peptides and manufacturing of the JY19#1 standard containing ten RT peptides, performance monitoring was significantly improved. RTs were largely stable over time and only changed upon technical intervention. In contrast, RT peptides and JY-derived peptides were subject to strong fluctuations on the level of both PSMs and intensities that could not be explained. Renewal of solvents (mobile phase), trapping and/or separation columns (stationary phase) were identified as major factors influencing the performance of both the Orbitrap Fusion Lumos (Figure 47) and the LTQ Orbitrap XL (Figure 48). Detection of RT0032 evolved as marker of top performance for both devices, as this peptide was only present when the other nine were as well.

**A**
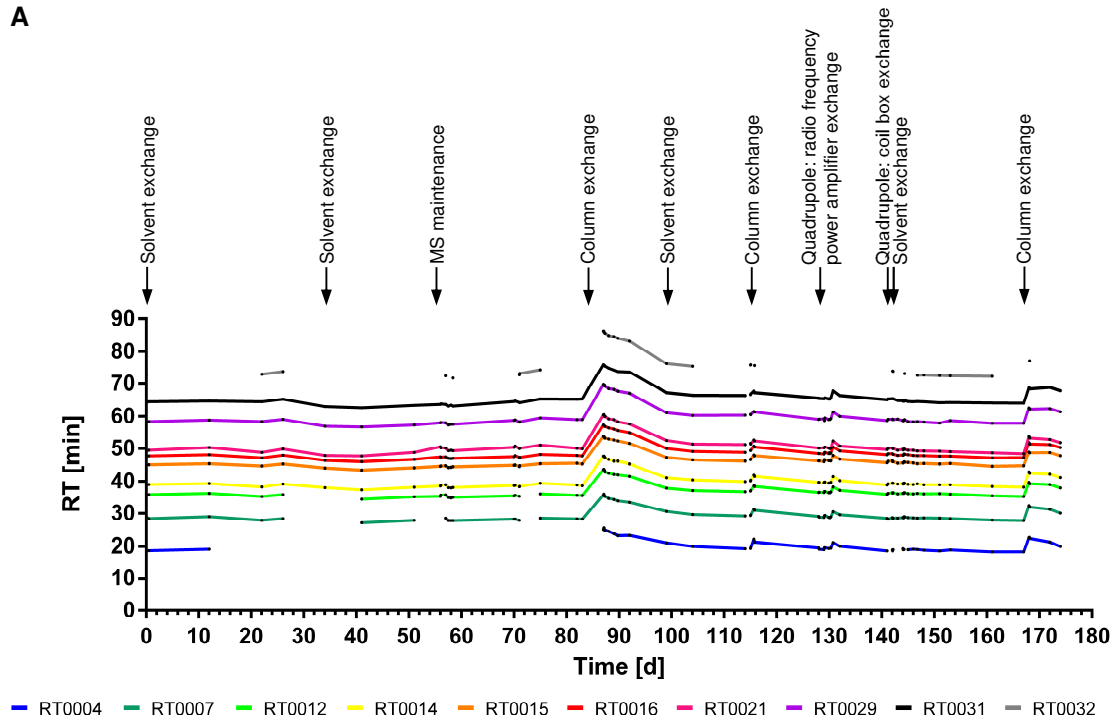


**B**
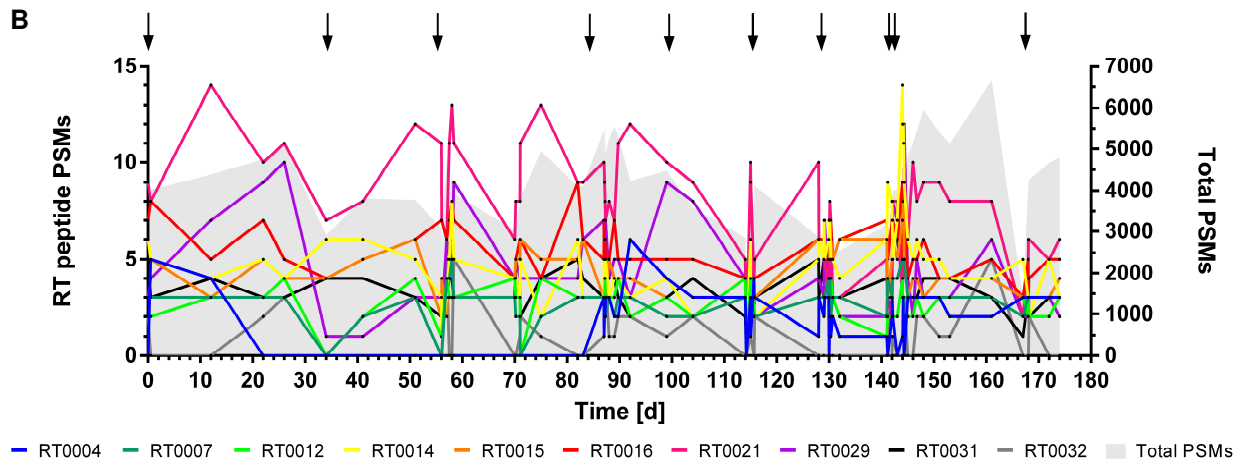


**C**
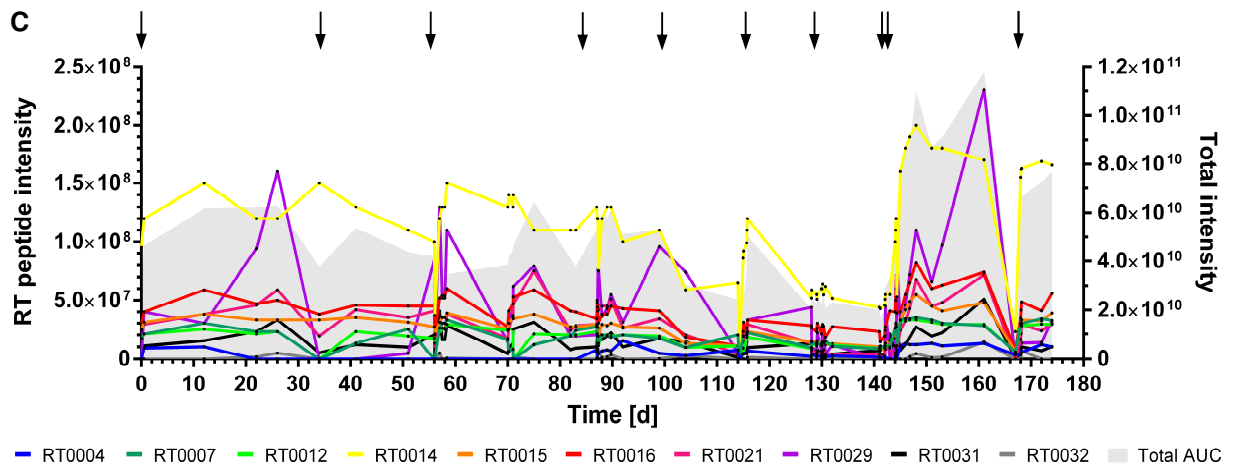


**Figure 47. Performance of the Orbitrap Fusion Lumos.** LC-MS/MS performance was monitored over almost half a year (19.06.2019 – 09.12.2019) by analyzing **(A) RTs of spike-in peptides**, **(B) total and RT peptide-derived PSMs**, and **(C) intensities of RT peptides and summed AUC of all peptide identifications.** Reported RTs and intensities refer to the best scoring PSM each.
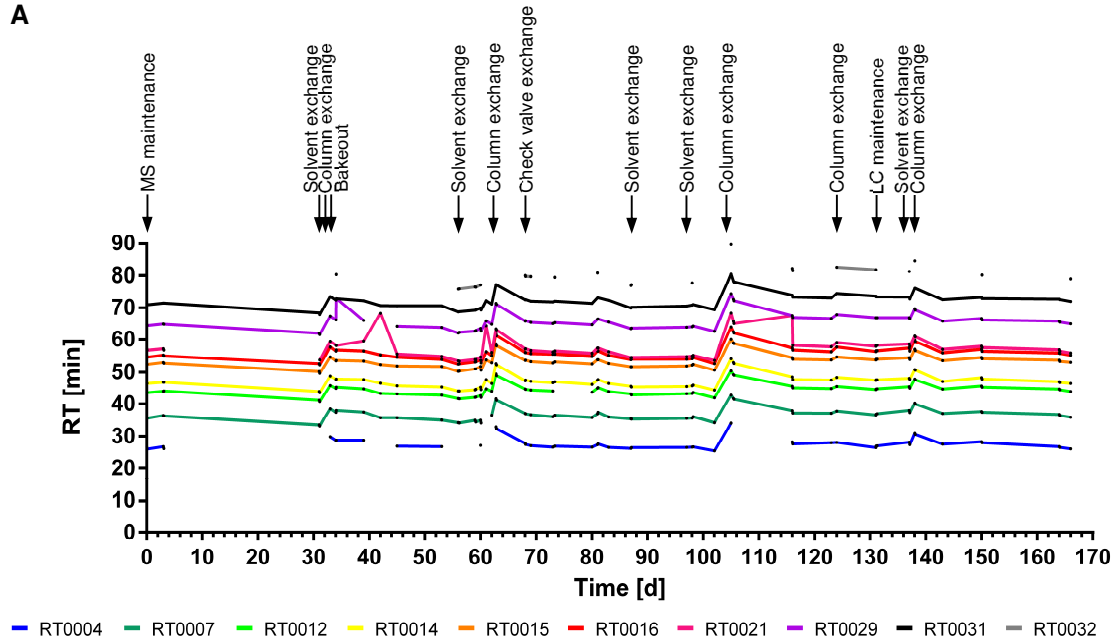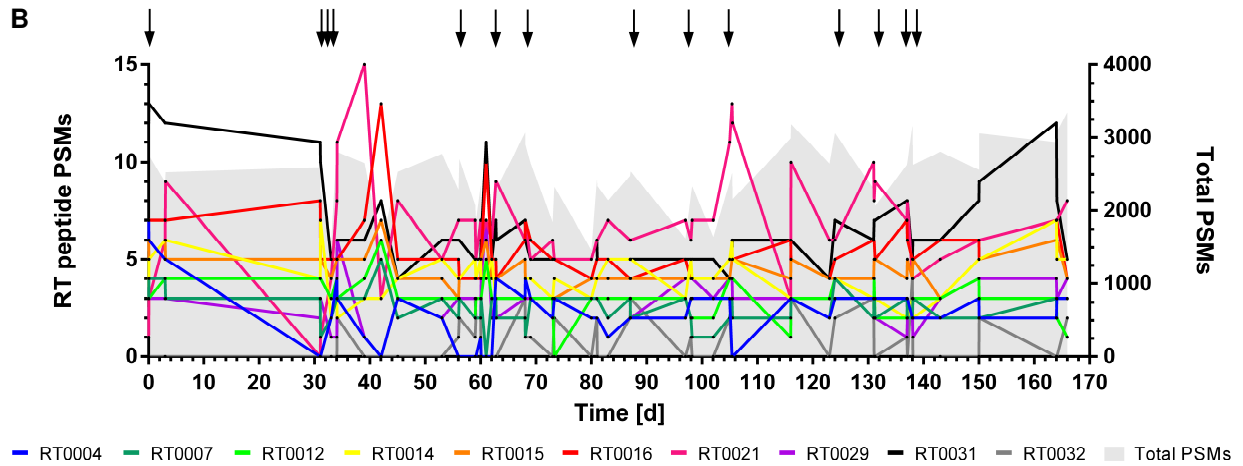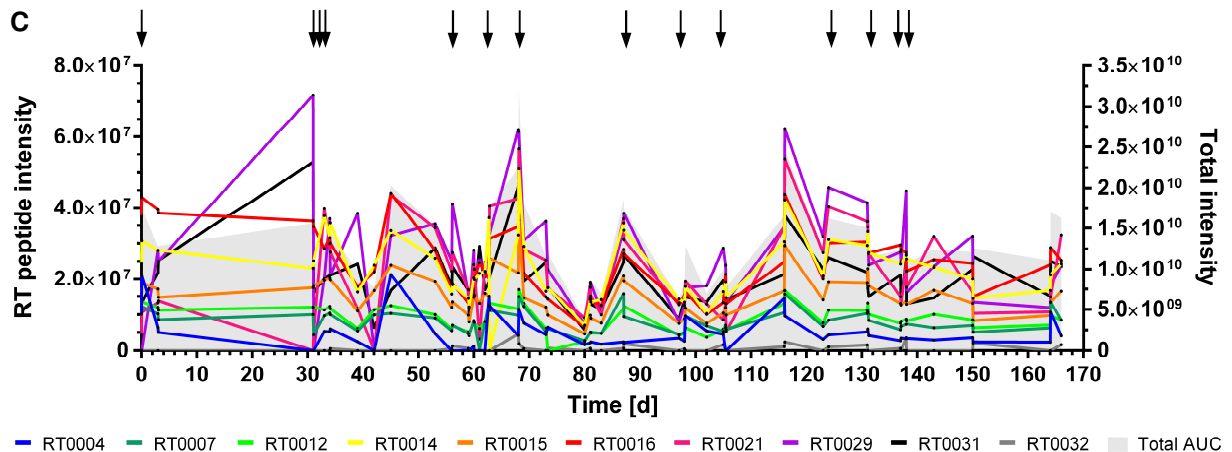
**Figure 48. Performance of the LTQ Orbitrap XL**. LC-MS/MS performance was monitored over almost half a year (14.06.2019 – 27.11.2019) by analyzing **(A) RTs of spike-in peptides**, **(B) total and RT peptide-derived PSMs**, and **(C) intensities of RT peptides and summed AUC of all peptide identifications.** Reported RTs and intensities refer to the best scoring PSM each.

## 4.4 Comparison of LC-MS/MS systems for a procurement measure

In the course of a procurement measure for a new LC-MS/MS system, standardized samples of JY-derived HLA class I peptides and ten heavy isotope-labeled RT peptides were measured on five devices coming into question. The highest concentrated sample contained 0.2 fmol/peptide/µl and was serially diluted with $A_{Load}$ to 0.1 / 0.05 / 0.025 / 0.0125 fmol/peptide/µl. Data acquisition was performed on an Orbitrap Fusion Lumos, an Orbitrap Fusion, a Q Exactive HF, a TimsTOF Pro with Parallel Accumulation Serial Fragmentation acquisition, and on a TripleTOF 6600. Overall, the Orbitrap Fusion Lumos and the Orbitrap Fusion, which are both operated in immunopeptidomic laboratories, performed best for the titration series. On the level of unique peptide identifications, data generated by the Q Exactive HF or by the TimsTOF Pro were comparable, whereby RT peptides were identified more reliably by the TimsTOF Pro (all 10 RT peptides at the two highest and 8 RT peptides at the two lowest concentration). The sensitivity of the Orbitrap Fusion was similar to the Orbitrap Fusion Lumos, both detecting 7 RT peptides at 12.5 amol/µl (Figure 49 A). From the third titration stage, the Q Exactive HF showed a clear decline in performance. The TripleTOF 6600 performed relatively well in RT peptide detection at high concentrations, whereas JY-derived HLA class I ligand identification and annotation of peptide intensities was severely impaired or even impossible. Peptide intensities were highest for the three Orbitrap instruments and reported reliably up to the lowest concentration stage (Figure 49 B and C).

**A**



| Orbitrap Fusion Lumos | Orbitrap Fusion | Q Exactive HF |
| TimsTOF Pro / 60 min gradient | TimsTOF Pro / 30 min gradient | TripleTOF 6600 |

**B**



**C**

**0.0125 fmol/µl**
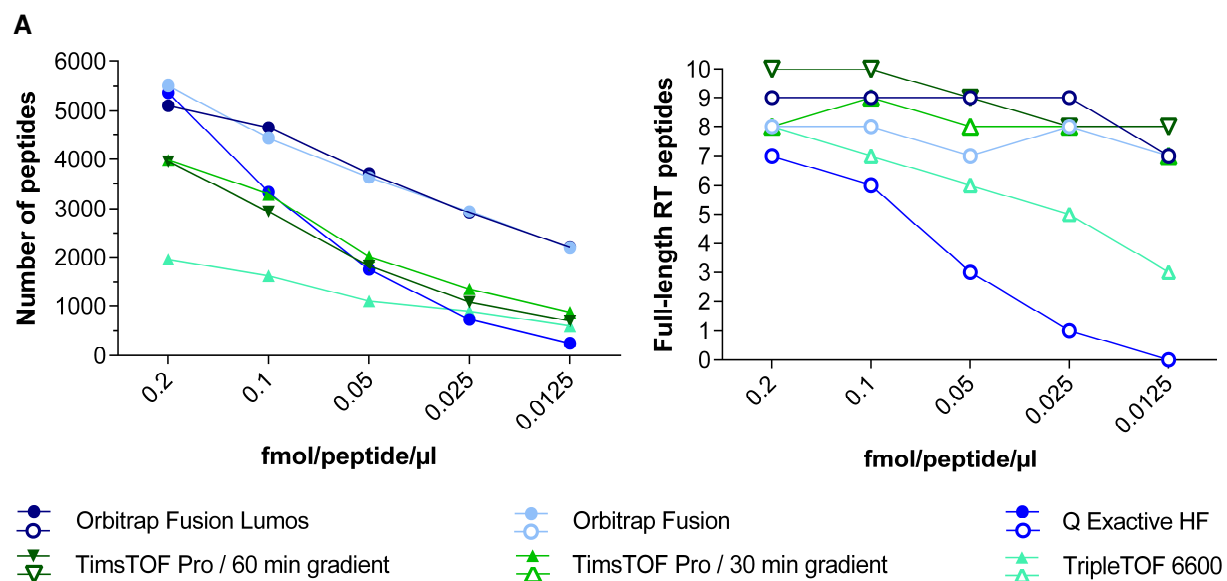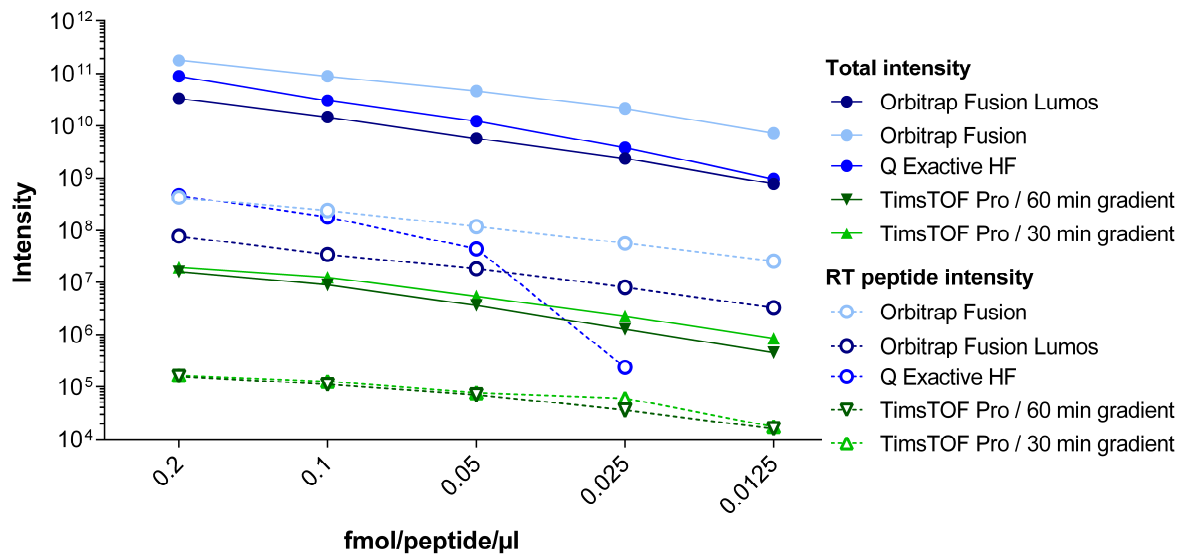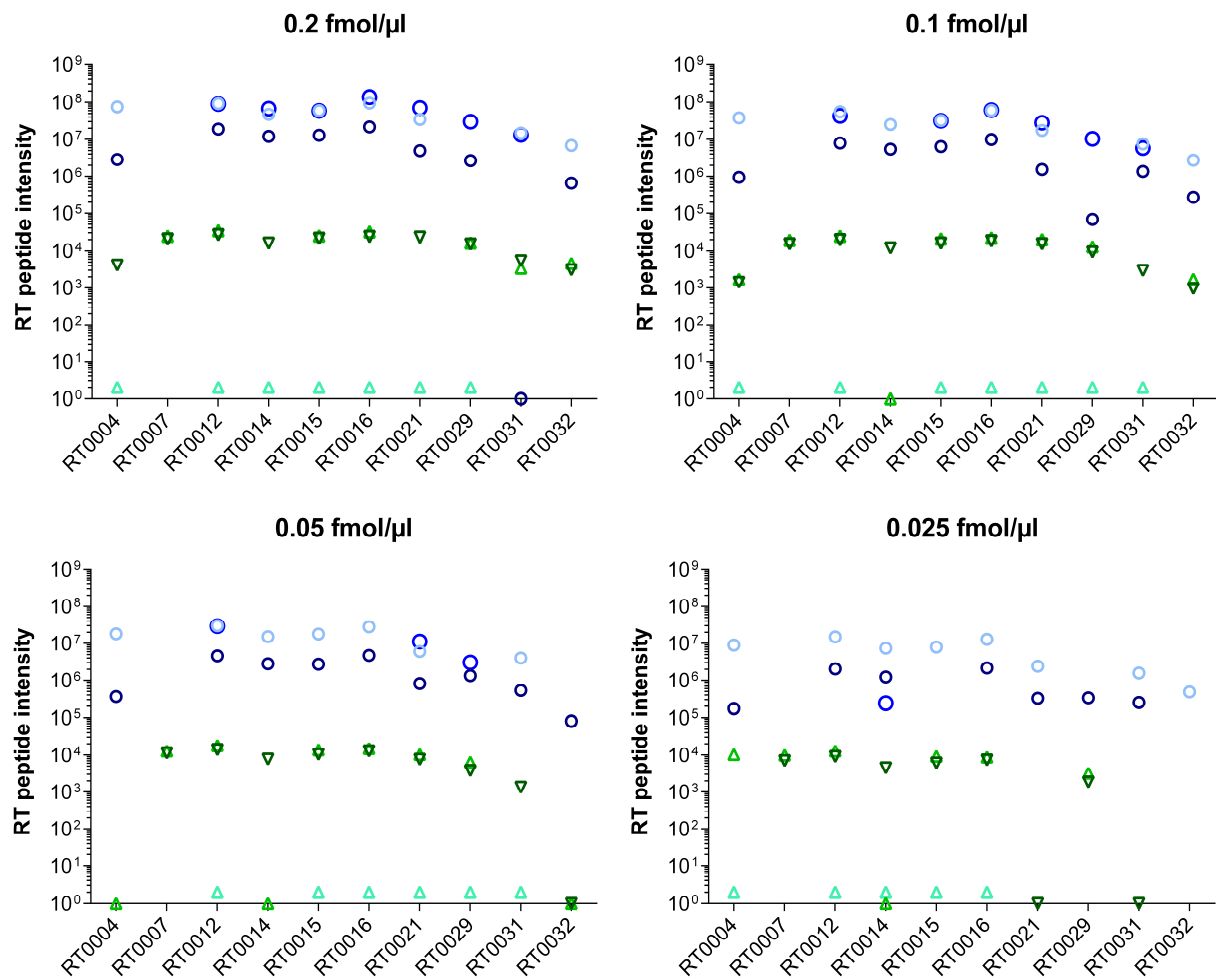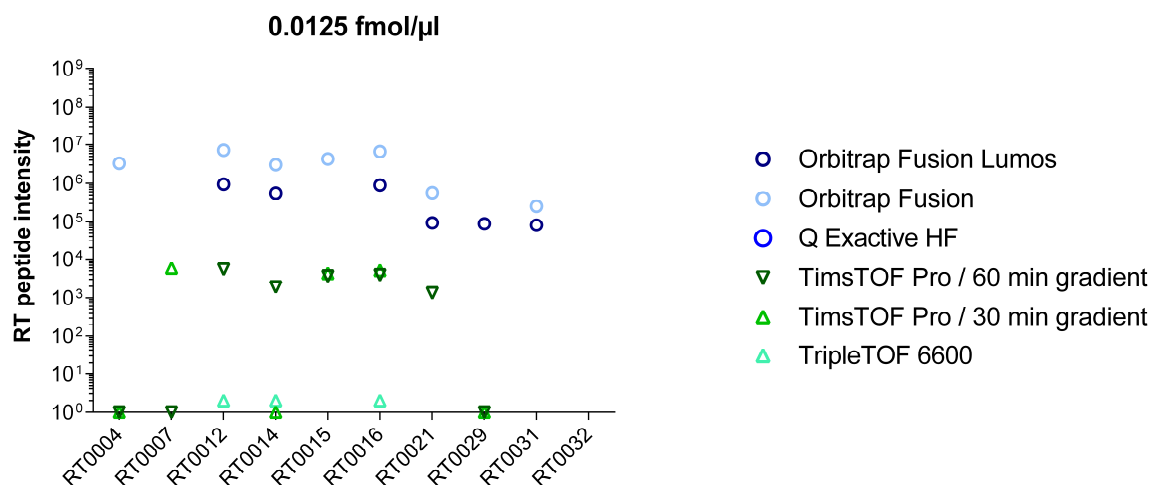
**Figure 49. Performance of five LC-MS/MS systems with immunopeptidomic samples.** LC-MS/MS systems coming into question in the course of a procurement measure were evaluated by data acquisition from a standardized titration series of RT peptides spiked into a matrix of HLA class I peptides eluted from JY cells. Raw data were kindly analyzed by Ana Marcu (Department of Immunology, University of Tübingen) and by Bruker Daltonics (TimsTOF Pro) using the PEAKS software. Overall, the Orbitrap Fusion Lumos and the Orbitrap Fusion, which are both operated in immunopeptidomic laboratories, performed best for the titration series. **(A) Total number of unique peptide identifications and number of identified RT peptides.** Even at the highest concentration, the TripleTOF 6600 reached less than 40% of the peptide identification rate achieved by Thermo Fisher Scientific instruments. Of note, gradient duration hardly affected the peptide yields achieved by the TimsTOF Pro, whereas RT peptide identification benefited from using the longer gradient. **Intensities (B) summed for all peptides or RT peptides** and **(C) of every individual RT peptide across all concentration stages.** Recorded intensities were largely comparable for the three Orbitrap instruments, followed by the TimsTOF Pro. At the lowest concentration of 12.5 amol/µl, the detection rate of the TimsTOF Pro was best, whereby the Orbitrap Fusion and the Orbitrap Fusion Lumos were superior regarding annotation of peptide intensities. Since corresponding peptide intensities could not be retrieved from TripleTOF 6600 data, intensities were not reported in (B) and with a value of 2 in (C).

# 5  Discussion

Synthetic peptide spike-ins have a wide range of applications including constant monitoring of chromatographic and mass spectrometric performance, contemporary data acquisition strategies and quantitative approaches with enhanced identification and reproducibility rates, as well as comparison and calibration of collision energies between two mass spectrometers.[1,3,4,6] However, such standardized peptide mixtures have so far not been introduced to immunopeptidomics. PROCAL, a mix of 40 synthetic peptides, was developed for proteomic purposes with tryptic digests as analyte. We found 34 of these peptides to be detectable with our LC-MS/MS method for HLA class I ligands. According to the manufacturer's instructions, the six most hydrophobic peptides elute at 33.7-39.9% AcN from the columns. Thus, these were not expected to elute over the course of the applied 90 min gradient ranging from 3 to 40% nano pump solvent B (equal to 2.4-32% AcN), but only during the wash peak reaching 95% solvent B (76% AcN) in min 106. In addition, the manufacturer's instructions claim that dissolution of the peptide mix in aqueous solvents other than 100% DMSO or 30% AcN results in loss of the six most hydrophobic peptides.[1] To evaluate the compatibility of PROCAL peptides with immunopeptidomic LC-MS/MS data acquisition, we deliberately

dissolved the peptides in $A_{Load}$, the standard solvent for HLA ligand extracts. Hence, this represents another reason for losing some of the PROCAL peptides. However, failure to detect RT0010 and (almost) RT0019 can neither be explained by inadequate dissolution nor by an insufficient AcN content of nano pump solvents. Both of these peptides were not within the applied mass window of 400-650 m/z when having two positive charges. This allows the presumption that present solvent compositions in combination with the peptide's ability to take up protons did not favor the formation of detectable triply positively charged precursor ions.

Based on this pilot experiments employing PROCAL peptides, 12 sequences were selected for in-house synthesis with heavy isotope-labeled leucine. Two of these were not synthesizable reducing the number to ten RT peptides. These were titrated for both LC-MS/MS systems currently operated at the Department of Immunology at the University of Tübingen. Following titration, ready-to-use standards composed of a complex matrix of HLA class I peptides eluted from JY cells as well as ten spike-in RT peptides were manufactured. These standards are constantly used to monitor chromatographic and mass spectrometric performance and are sufficient for a total of 2,500 and 1,500 standard injections on the Orbitrap Fusion Lumos or the LTQ Orbitrap XL, respectively. During the course of almost half a year, LC-MS/MS performance was shown to be significantly influenced by renewal of solvents (mobile phase) as well as of trapping and/or separation columns (stationary phase). However, major fluctuations affecting peptide intensities and the number of PSMs, but not retention times, appeared independent of any technical intervention on both devices and can so far not be explained. Besides performance monitoring, RT peptides are of great value for troubleshooting by revealing e.g. retention time shifts, selective loss of peptide identifications at a specific retention time, reduced peptide intensities, impaired fragmentation quality, or a decrease in mass accuracy.

During the course of a procurement measure, the mix of ten RT peptides was used to compare five LC-MS/MS systems coming into question – especially regarding sensitivity – thus guiding device selection. Overall, the Orbitrap Fusion Lumos and the Orbitrap Fusion performed best for the entire dilution series of HLA class I ligands eluted from JY cells with spike-in RT peptides at equal concentration. These results are to some extent biased, as both machines were operated in immunopeptidomic laboratories with highly optimized LC-MS/MS methods for the detection of HLA-presented peptides. Assuming that the performance of non-immunopeptidomic LC-MS/MS systems can be enhanced by optimizing methods to this special kind of analytes, the TimsTOF Pro offers promising prospects by introducing the trapped ion mobility separation-based detection technology which is complementary to our present Orbitrap instruments.[7] Regarding the detection of RT peptides, the TimsTOF Pro even outperformed all other LC-MS/MS systems with a detection limit of < 12.5 amol/µl for eight spike-in peptides. At the highest concentration, the number of unique peptide identifications achieved by the Q Exactive HF was comparable to the Orbitrap Fusion and the Orbitrap Fusion Lumos. For subsequent dilution stages, a clear decline in performance was observed, whereby technical issues during acquisition appear the most probable cause. The Q Exactive HF is nearly identical in construction with the Orbitrap Fusion and the Orbitrap Fusion Lumos, while being less expensive.[8] Hence, it should be possible to push it at the same performance level by strictly optimizing both chromatographic and mass spectrometric parameters.

The implemented set of ten heavy isotope-labeled RT peptides represents an essential tool in method development including optimization of collision energies for these newly acquired LC-MS/MS systems used for immunopeptidomic research. Further, these RT peptides open up new avenues to innovative data acquisition strategies in the field of immunopeptidomics. Data-independent acquisition and more accurate quantification strategies will enable deeper explorations of immunopeptidomes comprising so far underrepresented species such as neo-antigenic or cryptic epitopes. RT peptides may also facilitate the transfer of spectral libraries required for the analysis of data-independently acquired MS/MS spectra from one to another platform by allowing a comparison of fragmentation characteristics.[1,3,4,6] Of note, all RT peptides are also covered by the mass window applied to HLA class II peptide eluates.

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# CHAPTER 7

**General discussion and perspective**

# 1   General discussion and perspective

With the great success of checkpoint inhibitors, cancer immunotherapy has started to evolve as novel pillar in multimodal anti-tumor therapies.[1-4] Immune checkpoint blockade appears effective in metastatic melanoma or non-small cell lung carcinoma,[5-8] while patients suffering from other types of tumors including intracranial neoplasia do not clinically benefit.[9,10] The reason for this was found out later when response to checkpoint inhibition was shown to positively correlate with mutational burden,[7,11,12] which is low among brain tumors excepting MMR-deficient ones and recurrent glioblastomas exhibiting a hypermutation phenotype[13-16] Cancer immunotherapy aims at breaking immune evasion by provision of the (appropriate) antigen (1), supply of T-cell co-stimulation or blockade of T-cell co-inhibition (2), and transfer of effector cells (3).[17,18] As the second strategy alone has shown to be ineffective in tumors with low mutational burden and lacking pre-existing immunity, one should go for providing the appropriate antigen activating the patient's T cells or directly providing (antigen-specific) effector cells. Combined with checkpoint inhibition this might then elicit clinically relevant anti-tumoral immune responses.[2,17,19] In the case of glioblastoma, concomitant administration of anti-PD-1 or anti-PD-L1 antibodies might be promising, as PD-L1 is expressed in 72% of recurrent and 88% of primary glioblastomas.[20] Medulloblastomas and meningiomas, in turn, are rarely PD-L1 positive,[21,22] suggesting to target the PD-1–PD-L1 axis only *via* PD-1 or to use other checkpoint antibodies blocking e.g. CTLA4, TIGIT, LAG-3, and VISTA on T cells or B7-H3 expressed on tumor cells.[2,23,24]

Immunopeptidomics has proven as state-of-the art and method of choice to define candidate targets for cancer immunotherapy as it offers an unbiased and comprehensive view on the pathophysiologically relevant antigenic landscape.[25] We proved glioblastoma and, most importantly, also medulloblastoma and meningioma to be compatible with HLA peptidome-centric target definition yielding both HLA class I- and II-restricted peptides in considerable numbers. Remarkably, even with scarce amounts of tissue being available (less than 100 mg), we achieved several thousand peptide identifications (e.g. GBM20R: 3,213 HLA class I ligands and 3,957 HLA class II-restricted peptides isolated from 35 mg of tissue) using an optimized protocol including consequent avoidance of sample loss during lysate preparation and HLA-IP as well as direct injection for LC-MS/MS. Future approaches may even encompass the analysis of circulating neoplastic cells as well as brain tumor-derived soluble HLA-peptide complexes and extracellular vesicles isolated from blood or CSF. Besides representing a substitute when patient-derived tumor tissue is not available or facilitating the investigation of subclones and their effusions, these may serve as liquid biopsy to diagnose and monitor disease or even as predictive biomarker for response to therapy and prognosis.[25-33] The repertoire of HLA-presented antigens, which have so far been exploited for immunotherapeutic approaches comprises viral antigens, non-mutated differentiation antigens, TAAs, and CTAs as well as neo-antigens arising from non-synonymous mutations.[25,34-41] Epitopes derived from oncogenic viruses or mutation-bearing proteins represent prime candidates for antigen-specific immunotherapies as they offer maximum tumor specificity and do not underlie central tolerance to self-antigens that has to be overcome for anti-tumor responses.[42] A viral etiology of common intracranial neoplasms has, however, remained under controversial discussion.[43-46] Although we succeeded in proving two neo-antigenic peptides to be naturally presented on

HLA molecules of glioblastoma cells for the first time, the verification of mutated peptides by LC-MS/MS has remained anecdotally – even in tumors with higher mutational burden as compared with brain tumors. In addition to extremely low confirmation rates of predicted neo-epitopes, around 99% of somatic mutations are not being recognized by T cells greatly supporting to target non-mutated tumor-associated and tumor-specific antigens by immunotherapy instead.[14,25,47-51]

During the course of this thesis, I analyzed 123 brain tumor specimens gaining a deep insight into the immunopeptidomic landscape of intracranial neoplasms. We identified a total of 485 candidate target antigens exploitable for immunotherapeutic efforts. Among these, two proved to be pan-brain tumor antigens (WNT5A and ESCO1) characterized by frequent and tumor-exclusive HLA presentation on glioblastomas, medulloblastomas, and meningiomas. This is in line with previous findings in hematological malignancies (multiple myeloma, acute myeloid, chronic myeloid, and chronic lymphocytic leukemia)[52] and strongly emphasizes that the repertoire of naturally presented tumor antigens is highly entity-specific encouraging and requiring immunopeptidomic studies of every tumor type individually. In turn, we found a large fraction of meningioma-associated antigens and peptides to be presented across all WHO grades as well as candidate targets for medulloblastoma to be shared by WNT-activated, non-WNT/non-SHH, and SHH-activated as well as by childhood and adult tumors. Moreover, the data presented herein demonstrate that established TAAs and CTAs – independent of whether these are published as pan-tumor or entity-specific antigens – are not recommended for immunotherapeutic purposes as being either broadly presented on both benign and neoplastic tissues or exhibiting infrequent albeit tumor-exclusive HLA presentation. While meningiomas are supplied by vascular branches of the external carotid artery,[53,54] the blood supply of glioblastomas and medulloblastomas arising within the brain parenchyma itself is accomplished by branches of the internal carotid artery.[55-57] Targeting such intra-axial lesions located beyond the blood-brain barrier (neurovascular unit) inevitably requires epitopes to be tumor-specific and not to be presented on other CNS cells.[58-60] Although our target discovery approach focused on tumor-exclusively presented antigens lacking CNS-associated expression, an intense validation process is required to warrant an excellent safety profile of selected antigens excluding on-target off-tumor effects within the CNS. This is particularly important as the benign HLA peptidome dataset subtracted during the course of comparative profiling comprising n=12 brains, n=11 cerebella, and n=1 spinal cord neither covers all HLA allotypes nor achieves 100% saturation of the benign CNS peptidome.

Apart from the aforementioned TAAs and TSAs there is another class of HLA ligands, which has so far not been exploited for immunotherapeutic efforts: cryptic peptides. These arise from antisense transcripts, novel unannotated open reading frames, non-coding regions (5' and 3' UTRs, introns, intergenic regions), non-canonical reading frames of protein-coding regions, or unconventional (proteasomal) splicing events. They have been proven to be immunogenic and estimated to contribute with 6.5-13% to the entirety of HLA-presented peptides.[25,61-64] Thus, the chance to identify tumor-associated cryptic peptides appears a lot more promising than the search for neo-antigenic HLA ligands arising from non-synonymous mutations.[65-68] However, cryptic peptides may also be neo-antigenic with the majority of somatic mutations affecting non-coding regions (Figure 21).[25,64,69] Data-independent acquisition and more accurate

quantification strategies – enabled by the use of retention time standard peptides presented herein – as well as the development and usage of technically complementary LC-MS/MS systems, innovative bioinformatic algorithms, and computational tools will foster deeper explorations of tumor and benign immunopeptidomes.[65-68] I am convinced that cryptic peptides will be subject of future target discovery approaches for cancer immunotherapy.[25,69,70] Addressing non-mutated cryptic peptides in intracranial neoplasias is possible by re-analyzing the acquired LC-MS/MS data using the *de novo* sequencing workflow Peptide-PRISM, which is currently being developed for immunopeptidomic datasets by the group of Andreas Schlosser at the University of Würzburg (unpublished data by Erhard *et al.*, manuscript submitted 2020). In addition, one could re-measure remaining peptide eluates employing more sophisticated protocols such as data-independent acquisition with spiked RT peptides possibly using multiple complementary LC-MS/MS systems to maximize the number of unique peptide identifications per sample. A comprehensive search for mutation-bearing cryptic peptides, however, requires whole genome sequencing.[64] This is possible for a large fraction of the glioblastoma cohort, while germline DNA essential for somatic variant calling is not available from medulloblastoma and meningioma patients.

As recently shown in the GAPVAC-101 trial, only one out of six WT antigens employed in APVAC2 was immunogenic. This strongly argues against the use of naturally presented tumor-associated peptides without prior evaluation of immunogenicity for vaccination approaches.[14] For a selected set of glioblastoma-, medulloblastoma-, and meningioma-associated peptides presented herein, these experiments are currently being performed by Dr. Konstantina Kapolou, Dr. med. Julia Velz, and Gioele Medici in the Laboratory for Molecular Neuro-Oncology at the University of Zürich. These include IFN-γ ELISpots, naïve T-cell primings, and killing assays – if possible, in an autologous setting using TILs or PBMCs and an established tumor cell line from the same patient. Immunological characterization of TILs isolated from fresh glioblastoma tissue further comprises mass cytometry and ultra-deep TCR sequencing. A research group from the Washington University School of Medicine headed by Robert Schreiber has recently postulated that effective anti-tumor immune responses induced by immunotherapies require one MHC class I- and class II-presented (neo-)antigen each. Of note, this is even the case for MHC class II-negative tumors. In an ideal scenario of peptide vaccination, long peptides harboring both an immunogenic CD8 and CD4 epitope are applied inducing T-cell activation at the site of disease in the TME.[71] However, this conclusion was drawn from a monoclonal sarcoma model, while naturally grown human tumors are constituted of several subclonal populations of neoplastic cells.[60,71,72] Against the background of intratumoral heterogeneity – which is particularly high in glioblastoma[60,72] – it is not possible to elicit clinically relevant anti-tumor responses by targeting a single antigen. Instead, a mix of targetable CD4 and CD8 epitopes is required.[2,71,73] Herein, we provide such a mixture of both HLA class I- and II-restricted candidate target peptides for glioblastoma, medulloblastoma, and meningioma immunotherapy.

Besides target specificity, adaptability and durability of anti-tumor immune responses are crucial for clinical efficacy.[74] To not only prime, but also amplify tumor-directed immunity, (peptide) vaccines should be administered several times, whereby the exact dosage schedule is subject of ongoing research.[73] Moreover, the use of an appropriate adjuvant and

administration route have a decisive impact on the induction of clinically relevant responses which have stayed out in 95% of cancer vaccine administrations.[73,75,76] The treatment of intra-axial neoplasms represents a prime example of needed co-stimulation as T cells have to be empowered to enter the neurovascular unit in addition.[58-60] Besides the application of vaccine adjuvants, great potential is seen for the development of combination therapies complementing each other and unleashing synergistic effects. Radiotherapy, for instance, disrupts the blood-brain barrier, induces local neuroinflammation and antigen release thus guiding immune effector cells to the site of disease.[77-80] For peptide vaccination in glioblastoma, it has recently been shown that concomitant medication such as dexamethasone shapes and can even impede the response to immunotherapy.[81] Likewise, checkpoint blockade is of different effectiveness when administered in an adjuvant or neoadjuvant setting. It has been suggested that the larger degree of antigenic diversity before surgery for recurrent glioblastoma favors the expansion of (polyclonal) anti-tumor T cells upon checkpoint inhibition.[82] Consequently, not only the composition, dosage, and timing of antigen-specific immunotherapeutic agents themselves, but also of concomitant medication and therapies have a decisive impact on patient outcomes and should therefore be subject of in-depth investigations.

In conclusion, there is tremendous potential for the implementation of cancer immunotherapy as additional pillar of multimodal therapies for intracranial neoplasias. Being convinced that non-mutated antigens will coin peptide-specific approaches with immunopeptidomics-based target definition in the short to medium term, we defined a large set of naturally, frequently, and tumor-exclusively presented antigens and peptides for glioblastoma, medulloblastoma, and meningioma immunotherapy. Apart from peptide vaccination, these may also guide the development of DC- and T cell-based strategies. With antigen-specific immunotherapy for intracranial neoplasms, we envision to replace radiotherapy in the treatment of childhood tumors thus reducing long-term sequelae, to offer therapeutic options for the management of disease recurrence, and to improve patient outcomes.

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# Appendix

## Abbreviations

| | |
|---|---|
| α-KG | α-ketoglutarate |
| AA | Amino acid |
| AAST | Anaplastic astrocytoma |
| AcN | Acetonitrile |
| ACTL8 / CT57 | Actin-like protein 8 |
| ADCC | Antibody-dependent cellular cytotoxicity |
| AIM2 | Absent in melanoma 2 |
| ALECSAT | Autologous Lymphoid Effector Cells Specific Against Tumour Cells |
| AMPA | α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid |
| ANR40 | Ankyrin repeat domain-containing protein 40 |
| APC | Antigen-presenting cell |
| ARF / p14$^{ARF}$ | Alternative open reading frame |
| ARG1 | Arginase-1 |
| ARID1A/2 | AT-rich interactive domain-containing protein 1A/2 |
| ASPM | Abnormal spindle-like microcephaly-associated protein |
| ATAT | Alphatubulin N-acetyltransferase 1 |
| ATG9B | Autophagy-related protein 9 |
| ATRT | Atypical teratoid rhabdoid tumor |
| AUC | Area under the curve |
| β$_2$m | β$_2$ microglobulin |
| B7-H3 / CD276 | B7 homolog 3 protein |
| BAP1 | BRCA-associated protein-1 |
| B-FABP | Brain-type fatty acid-binding protein |
| BH | Benjamini-Hochberg |
| BIRC5 | Baculoviral IAP repeat-containing protein 5 / survivin |
| BiTe | Bi-specific antibody for T-cell redirection and activation |
| BRCA2 | Breast cancer type 2 susceptibility protein |
| BRPF1 | Peregrin |
| BSG | Brain stem glioma |
| BTLA | B- and T-cell lymphocyte attenuator |
| CAR | Chimeric antigen receptor |
| CD | Cluster of differentiation |
| CD19t | Truncated CD19 |
| CDK2/4/6 | Cyclin-dependent kinase 4 / 6 |
| CDKN2A / p16$^{Ink4a}$ | Cyclin-dependent kinase inhibitor 2A |
| CDKN2B / p15$^{Ink4b}$ | Cyclin-dependent kinase 4 inhibitor 2B |
| CerS1 | Ceramide synthase 1 |
| CGE | Cobalt gray equivalent |
| CHAPS | [(3-cholamidopropyl) dimethyl-ammonio]-1-propanesulfonate |
| CI | Confidence interval |
| CID | Collision-induced dissociation |
| CLIP | Class II-associated invariant chain peptide |
| CLUS | Clusterin |
| c-Met | Met proto-oncogene / hepatocyte growth factor receptor |
| CNBr | Cyanogen bromide |
| CNS | Central nervous system |
| COSMIC | Catalogue Of Somatic Mutations In Cancer |
| CpG ODN | Deoxycytidyl-deoxyguanosin oligodeoxynucleotide |
| CR | Complete response |
| CREBBP | CREB-binding protein |
| CRUM1 | Protein crumbs homolog 1 |
| CSF | Cerebrospinal fluid |
| CSF1 | Colony stimulating factor 1 |
| CSI | Craniospinal irradiation |
| CSPG4 | Chondroitin sulfate proteoglycan 4 |
| CSPG7 | Chondroitin sulfate proteoglycan 7 / brevican core protein |
| CSRP2 | Cysteine and glycine-rich protein 2 |
| CTA | Cancer-testis antigen |

**Appendix:** Abbreviations

| | |
|---|---|
| CTCFL / CT27 | Transcriptional repressor CTCFL |
| CTL | Cytotoxic T lymphocyte |
| CTLA4 | Cytotoxic T-lymphocyte-associated antigen 4 |
| CTNNB1 | Catenin beta-1 |
| D2HG | D-2-hyroxyglutarate |
| DC | Dendritic cell |
| DCLK2 | Serine/threonine-protein kinase DCLK2 |
| DDA | Date-dependent acquisition |
| DDX3X | X-chromosomal ATP-dependent RNA helicase DEAD box protein 3 |
| DJC25 | DnaJ homolog subfamily C member 25 |
| DMSO | Dimethyl sulfoxide |
| DOCK7 | Dedicator of cytokinesis protein 7 |
| DP2.5 / APC | Deleted in polyposis 2.5 / adenomatous polyposis coli protein |
| DPBS | Dulbecco's phosphate-buffered saline |
| DRP-4 | Dihydropyrimidinase-related protein 4 |
| DTH | Delayed-type hypersensitivity |
| EAA4 | Excitatory amino acid transporter 4 |
| EBV | Epstein-Barr Virus |
| EFHC1 | EF-hand domain-containing protein 1 |
| EGFR | Epidermal growth factor receptor |
| EGFRt | Truncated EGFR |
| EGFRvIII | Epidermal growth factor receptor variant III |
| eIF4E | Eukaryotic translation initiation factor 4E |
| ELISpot | Enzyme-linked immunospot assay |
| ELOV2 | Elongation of very long chain fatty acids protein 2 |
| EMT | Epithelial-to-mesenchymal transition |
| EphA2 | Ephrin type-A receptor 2 |
| ER | Endoplasmic reticulum |
| ERAAP | ER aminopeptidase associated with antigen processing |
| EVS | Empirical variant scoring |
| F120C | Constitutive coactivator of PPAR-gamma-like protein 2 |
| FA | Formic acid |
| FADS2 | Acyl-CoA 6-desaturase |
| Fas | Fas cell surface death receptor |
| FasL | Fas ligand |
| FBXW7 | F-box/WD repeat-containing protein 7 |
| fc | Fold change |
| FDA | U.S. Food and Drug Administration |
| FDR | False discovery rate |
| FLT3 | FMS-like tyrosine kinase 3 |
| FPRP | Prostaglandin F2 receptor negative regulator |
| FTMS | Fourier Transform Mass Spectrometry (refers to Orbitrap detection) |
| GABA | Gamma-aminobutyric acid |
| GAGE-1 / CT4.1 | G antigen 1 |
| GAPVAC | The Glioma Actively Personalized VAccine Consortium |
| Gb | Giga base |
| GBM | Glioblastoma |
| G-CIMP | Glioma CpG island methylator phenotype |
| GITR | Glucocorticoid-induced tumor necrosis factor receptor |
| GluK3 | Ionotropic glutamate receptor kainate 3 |
| GluR4 | Glutamate receptor 4 |
| GM-CSF | Granulocyte-macrophage colony-stimulating factor |
| GMP | Good manufacturing practice |
| GO | Gene Ontology |
| gp100 | Melanocytes lineage-specific antigen gp100 |
| GPC1/2 | Glypican-1/2 |
| GPM6B | Neuronal membrane glycoprotein M6-b |
| GSE1 | Genetic suppressor element 1 |
| GTEx | Genotype-Tissue Expression database |
| HCD | Higher-energy induced dissociation |
| HCMV | Human cytomegalovirus |
| HEAT1 | HEAT repeat-containing protein 1 |

| | |
|---|---|
| HEPACAM | Hepatocyte cell adhesion molecule |
| HER2 | Receptor tyrosine-protein kinase erbB-2 |
| HFE | Human hemochromatosis protein |
| HHV | Human herpesvirus |
| HLA | Human leukocyte antigen |
| HLA-IP | HLA-immunoprecipitation |
| HO-1 | Heme oxygenase-1 |
| HPLC | High-performance liquid chromatography |
| HS2ST | Heparan sulfate 2-O-sulfotransferase 1 |
| HyTK | Hygromycin phosphotransferase-herpes simplex virus 1 thymidine kinase |
| i.d. | Intradermal |
| i.n. | Intranodal |
| i.t. | Intratumoral |
| i.v. | Intravenous |
| IDH1/2 | Isocitrate dehydrogenase 1/2 |
| IDH$^{mut}$ | Mutated IDH1/2 genes |
| IDH$^{WT}$ | Non-mutated / wild-type IDH1/2 genes |
| IDO1 | Indoleamine 2,3-dioxygenase-1 |
| IEDB | Immune Epitope Database |
| IF2BP3 | Insulin-like growth factor 2 mRNA binding protein 3 |
| IFN | Interferon |
| IFN-α | Interferon alpha / Type I interferon |
| Ii | Invariant chain |
| IL13Rα2 | IL-13 receptor alpha 2 |
| IL-2/10/12/15/21 | Interleukin 2/10/12/15/21 |
| ILT | Inhibitory Ig-like transcript |
| ImmTAC | Immune-mobilizing monoclonal T-cell receptors against cancer |
| Indel | Insertion or deletion |
| iRT | Indexed retention time |
| ITA7 | Integrin alpha-7 |
| JAK2 | Janus kinase 2 |
| JY | B-lymphoblastoid cell line |
| KANSL1 | KAT8 regulatory NSL complex subunit 1 |
| KBTBD4 | Kelch repeat and BTB domain-containing protein 4 |
| KCJ10 | ATP-sensitive inward rectifier potassium channel 10 |
| KDM6A | Lysine-specific demethylase 6A |
| KLF4 | Krueppel-like factor 4 |
| KMT2C/D | Histone-lysine N-methyltransferase 2C/2D |
| LAG-3 | Lymphocyte activation gene-3 |
| LC-MS/MS | (Reversed-phase) Liquid chromatography coupled with tandem mass spectrometry |
| LFQ | Label-free quantitation |
| LMP | Low molecular weight protein |
| LTQ | Linear trap quadrupole |
| LZTS1 | Leucine zipper putative tumor suppressor 1 |
| m/z | Mass-to-charge ratio |
| MAGE-A1 / CT1.1 | Melanoma-associated antigen 1 |
| MAGE-A3 / CT1.3 | Melanoma-associated antigen 3 |
| MAGE-A4 / CT1.4 | Melanoma-associated antigen 4 |
| MAGE-A6 / CT1.6 | Melanoma-associated antigen 6 |
| MAGE-C1 / CT7.1 | Melanoma-associated antigen C1 |
| MAGE-C2 / CT10 | Melanoma-associated antigen C2 |
| MAGE-F1 | Melanoma-associated antigen F1 |
| MAGI2 | Membrane-associated guanylate kinase inverted 2 |
| MAPK | Mitogen-activated protein kinase |
| MB | Medulloblastoma |
| MDM2 | Mouse double minute 2 homolog |
| MDSC | Myeloid-derived suppressor cells |
| MGMT | $O^6$-methylguanine-DNA methyltransferase |
| MHC | Major histocompatibility complex |
| MIC | MHC class I polypeptide-related sequence protein |
| MIC25 | MICOS complex subunit MIC25 |
| MIIC | Multivesicular late endosomal-lysosomal MHC class II antigen-processing compartment |

| | |
|---|---|
| MLH1 | MutL homolog 1 |
| MMR | Mismatch repair |
| MNG | Meningioma |
| MS | Mass spectrometry |
| MS/MS | Tandem mass spectrometry |
| MSH2/6 | MutS homolog 2/6 |
| MTSS2 | Protein MTSS 2 |
| n.a. | Not available |
| n.d. | Not determined |
| NF1/2 | Neurofibromin-1/-2 |
| NF-κB | Nuclear factor kappa-light-chain-enhancer of activated B cells |
| NIC | Nanoscale immunoconjugate |
| NK cell | Natural killer cell |
| NLGNX/Y | X-/Y-linked neuroligin-4 |
| NMRL1 | NmrA-like family domain-containing protein 1 |
| NRCAM | Neuronal cell adhesion molecule |
| NY-ESO-1 / CT6.1 | New York esophageal squamous cell carcinoma-1 |
| OIP5 / CT86 | Opa-interacting protein 5 / protein Mis18-beta |
| OLIG2 | Oligodendrocyte transcription factor 2 |
| OR | Odds ratio |
| ORML1 | ORM1-like protein 1 |
| OS | Overall survival |
| P4HTM | Transmembrane prolyl 4hydroxylase |
| PAI1 | Plasminogen activator inhibitor 1 |
| PALB2 | Partner and localizer of BRCA2 |
| PAX6 | Paired box protein Pax6 |
| PBMCs | Peripheral blood mononuclear cell |
| PBS | Phosphate-buffered saline |
| PCDGM | Protocadherin gamma-C5 |
| PCX3 | Pecanex-like protein 3 |
| PD | Progressive disease |
| PD-1 | Programmed cell death protein 1 |
| PDGFR / PDGFRα | Platelet-derived growth factor receptor / alpha |
| PD-L1 / PD-L2 | Programmed cell death ligand 1 / 2 |
| PEG | Polyethylene glycol |
| PFS | Progression-free survival |
| pfu | Plaque-forming units |
| PI3K | Phosphatidylinositol-3-OH kinase |
| PJA2 | E3 ubiquitin-protein ligase Praja-2 |
| PKHA4 | Pleckstrin homology domain-containing family A member 4 |
| PLC | Peptide-loading complex |
| PMS2 | Postmeiotic segregation increased 2 |
| PNET | Primitive neuroectodermal tumor |
| POLR2A | RNA polymerase II subunit A |
| Poly-ICLC | Polyinosinic-polycytidylic acid stabilized by polylysine and carboxymethylcellulose |
| PR | Partial response |
| PRAME | Melanoma antigen preferentially expressed in tumors |
| PRiME | PEP-CMV in Recurrent Medulloblastoma/Malignant Glioma |
| PROCAL | ProteomeTools Calibration Standard |
| PSM | Peptide spectrum match |
| PTCH1 | Protein patched homolog 1 |
| PTEN | Phosphatase and tensin homolog |
| PTM | Post-translational modification |
| PTPRZ | Receptor-type tyrosine-protein phosphatase zeta |
| RAD54B | DNA repair and recombination protein RAD54B |
| RasGAP | Ras GTPase activating protein |
| RFTN2 | Raftlin-2 |
| RIN1 | Ras and Rab interactor 1 |
| RL7A | 60S ribosomal protein L7a |
| RPKM | Reads per kilobase per million mapped reads |
| rpm | Revolutions per minute |
| RR | Radiological response |

**Appendix:** Abbreviations

| | |
|---|---|
| RT | Retention time |
| S | Svedberg unit |
| s.c. | Subcutaneous |
| S/N | Signal-to-noise ratio |
| SD | Stable disease |
| SE6L1 | Seizure 6-like protein |
| SH2 | Src homology 2 |
| SHH | Sonic Hedgehog |
| SIA8E | Alpha-2,8-sialyltransferase 8E |
| Siglec-15 | Sialic acid-binding immunoglobulin-like lectin 15 |
| siRNA | Small interfering RNA |
| SMARCA4 | ATP-dependent helicase SMARCA4 |
| SMARCB1 / SMARCE1 | SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B / E member 1 |
| SMC4 | Structural maintenance of chromosomes protein 4 |
| SMO | Smoothened homolog |
| SNV | Single nucleotide variant |
| SO6A1 / CT48 | Solute carrier organic anion transporter family member 6A1 |
| SPA17 / CT22 | Sperm autoangiogenic protein 17 |
| SUFU | Suppressor of fused homolog |
| SV40 | Simian virus 40 |
| SYCP1 / CT8 | Synaptonemal complex protein 1 |
| T255A | Transmembrane protein 255A |
| TAA | Tumor-associated antigen |
| TACC3 | Transforming acidic coiled-coil-containing protein 3 |
| TAP | Transporter associated with antigen processing |
| TCF4 | Transcription factor 4 |
| TCR | T-cell receptor |
| TERT | Telomerase reverse transcriptase |
| TET2 | Tet methylcytosine dioxygenase 2 |
| TFA | Trifluoroacetic acid |
| TGFB2 | Transforming growth factor beta-2 proprotein |
| TGF-β | Transforming growth factor β |
| Th cell | CD4$^+$ T helper cell |
| Th1 / Th2 | Type 1 / Type 2 CD4$^+$ T helper cell |
| TIGIT | T-cell immunoglobulin and ITIM domain |
| TILs | Tumor-infiltrating lymphocytes |
| TIM-3 | T-cell immunoglobulin and mucin-domain containing-3 |
| TLR1/2/3/7/8/9 | Toll-like receptor 1/2/3/7/8/9 |
| TM231 | Transmembrane protein 231 |
| TME | Tumor microenvironment |
| TMZ | Temozolomide |
| TN-C | Tenascin C |
| TNF / TNF-α | Tumor necrosis factor / alpha |
| TP53 | Tumor suppressor p53 |
| TRAF7 | TNF receptor-associated factor 7 |
| TRAIL | Tumor necrosis factor-related apoptosis inducing ligand |
| TRAILR | TRAIL receptor |
| Treg | CD4$^+$CD25$^+$ regulatory T cell |
| TRP2 | Tyrosinase-related protein 2 |
| TSA | Tumor-specific antigen |
| TTF | Tumor treating fields |
| TTP | Time to progression |
| TTRNA | Total tumor RNA |
| T-Vec | Talimogene laherparepvec |
| UBP11 | Ubiquitin carboxyl-terminal hydrolase 11 |
| US | United States |
| UTR | Untranslated region |
| VAF | Variant allele frequency |
| VEGF | Vascular endothelial growth factor |
| VEGFR | Vascular endothelial growth factor receptor |
| VISTA | V-domain immunoglobulin suppressor of T-cell activation |
| VP13B | Vacuolar protein sorting-associated protein 13B |

**Appendix:** Abbreviations

| | |
|---|---|
| WHO | World Health Organization |
| Wnt | Wingless and Int-1 |
| WT | Wild-type |
| WT1 | Wilms' tumor gene 1 |
| XAGE3 / CT12.3 | X antigen family member 3 |
| xALT | *Ex vivo* expanded Autologous Lymphocyte Transfer |
| ZIC1 | Zinc finger protein ZIC 1 |
| ZMYM3 | Zinc finger MYM-type protein 3 |
| ZNF3 | Zinc finger protein 3 |

# Supplementary Figures and Tables

## Supplement of CHAPTER 2

**Supplementary Table 1. HLA class I allotype and allele frequencies in the glioblastoma collective comprising n=40 patients.** The top three ranking HLA-A, -B, and -C allotypes within the study cohort are marked in bold.

| HLA allotype | Positive patients | Allele frequency | HLA allotype | Positive patients | Allele frequency |
|---|---|---|---|---|---|
| **A*01:01** | **25%** | 14% | B*41:01 | 3% | 1% |
| **A*02:01** | **63%** | 40% | **B*44:02** | **28%** | 14% |
| A*02:05 | 8% | 4% | B*44:03 | 13% | 6% |
| **A*03:01** | **28%** | 15% | B*44:05 | 3% | 1% |
| A*11:01 | 13% | 6% | B*47:01 | 3% | 1% |
| A*23:01 | 3% | 1% | B*49:01 | 3% | 1% |
| A*24:02 | 13% | 8% | **B*51:01** | **15%** | 8% |
| A*29:02 | 5% | 3% | B*51:26 | 3% | 1% |
| A*31:01 | 8% | 4% | B*55:01 | 3% | 1% |
| A*32:01 | 3% | 1% | B*56:01 | 3% | 1% |
| A*33:01 | 5% | 3% | B*57:01 | 10% | 5% |
| A*33:05 | 3% | 1% | B*58:01 | 3% | 1% |
| A*68:01 | 3% | 1% | C*01:02 | 20% | 10% |
| **B*07:02** | **33%** | 19% | C*02:02 | 10% | 5% |
| **B*08:01** | **15%** | 8% | C*03:03 | 5% | 3% |
| B*13:02 | 8% | 4% | C*03:04 | 5% | 3% |
| B*14:02 | 8% | 4% | C*04:01 | 18% | 9% |
| B*15:01 | 8% | 4% | C*05:01 | 18% | 9% |
| B*15:03 | 3% | 1% | **C*06:02** | **20%** | 10% |
| B*15:17 | 3% | 1% | **C*07:01** | **30%** | 15% |
| B*18:01 | 5% | 3% | **C*07:02** | **30%** | 18% |
| B*27:05 | 8% | 4% | C*07:04 | 10% | 5% |
| B*35:01 | 5% | 3% | C*08:02 | 8% | 4% |
| B*35:02 | 3% | 1% | C*12:03 | 10% | 5% |
| B*35:03 | 8% | 4% | C*15:02 | 5% | 3% |
| B*39:10 | 3% | 1% | C*15:06 | 3% | 1% |
| B*40:01 | 3% | 1% | C*16:01 | 5% | 3% |
| B*40:02 | 3% | 1% | | | |

**frequency of positive glioblastoma patients [%]**

0　　　　　　　　　　　32　　　　　　　　　　　64
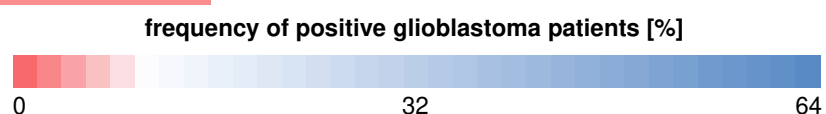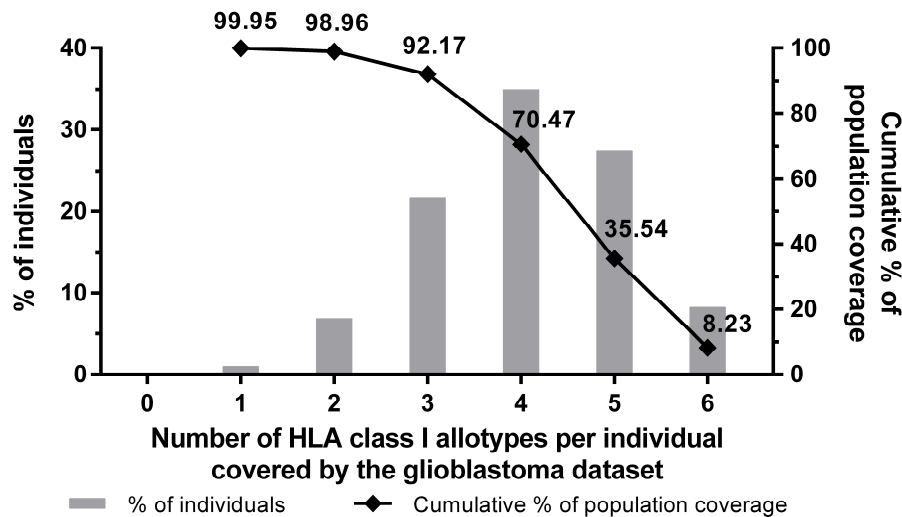
**Supplementary Figure 1. HLA class I allotype population coverage.** Using the population coverage tool provided by the IEDB Analysis Resource, the world population coverage of the 55 distinct HLA-A, -B, and -C allotypes was calculated. The percentage of individuals positive for a specific number of HLA class I allotypes (max. of 6) is indicated by bar charts (associated with the left y-axis). The line diagram (associated with the right y-axis) shows the cumulative percentage of population coverage. The HLA class I allotypes of the glioblastoma cohort cover 99.95% of the world population (first diamond on line diagram counted from the left) meaning that only 0.05% of all individuals are negative for all HLA-A, -B, and -C allotypes included in the present study (first bar counted from the left). 92.17% of all individuals are positive for at least three HLA class I allotypes (third diamond on line diagram counted from the left).
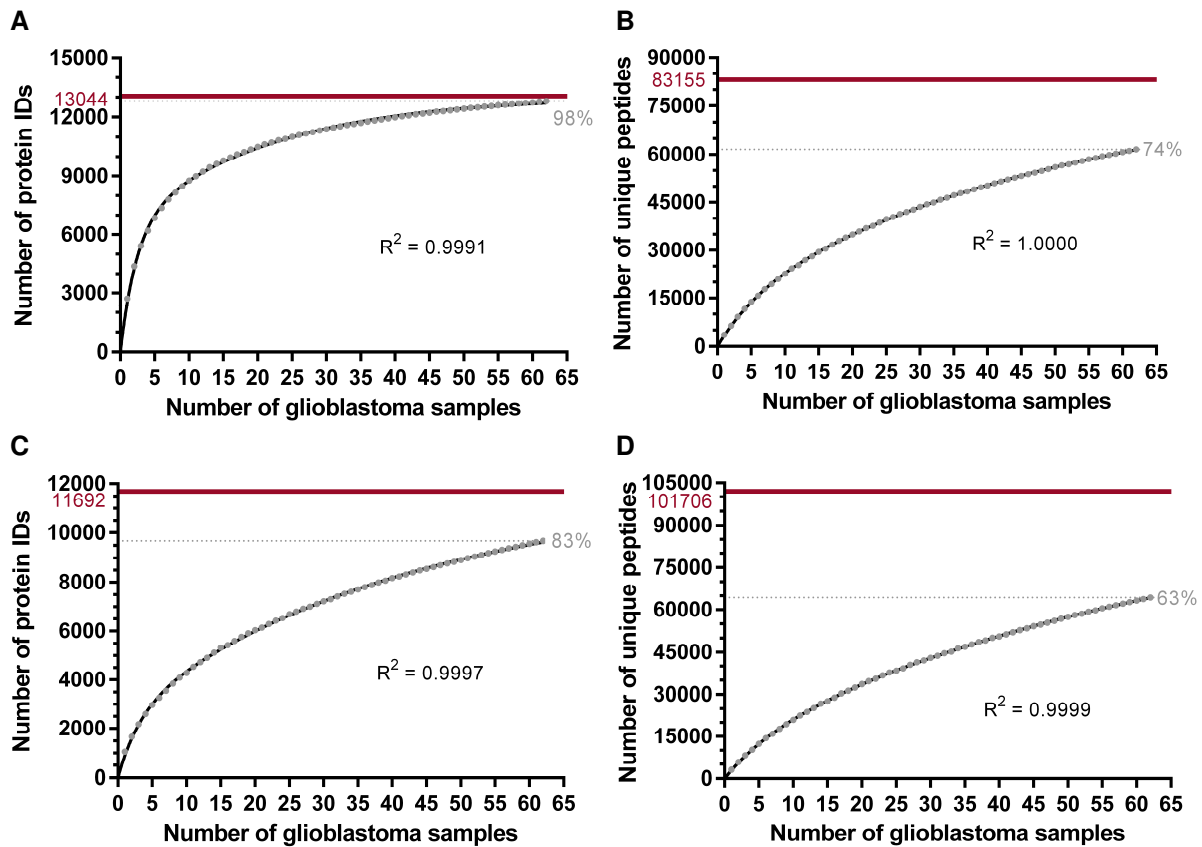


**Supplementary Figure 2. Saturation analysis for the identification of HLA-presented antigens and peptides in glioblastoma tissue.** For each source count, the mean number of **(A) HLA class I-presented antigens**, **(B) HLA class I ligands**, **(C) HLA class II-presented antigens**, and **(D) HLA class II-restricted peptides** was calculated by 1,000 random samplings. Using non-linear regression,

exponential functions with a forced y-intercept of 0 (internal data created by Daniel Kowalewski at the Department of Immunology, University of Tübingen indicate that subjecting cell-free lysis buffer to HLA-IP does not result in peptide identifications) were fitted. For all models, the goodness of fit was in the uppermost range ($R^2 = 0.9991$, $R^2 = 1.0000$, $R^2 = 0.9997$ and $R^2 = 0.9999$). Based on these curves, the maximum attainable number of distinct source proteins and peptides was estimated (highlighted as solid lines). With the available number of 62 glioblastoma samples, 98% or 83% of the estimated maximum attainable amount of distinct HLA class I- and II-presented proteins as well as 74% or 63% of the estimated maximum attainable amount of distinct HLA class I- or II-restricted peptides had been identified, respectively.

**Supplementary Table 2. Glioblastoma-associated HLA class I- and II-presented antigens identified on both primary and recurrent tumors of at least five different patients.** GTEx profiles were assessed from all available datasets excepting EBV-transformed lymphocytes and cultured fibroblasts. Glioblastoma-exclusive antigens with a brain-specific expression profile > 10 TPM were not reported (HLA class I: n=29; HLA class II: n=6) as candidate targets. Color codes were defined as follows: ■ < 10 TPM in any tissue, ♦ > 10 TPM in testes and < 10 TPM in other tissues (CTA-like expression profile), ■ 10-20 TPM in any tissue, ■ 20-30 TPM in any tissue, ■ > 30 TPM in any tissue. The number of positive tumors other than glioblastoma was based on n=824 HLA class I and n=585 HLA class II peptidome datasets. HLA restrictions not passing manual assessment as quality control as well as one highly repetitive sequence annotated to IGFBP2 are indicated in italic. These peptides were excluded from downstream analyses such as calculation of peptides matching per patient worldwide. HLA class II-presented proteins neither identified with peptides exceeding a length of twelve AA nor with different sequences across patients were not considered for this listing of candidate target antigens. NOP16, ESCO1, MFHAS1, TRIM58, CDC26, ONECUT1, AFTPH, and RBFA were detected with only one HLA class II peptide sequence across all patients. Frequencies of positive tumors are given separately for n=38 primary (P) and n=24 recurrent (R) glioblastomas.

| Antigen | Frequency of positive tumors | Peptide sequence | HLA restriction | UniProt accession GTEx profile | Positive non-GBM tumors |
|---|---|---|---|---|---|
| **Glioblastoma-associated HLA class I antigens** | | | | | |
| Protein TANC2 (TANC2) 1 peptide multi-maps to non-GBM-exclusive TANC1 (Q9C0D5) | 50% P / 38% R | | | Q9HCD6 ■ | 62 |
| | GBM3P | ALMDKEGLTAL | A*02:01 | | |
| | | DEHIFQAI | B*18:01 | | |
| | GBM4P | APYRPPDISL | B*07:02 | | |
| | GBM4R | APYRPPDISL | B*07:02 | | |
| | GBM5P | GIIATLTSY | B*15:01 | | |
| | | YAGESSKEL | C*03:03 | | |
| | | YSENVERTKY | A*01:01 | | |
| | GBM5R | GIIATLTSY | B*15:01 | | |
| | GBM6P | AIISRLVAL | A*02:05 | | |
| | | LVPEFVHNV | A*02:05 | | |
| | | MKFPTQSSF | B*15:03 | | |
| | GBM7P | APYRPPDISL | B*07:02 | | |
| | GBM8P | TTESVFVGR | A*68:01 | | |
| | GBM9P | AIISRLVAL | B*08:01 | | |
| | | DLQAYILHR | A*33:05 | | |
| | GBM10R | ALMDKEGLTAL | A*02:01 | | |
| | | APYRPPDISL | B*07:02 | | |
| | | RPSQGLPVI | B*07:02 | | |
| | GBM11P | ALMDKEGLTAL | A*02:01 | | |
| | | LVPEFVHNV | A*02:01; A*02:05 | | |
| | GBM12R | GIIATLTSY | B*15:01 | | |
| | GBM13P | AIISRLVAL | A*02:01; B*08:01 | | |
| | GBM16P | AIISRLVAL | B*08:01 | | |
| | | APYRPPDISL | B*07:02 | | |
| | | RPSQGLPVI | B*07:02 | | |
| | GBM16R | AIISRLVAL | B*08:01 | | |
| | | APYRPPDISL | B*07:02 | | |
| | GBM17R | NEAEFHKPDY | B*44:03 | | |
| | | SESGLTPLGY | B*44:03 | | |
| | GBM18R | AIISRLVAL | B*08:01 | | |
| | | APYRPPDISL | B*07:02 | | |
| | GBM22P | SEIQNNISL | B*40:01 | | |
| | GBM22R | SEIQNNISL | B*40:01 | | |
| | ZH613 | LVPEFVHNV | A*02:05 | | |

| | | | | | |
|---|---|---|---|---|---|
| | ZH617 | AIISRLVAL | A*02:01; B*08:01 | | |
| | | YSENVERTKY | A*01:01 | | |
| | ZH631 | GQQQGVFKK | A*11:01 | | |
| | | KMIGKFPSW | A*32:01 | | |
| | ZH654 | RTLPVAQAY | B*57:01 | | |
| | | YSENVERTKY | A*01:01 | | |
| | ZH678 | GQQQGVFKK | A*11:01 | | |
| | | NEAEFHKPDY | B*44:03 | | |
| | ZH681 | ALMDKEGLTAL | A*02:01 | | |
| | | RTLPVAQAY | B*57:01 | | |
| | ZH753 | AIISRLVAL | A*02:01; B*08:01 | | |
| | ZH761 | NEAEFHKPDY | B*44:02 | | |
| | ZH802 | DLQAYILHR | A*33:01 | | |
| Fatty acid 2-hydroxylase (FA2H) | 32% P / 63% R | | | Q7L5A8 ■ | 13 |
| | GBM2P | APAPPPAASF | B*07:02 | | |
| | GBM2R | APAPPPAASF | B*07:02 | | |
| | | RLFTSFTTEY | B*15:01 | | |
| | GBM4P | APAPPPAASF | B*07:02 | | |
| | GBM4R | APAPPPAASF | B*07:02 | | |
| | | APFDGSRLVF | B*07:02 | | |
| | GBM5P | HMKPPSDSY | B*15:01 | | |
| | GBM5R | HMKPPSDSY | B*15:01 | | |
| | | RLFTSFTTEY | B*15:01 | | |
| | GBM6R | APAPPPAASF | B*35:01 | | |
| | | APFDGSRLVF | B*35:01 | | |
| | | FAQGNVRLF | C*12:03 | | |
| | | LPEAVGGTVF | B*35:01 | | |
| | GBM7P | APAPPPAASF | B*07:02 | | |
| | | FAQGNVRLF | C*02:02 | | |
| | GBM7R | APAPPPAASF | B*07:02 | | |
| | GBM10R | APAPPPAASF | B*07:02 | | |
| | | APFDGSRLVF | B*07:02 | | |
| | GBM11R | APAPPPAASF | B*07:02 | | |
| | GBM12R | APAPPPAASF | B*35:01 | | |
| | | APFDGSRLVF | B*35:01 | | |
| | | HMKPPSDSY | B*15:01 | | |
| | | LPEAVGGTVF | B*35:01 | | |
| | | SPHKGSYLY | B*35:01 | | |
| | GBM16P | APAPPPAASF | B*07:02 | | |
| | GBM16R | APAPPPAASF | B*07:02 | | |
| | | APFDGSRLVF | B*07:02 | | |
| | GBM17R | RTFAQGNVRLF | B*57:01 | | |
| | GBM18R | APAPPPAASF | B*07:02 | | |
| | GBM22R | FAQGNVRLF | C*12:03 | | |
| | GBM23R | LPEAVGGTVF | B*35:02; *C*04:01* | | |
| | ZH613 | RTFAQGNVR | A*31:01 | | |
| | ZH654 | RTFAQGNVR | A*31:01 | | |
| | | RTFAQGNVRLF | B*57:01 | | |
| | ZH681 | APAPPPAASF | B*07:02 | | |
| | | RTFAQGNVRLF | B*57:01 | | |
| | ZH753 | FAHQKSGF | B*08:01; C*15:02 | | |
| | ZH757 | APAPPPAASF | B*07:02 | | |
| | ZH761 | FAQGNVRLF | C*16:01 | | |
| | ZH784 | APAPPPAASF | B*07:02 | | |
| | ZH791 | APAPPPAASF | B*07:02 | | |
| | ZH802 | RTFAQGNVR | A*31:01 | | |
| Bestrophin-1 (BEST1) | 24% P / 54% R | | | O76090 ■ | 5 |
| | GBM2P | SPTNIHTTL | B*07:02 | | |
| | GBM2R | SPTNIHTTL | B*07:02 | | |
| | | TQVVTVAVY | B*15:01 | | |
| | GBM4P | SPTNIHTTL | B*07:02 | | |
| | GBM4R | SPTNIHTTL | B*07:02 | | |
| | GBM5R | TQVVTVAVY | B*15:01 | | |
| | GBM7P | SPTNIHTTL | B*07:02 | | |
| | GBM7R | SPTNIHTTL | B*07:02 | | |
| | GBM9R | DAHAGIIGR | A*33:05 | | |
| | GBM10P | SPTNIHTTL | B*07:02 | | |
| | GBM10R | SPTNIHTTL | B*07:02 | | |
| | GBM11R | SPTNIHTTL | B*07:02 | | |
| | GBM14R | DAHAGIIGR | A*33:01 | | |
| | GBM16P | SPTNIHTTL | B*07:02 | | |
| | GBM16R | SPTNIHTTL | B*07:02 | | |
| | GBM18R | SPTNIHTTL | B*07:02 | | |
| | GBM22R | SPTNIHTTL | B*35:03 | | |
| | ZH616 | SPTNIHTTL | B*07:02 | | |
| | ZH681 | SPTNIHTTL | B*07:02 | | |

| | | | | | |
|---|---|---|---|---|---|
| | ZH753 | ALMEHPEVSQV | A*02:01 | | |
| | ZH757 | ALMEHPEVSQV | A*02:01 | | |
| | | SPTNIHTTL | B*07:02 | | |
| | ZH784 | SPTNIHTTL | B*07:02 | | |
| | ZH802 | DAHAGIIGR | A*33:01 | | |
| | | RYANLGNVLILR | A*31:01 | | |
| Oligodendrocyte transcription factor 3 (OLIG3) Peptides multi-map to GBM-exclusive OLIG2 (Q13516) with brain-associated expression | 34% P / 33% R | | | Q7RTU3 ■ | 3 |
| | GBM2R | MPYAHGPSV | B*07:02 | | |
| | GBM4P | MPYAHGPSV | B*07:02 | | |
| | GBM4R | MPYAHGPSV | B*07:02 | | |
| | GBM6R | MPYAHGPSV | B*35:01 | | |
| | GBM7P | MPYAHGPSV | B*07:02; B*56:01 | | |
| | GBM7R | MPYAHGPSV | B*07:02; B*56:01 | | |
| | GBM8P | EVMPYAHGPSVR | A*68:01 | | |
| | GBM10R | MPYAHGPSV | B*07:02 | | |
| | GBM16P | MPYAHGPSV | B*07:02 | | |
| | GBM16R | MPYAHGPSV | B*07:02 | | |
| | GBM18R | MPYAHGPSV | B*07:02 | | |
| | ZH613 | MPYAHGPSV | B*51:01 | | |
| | ZH631 | MPYAHGPSV | B*51:01 | | |
| | ZH645 | MPYAHGPSV | B*51:01 | | |
| | ZH654 | MPYAHGPSV | B*51:01 | | |
| | ZH678 | MPYAHGPSV | B*51:01 | | |
| | ZH681 | MPYAHGPSV | B*07:02 | | |
| | ZH753 | MPYAHGPSV | B*51:01 | | |
| | ZH757 | MPYAHGPSV | B*07:02 | | |
| | ZH761 | LARNYILML | C*16:01 | | |
| | | MPYAHGPSV | B*51:26 | | |
| | ZH829 | MPYAHGPSV | B*55:01 | | |
| Transcription factor SOX-2/21 (SOX2/21) 4 peptides multi-map to GBM-exclusive SOX1 (O00570) and to non-GBM-exclusive SOX14 (O95416) | 34%/26% P 29%/21% R | | | P48431 ■ Q9Y651 ■ | 5 3 |
| | GBM14R | DEAKRLRAL (SOX1/2) | B*14:02 | | |
| | GBM14R | KMAQENPKM (SOX1/2/14/21) | A*02:01 | | |
| | GBM20R | SEISKRLGAEW (SOX1/2/14/21) | B*44:02 | | |
| | ZH654 | ISKRLGAEW (SOX1/2/14/21) | B*57:01 | | |
| | ZH681 | ISKRLGAEW (SOX1/2/14/21) | B*57:01 | | |
| | | KMAQENPKM (SOX1/2/14/21) | A*02:01 | | |
| | GBM4P | YPQHPGLNA (SOX2) | B*07:02 | | |
| | GBM4R | YPQHPGLNA (SOX2) | B*07:02 | | |
| | GBM5P | AQMQPMHRY (SOX2) | B*15:01 | | |
| | GBM6P | AQMQPMHRY (SOX2) | B*15:03 | | |
| | GBM6R | AQMQPMHRY (SOX2) | B*15:03 | | |
| | GBM7P | HPGLNAHGA (SOX2) | B*56:01 | | |
| | | QPMHRYDVSA (SOX2) | B*07:02; B*56:01 | | |
| | | YPQHPGLNA (SOX2) | B*07:02; B*56:01 | | |
| | GBM7R | QPMHRYDVSA (SOX2) | B*07:02; B*56:01 | | |
| | | YPQHPGLNA (SOX2) | B*07:02; B*56:01 | | |
| | ZH616 | YPQHPGLNA (SOX2) | B*07:02 | | |
| | ZH681 | YPQHPGLNA (SOX2) | B*07:02 | | |
| | ZH757 | YPQHPGLNA (SOX2) | B*07:02 | | |
| | ZH829 | YPQHPGLNA (SOX2) | B*55:01 | | |
| Uncharacterized protein C2orf72 (C2orf72) | 29% P / 29% R | | | A6NCS6 ■ | 8 |
| | GBM2R | RPAEPPFQAL | B*07:02 | | |
| | GBM4P | IRSPLVFVL | C*07:02 | | |
| | | RPAEPPFQAL | B*07:02 | | |
| | GBM4R | IRSPLVFVL | C*07:02 | | |
| | | RPAEPPFQAL | B*07:02 | | |
| | GBM7P | RPAEPPFQAL | B*07:02 | | |
| | GBM7R | RPAEPPFQAL | B*07:02 | | |
| | GBM10R | RPAEPPFQAL | B*07:02 | | |
| | GBM11P | RPAEPPFQAL | B*07:02 | | |
| | GBM13P | IRSPLVFVL | C*06:02; C*07:01 | | |
| | GBM16P | IRSPLVFVL | C*07:01; C*07:02 | | |
| | | RPAEPPFQAL | B*07:02 | | |
| | GBM16R | IRSPLVFVL | C*07:01; C*07:02 | | |
| | | RPAEPPFQAL | B*07:02 | | |
| | GBM18P | RPAEPPFQAL | B*07:02 | | |
| | GBM18R | IRSPLVFVL | C*07:01; C*07:02 | | |
| | | RPAEPPFQAL | B*07:02 | | |
| | ZH616 | RPAEPPFQAL | B*07:02 | | |

| | ZH654 | IRSPLVFVL | C*06:02 | | |
| | ZH681 | RPAEPPFQAL | B*07:02 | | |
| | ZH720 | RPAEPPFQAL | B*39:10 | | |
| | ZH757 | RPAEPPFQAL | B*07:02 | | |
| | ZH784 | RPAEPPFQAL | B*07:02 | | |
| Transmembrane protein 255A (TMEM255A) | <u>34% P / 17% R</u> | | | Q5JRV8 ■ | 6 |
| | GBM3P | YAHPQVASY | C*02:02 | | |
| | GBM4P | YYPGVILGF | C*07:02 | | |
| | GBM6P | ATILNIVGL | A*02:05; C*12:03 | | |
| | | YAHPQVASY | C*12:03 | | |
| | GBM7P | YAHPQVASY | C*02:02 | | |
| | GBM9P | DLKPLYANR | A*33:05 | | |
| | GBM14R | DLKPLYANR | A*33:01 | | |
| | GBM16P | YYPGVILGF | C*07:02 | | |
| | GBM16R | YYPGVILGF | C*07:02 | | |
| | GBM19P | YYPGVILGF | A*24:02 | | |
| | GBM22P | YYPGVILGF | A*24:02 | | |
| | GBM22R | YYPGVILGF | A*24:02 | | |
| | GBM23P | YYPGVILGF | A*23:01 | | |
| | GBM23R | YYPGVILGF | A*23:01 | | |
| | ZH613 | ATILNIVGL | A*02:05; C*07:01 | | |
| | ZH750 | YYPGVILGF | A*24:02 | | |
| | ZH802 | DLKPLYANR | A*33:01 | | |
| | ZH810 | YYPGVILGF | A*24:02 | | |
| G patch domain-containing protein 11 (GPATCH11) | <u>34% P / 17% R</u> | | | Q8N954 ■ | 15 |
| | GBM3P | YMSDSFINV | A*02:01 | | |
| | GBM6P | YMSDSFINV | A*02:05 | | |
| | GBM10R | YMSDSFINV | A*02:01 | | |
| | GBM11P | YMSDSFINV | A*02:01; A*02:05 | | |
| | GBM11R | YMSDSFINV | A*02:01; A*02:05 | | |
| | GBM13P | YMSDSFINV | A*02:01 | | |
| | GBM13R | YMSDSFINV | A*02:01 | | |
| | GBM14R | YMSDSFINV | A*02:01 | | |
| | GBM15P | AEEKAAEQF | B*44:02; B*44:03 | | |
| | GBM16P | NLKNRQKSL | B*08:01 | | |
| | GBM23P | RDIGLKNAL | B*47:01 | | |
| | ZH617 | YMSDSFINV | A*02:01 | | |
| | ZH645 | YMSDSFINV | A*02:01 | | |
| | ZH681 | YMSDSFINV | A*02:01 | | |
| | ZH757 | YMSDSFINV | A*02:01 | | |
| | ZH829 | AEEKAAEQF | B*44:05 | | |
| Collagen alpha-2(IX) chain (COL9A2) | <u>24% P / 25% R</u> | | | Q14055 ■ | 17 |
| | GBM4P | SPRSLLVLLQ | *B*07:02* | | |
| | GBM4R | SPRSLLVLLQ | *B*07:02* | | |
| | GBM7P | SPRSLLVLLQ | *B*07:02* | | |
| | GBM8P | SPRSLLVLLQ | *B*07:02* | | |
| | GBM10R | SPRSLLVLLQ | *B*07:02* | | |
| | GBM16P | SPRSLLVLLQ | *B*07:02* | | |
| | GBM16R | SPRSLLVLLQ | *B*07:02* | | |
| | GBM18R | SPRSLLVLLQ | *B*07:02* | | |
| | ZH613 | TASPRSLLV | B*51:01 | | |
| | ZH654 | TASPRSLLV | B*51:01; C*15:06 | | |
| | ZH678 | TASPRSLLV | C*15:02 | | |
| | ZH681 | SPRSLLVLLQ | *B*07:02* | | |
| | ZH753 | TASPRSLLV | C*15:02 | | |
| | ZH757 | KMLQEQLAEV | A*02:01 | | |
| | | SPRSLLVLLQ | *B*07:02* | | |
| | ZH784 | SPRSLLVLLQ | *B*07:02* | | |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4-like 2 (NDUFA4L2) | <u>26% P / 8% R</u> | | | Q9NRX3 ■ | 68 |
| | GBM18P | ASLGARFYR | A*11:01 | | |
| | GBM18R | GSAALYLLR | A*11:01 | | |
| | GBM20P | GASLGARFY | A*29:02 | | |
| | GBM20R | GASLGARFY | A*29:02 | | |
| | ZH613 | ASLGARFYR | A*31:03 | | |
| | | DQYKFLAV | B*51:01 | | |
| | ZH631 | DQYKFLAV | B*51:01 | | |
| | ZH645 | DQYKFLAV | B*51:01 | | |
| | ZH654 | DQYKFLAV | B*51:01 | | |
| | ZH678 | DQYKFLAV | B*51:01 | | |
| | ZH761 | DQYKFLAV | B*51:01 | | |
| | ZH791 | GSAALYLLR | A*11:01 | | |
| | ZH802 | AGASLGARFYR | A*31:01 | | |

| Protein | Sample | Peptide | HLA | Accession | Count |
|---|---|---|---|---|---|
| Protein Wnt-5a (WNT5A) | 21% P / 13% R | | | P41221 ■ | 42 |
| | GBM2P | AMSSKFFLV | A*02:01 | | |
| | GBM3P | AMSSKFFLV | A*02:01 | | |
| | GBM10R | AMSSKFFLV | A*02:01 | | |
| | GBM11R | AMSSKFFLV | A*02:01 | | |
| | GBM14R | AMSSKFFLV | A*02:01 | | |
| | GBM20P | AMSSKFFLV | A*02:01 | | |
| | GBM21P | AMSSKFFLV | A*02:01 | | |
| | ZH616 | AMSSKFFLV | A*02:01 | | |
| | ZH617 | AMSSKFFLV | A*02:01 | | |
| | ZH681 | AMSSKFFLV | A*02:01 | | |
| | ZH757 | AMSSKFFLV | A*02:01 | | |
| Immunoglobulin superfamily member 11 (IGSF11) | 18% P / 17% R | | | Q5DX21 ■ | 3 |
| | GBM4P | SPQPRNIGL | B*07:02 | | |
| | | VPAQSRAGSLV | B*07:02 | | |
| | GBM4R | SPQPRNIGL | B*07:02 | | |
| | GBM7P | SPQPRNIGL | B*07:02 | | |
| | GBM10R | VPAQSRAGSLV | B*07:02 | | |
| | GBM16R | SPQPRNIGL | B*07:02 | | |
| | GBM17R | EEGIPRPTY | B*44:03 | | |
| | ZH645 | SESPGSIQV | B*49:01 | | |
| | ZH654 | LPDIGGRNI | B*51:01 | | |
| | ZH678 | EEGIPRPTY | B*44:03 | | |
| | | SEEGIPRPTY | B*44:03 | | |
| | ZH761 | SEEGIPRPTY | B*44:02 | | |
| | ZH802 | KTLVVTANR | A*31:01 | | |
| Mitochondrial 39S ribosomal protein L35 (MRPL35) | 18% P / 17% R | | | Q9NZE8 ■ | 15 |
| | GBM3P | YVDDPYQKY | A*01:01 | | |
| | GBM5P | YVDDPYQKY | A*01:01; B*15:01 | | |
| | GBM5R | YVDDPYQKY | A*01:01; B*15:01 | | |
| | GBM9P | YVDDPYQKY | A*01:01 | | |
| | GBM9R | YVDDPYQKY | A*01:01 | | |
| | GBM13P | YVDDPYQKY | A*01:01 | | |
| | GBM13R | YVDDPYQKY | A*01:01 | | |
| | GBM16P | YVDDPYQKY | A*01:01 | | |
| | GBM16R | YVDDPYQKY | A*01:01 | | |
| | ZH617 | YVDDPYQKY | A*01:01 | | |
| | ZH654 | YVDDPYQKY | A*01:01 | | |
| Transmembrane protein 87A (TMEM87A) | 18% P / 13% R | | | Q8NBN3 ■ | 33 |
| | GBM7P | HPSPLSFFSA | B*07:02; B*56:01 | | |
| | GBM7R | HPSPLSFFSA | B*07:02; B*56:01 | | |
| | GBM15P | AELLSAVKR | B*44:03 | | |
| | GBM17R | FKAEEVELY | C*06:02 | | |
| | ZH613 | DAPYIFIV | B*51:01 | | |
| | ZH645 | DAPYIFIV | B*51:01 | | |
| | ZH654 | DAPYIFIV | B*51:01 | | |
| | ZH678 | DAPYIFIV | B*51:01 | | |
| | ZH753 | DAPYIFIV | B*51:01 | | |
| | ZH761 | AAWLQVLPV | B*51:26; C*16:01 | | |
| | | DAPYIFIV | B*51:26 | | |
| 85/88 kDa calcium-independent phospholipase A2 (PLA2G6) | 18% P / 13% R | | | O60733 ■ | 3 |
| | GBM3P | ALWETEVYI | A*02:01 | | |
| | GBM7P | ALWETEVYI | A*02:01 | | |
| | GBM7R | ALWETEVYI | A*02:01 | | |
| | GBM13R | ALWETEVYI | A*02:01 | | |
| | GBM19P | ALWETEVYI | A*02:01 | | |
| | GBM22P | LPFYESSPQVL | B*35:03 | | |
| | ZH750 | ALWETEVYI | A*02:01 | | |
| | ZH757 | ALWETEVYI | A*02:01 | | |
| | ZH784 | ALWETEVYI | A*02:01 | | |
| | ZH829 | ALWETEVYI | A*02:01 | | |
| Protein shisa-9 (SHISA9) | 18% P / 13% R | | | B4DS77 ■ | 4 |
| | GBM7P | KVNDDFYTK | A*11:01 | | |
| | GBM10R | KVNDDFYTK | A*03:01 | | |
| | GBM16P | KVNDDFYTK | A*03:01 | | |
| | GBM16R | KVNDDFYTK | A*03:01 | | |
| | GBM18P | KVNDDFYTK | A*03:01; A*11:01 | | |
| | GBM18R | KVNDDFYTK | A*03:01; A*11:01 | | |
| | ZH631 | KVNDDFYTK | A*11:01 | | |
| | ZH678 | KVNDDFYTK | A*11:01 | | |
| | ZH761 | KVNDDFYTK | A*03:01 | | |

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|---|---|---|---|---|---|
| | | ZH791 | KVNDDFYTK | A*03:01; A*11:01 | |
| Leucine-rich repeat neuronal protein 3 (LRRN3) 2 peptides multi-map to non-GBM-exclusive LRRN1 (Q6UXK5) | 18% P / 13% R | GBM6P | GTIESLPNL | A*02:05; C*12:03 | Q9H3W5 ■ 5 |
| | | GBM6R | GTIESLPNL | A*02:05; C*12:03 | |
| | | GBM9P | DMNFKPLINLR | A*33:05 | |
| | | | DMPLRIHVL | B*08:01 | |
| | | GBM16P | DMPLRIHVL | B*08:01 | |
| | | GBM16R | DMPLRIHVL | B*08:01 | |
| | | GBM19P | LYPPLINLW | A*24:02 | |
| | | GBM22P | LYPPLINLW | A*24:02 | |
| | | GBM22R | LYPPLINL | A*24:02 | |
| | | ZH617 | DMPLRIHVL | A*02:01; B*08:01 | |
| | | ZH810 | LYPPLINLW | A*24:02 | |
| Neuronal migration protein doublecortin (DCX) Peptides multi-map to non-GBM-exclusive doublecortin-like kinase 1/2 (DCLK1/2; O15075/Q8N568) | 16% P / 17% R | GBM10R | VLTDITEAI | A*02:01 | O43602 ■ 8 |
| | | GBM13P | VLTDITEAI | A*02:01; *B*13:02* | |
| | | GBM13R | VLTDITEAI | A*02:01; *B*13:02* | |
| | | GBM14R | VLTDITEAI | A*02:01 | |
| | | GBM23P | TAHSFEQVL | B*35:02 | |
| | | GBM23R | TAHSFEQVL | B*35:02 | |
| | | ZH645 | VLTDITEAI | A*02:01 | |
| | | ZH681 | VLTDITEAI | A*02:01 | |
| | | ZH757 | VLTDITEAI | A*02:01 | |
| | | ZH761 | VLTDITEAI | A*02:01 | |
| Protein pitchfork (PIFO) | 13% P / 17% R | GBM3P | VRFKPIQK | B*27:05 | Q8TCI5 ■ 1 |
| | | | VRFKPIQKEM | B*27:05 | |
| | | GBM4P | RVAYLSLYY | A*03:01 | |
| | | GBM4R | RVAYLSLYY | A*03:01 | |
| | | GBM16P | RVAYLSLYY | A*03:01 | |
| | | GBM16R | RVAYLSLYY | A*03:01 | |
| | | GBM17R | RVAYLSLYY | A*03:01 | |
| | | GBM20R | AELSTDKDF | B*44:02; B*44:03 | |
| | | ZH616 | AELSTDKDF | B*44:02 | |
| | | ZH617 | AELSTDKDF | B*44:02 | |
| Uncharacterized aarF domain-containing protein kinase 1 (ADCK1) | 13% P / 17% R | GBM5P | TQISFSEAF | B*15:01 | Q86TW2 ■ 17 |
| | | GBM5R | TQISFSEAF | B*15:01 | |
| | | GBM9P | DFGAVRVGR | A*33:05 | |
| | | GBM9R | DFGAVRVGR | A*33:05 | |
| | | GBM13R | LTEEFRLNY | A*01:01 | |
| | | GBM14R | DFGAVRVGR | A*33:01 | |
| | | GBM16P | LTEEFRLNY | A*01:01 | |
| | | GBM23P | NYLPQISHL | A*23:01; C*06:02 | |
| | | ZH802 | DFGAVRVGR | A*33:01 | |
| SAC3 domain-containing protein 1 (SAC3D1) | 13% P / 17% R | GBM4P | KPRPPPSQL | B*07:02 | A6NKF1 ■ 14 |
| | | GBM4R | KPRPPPSQL | B*07:02 | |
| | | GBM7P | KPRPPPSQL | B*07:02 | |
| | | GBM10R | KPRPPPSQL | B*07:02 | |
| | | GBM16P | KPRPPPSQL | B*07:02 | |
| | | GBM16R | KPRPPPSQL | B*07:02 | |
| | | GBM18P | KPRPPPSQL | B*07:02 | |
| | | GBM18R | KPRPPPSQL | B*07:02 | |
| | | ZH681 | KPRPPPSQL | B*07:02 | |
| Low-density lipoprotein receptor class A domain-containing protein 3 (LDLRAD3) | 16% P / 8% R | GBM5P | ASEVGSPPSY | A*01:01 | Q86YD5 ■ 2 |
| | | | LLDQRPAWY | A*01:01; B*15:01 | |
| | | GBM9P | ASEVGSPPSY | A*01:01 | |
| | | GBM13R | ASEVGSPPSY | A*01:01 | |
| | | | LLDQRPAWY | A*01:01 | |
| | | GBM16P | ASEVGSPPSY | A*01:01 | |
| | | | LLDQRPAWY | A*01:01 | |
| | | GBM16R | LLDQRPAWY | A*01:01 | |
| | | ZH617 | ASEVGSPPSY | A*01:01; | |
| | | ZH645 | ASEVGSPPSY | B*44:02 A*01:01 | |
| | | ZH654 | LLDQRPAWY | A*01:01 | |

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|---|---|---|---|---|---|
| 6-pyruvoyl tetrahydrobiopterin synthase (PTS) | 16% P / 8% R | | | Q03393 ■ | 39 |
| | GBM3P | ETDNNIVVY | A*01:01 | | |
| | GBM5R | ETDNNIVVY | A*01:01 | | |
| | GBM6P | QKVLPVGVLY | *B*51:03* | | |
| | GBM13R | ETDNNIVVY | A*01:01 | | |
| | GBM15P | GEIDPATGM | B*44:02 | | |
| | GBM16P | ETDNNIVVY | A*01:01 | | |
| | ZH645 | ETDNNIVVY | A*01:01 | | |
| | ZH654 | ETDNNIVVY | A*01:01 | | |
| Probable G-protein coupled receptor 75 (GPR75) | 16% P / 8% R | | | O95800 ■ | 2 |
| | GBM4P | TPAASRLQL | B*07:02 | | |
| | GBM4R | TPAASRLQL | B*07:02 | | |
| | GBM7P | TPAASRLQL | B*07:02 | | |
| | GBM16P | TPAASRLQL | B*07:02 | | |
| | GBM16R | TPAASRLQL | B*07:02 | | |
| | GBM18P | TPAASRLQL | B*07:02 | | |
| | ZH613 | GLNPFIYSR | A*31:01 | | |
| | ZH654 | GLNPFIYSR | A*31:01 | | |
| POU domain, class 3, transcription factor 3 (POU3F3) 1 peptide multi-maps to GBM-exclusive POU3F2/4 (P20265/P49335) | 13% P / 13% R | | | P20264 ■ | 6 |
| | GBM4P | NPYLPGNSL | B*07:02 | | |
| | GBM4R | NPYLPGNSL | B*07:02 | | |
| | GBM7P | SPTSIDKIAA | B*56:01 | | |
| | GBM10R | LVRGDTPEL | A*02:01; B*07:02 | | |
| | | NPYLPGNSL | B*07:02 | | |
| | GBM16P | NPYLPGNSL | B*07:02 | | |
| | GBM16R | NPYLPGNSL | B*07:02 | | |
| | ZH613 | DVYSQVGTV | B*51:01 | | |
| | ZH631 | DVYSQVGTV | B*51:01 | | |
| Integral membrane protein DGCR2/IDD (DGCR2) | 13% P / 13% R | | | P98153 ■ | 13 |
| | GBM4P | VPKADSGAFL | B*07:02 | | |
| | GBM4R | VPKADSGAFL | B*07:02 | | |
| | GBM10R | VPKADSGAFL | B*07:02 | | |
| | GBM16P | VPKADSGAFL | B*07:02 | | |
| | GBM16R | VPKADSGAFL | B*07:02 | | |
| | GBM23P | FYDPADDDAF | C*04:01 | | |
| | ZH654 | DPSHFHAVNV | B*51:01 | | |
| | ZH681 | VPKADSGAFL | B*07:02 | | |
| Cannabinoid receptor 1 (CNR1) | 11% P / 17% R | | | P21554 ■ | 2 |
| | GBM5P | KLGGVTASF | B*15:01 | | |
| | GBM12R | FPQKFPLTSF | B*35:01 | | |
| | GBM14R | FPHIDETYL | B*35:03 | | |
| | | FPQKFPLTSF | B*35:03 | | |
| | | STDTSAEAL | *C*08:02* | | |
| | | TVNPIIYAL | A*02:01; *C*08:02* | | |
| | GBM16P | RPDQARMDIRL | B*07:02 | | |
| | GBM16R | RPDQARMDIRL | B*07:02 | | |
| | GBM22R | FPQKFPLTSF | B*35:03 | | |
| | | FYNKSLSSF | A*24:02 | | |
| | | TVNPIIYAL | C*03:04; C*12:03 | | |
| | ZH678 | SLAVADLLGSV | A*02:01 | | |
| | ZH720 | FIGSLAVADL | A*02:01 | | |
| Lipoma HMGIC fusion partner-like 2 protein (LHFPL2) | 8% P / 21% R | | | Q6ZUX7 ■ | 12 |
| | GBM10R | SPEPYHPTL | B*07:02 | | |
| | GBM14R | SPEPYHPTL | B*35:03; C*08:02 | | |
| | GBM16P | SPEPYHPTL | B*07:02 | | |
| | GBM16R | SPEPYHPTL | B*07:02 | | |
| | GBM18R | SPEPYHPTL | B*07:02 | | |
| | GBM21P | SPEPYHPTL | B*35:03 | | |
| | GBM23P | SPEPYHPTL | B*35:02 | | |
| | GBM23R | IRNPGVQHF | C*06:02 | | |
| | GBM23R | SPEPYHPTL | B*35:02 | | |
| Insulin-like growth factor-binding protein 2 (IGFBP2) | 16% P / 4% R | | | P18065 ■ | 8 |
| | GBM7P | *LPLPPPPLLP* | B*56:01 | | |
| | GBM12P | YPHPGSELPL | B*35:01 | | |
| | GBM16P | YPHPGSEL | B*07:02 | | |
| | GBM18R | YPHPGSEL | B*07:02 | | |
| | GBM21P | YPHPGSEL | B*35:03 | | |
| | GBM22P | YPHPGSEL | B*35:03; *C*03:04* | | |
| | | YPHPGSELPL | B*35:03 | | |
| | ZH645 | AEYGASPEQV | B*49:01 | | |
| Protein shisa-4 (SHISA4) | 16% P / 4% R | | | Q96DD7 ■ | 6 |
| | GBM23R | YPYPQDPKA | B*35:02 | | |
| | ZH613 | LAFSPKTI | B*51:01 | | |
| | ZH631 | LAFSPKTI | B*51:01 | | |

| Protein | Sample | Peptide | HLA | UniProt | # |
|---|---|---|---|---|---|
| | ZH645 | LAFSPKTI | B*51:01 | | |
| | ZH678 | LAFSPKTI | B*51:01 | | |
| | ZH757 | AAPLTAIAL | C*01:02 | | |
| | ZH761 | AAPLTAIAL | C*01:02 | | |
| IGF-like family receptor 1 (IGFLR1) | 13% P / 8% R | | | Q9H665 ■ | 45 |
| | GBM5P | GRADALRVL | C*06:02 | | |
| | GBM5R | GRADALRVL | C*06:02 | | |
| | GBM13P | GRADALRVL | C*06:02 | | |
| | GBM13R | GRADALRVL | C*06:02 | | |
| | GBM23P | GRADALRVL | C*06:02 | | |
| | ZH654 | GRADALRVL | C*06:02 | | |
| | ZH681 | GRADALRVL | C*06:02 | | |
| CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 4 (ST3GAL4) | 13% P / 8% R | | | Q11206 ■ | 15 |
| | GBM7P | SIPKNIQSL | C*01:02 | | |
| | GBM18R | DLLLRVLAI | B*08:01 | | |
| | GBM23R | RDQPIFLRL | B*47:01 | | |
| | ZH613 | SIPKNIQSL | A*02:05 | | |
| | ZH678 | AITSSSIPK | A*11:01 | | |
| | ZH757 | SIPKNIQSL | C*01:02 | | |
| | ZH761 | SIPKNIQSL | C*01:02 | | |
| Solute carrier family 25 member 53 (SLC25A53) | 13% P / 8% R | | | Q5H9E4 ■ | 7 |
| | GBM7P | STFLTFPIYK | A*11:01 | | |
| | GBM7R | STFLTFPIYK | A*11:01 | | |
| | GBM18P | STFLTFPIYK | A*11:01 | | |
| | GBM18R | STFLTFPIYK | A*11:01 | | |
| | ZH631 | STFLTFPIYK | A*11:01 | | |
| | ZH654 | AVSEAVRQLW | B*57:01 | | |
| | ZH678 | STFLTFPIYK | A*11:01 | | |
| Leucine-rich repeats and immunoglobulin-like domains protein 1 (LRIG1) | 13% P / 8% R | | | Q96JA1 ■ | 10 |
| | GBM3P | ARIHRKGWSF | B*27:05 | | |
| | GBM16P | LPRLTQLDL | B*07:02 | | |
| | GBM16R | RPVRGGLGA | B*07:02 | | |
| | GBM21P | FPEPDTHSV | B*35:03 | | |
| | GBM23P | FPEPDTHSV | B*35:02 | | |
| | GBM23R | FPEPDTHSV | B*35:02 | | |
| | ZH654 | AALPGDLPSW | B*57:01 | | |
| | | AFHPQPVSR | A*31:01 | | |
| Glycosyltransferase-like domain-containing protein 2 (GTDC2) | 11% P / 13% R | | | Q8NAT1 ■ | 12 |
| | GBM7P | ILNEAELLL | A*02:01 | | |
| | GBM10R | AMLPGMDLQYV | A*02:01 | | |
| | GBM11P | AMLPGMDLQYV | A*02:01 | | |
| | GBM11R | LILNEAELL | A*02:01; A*02:05 | | |
| | GBM13R | AMLPGMDLQYV | A*02:01 | | |
| | ZH681 | AMLPGMDLQYV | A*02:01 | | |
| | | ILNEAELLL | A*02:01 | | |
| | ZH757 | AMLPGMDLQYV | A*02:01 | | |
| Growth arrest and DNA damage-inducible proteins-interacting protein 1 (GADD45GIP1) | 11% P / 13% R | | | Q8TAE8 ■ | 12 |
| | GBM7P | SLWPSPEQL | A*02:01 | | |
| | GBM13R | SLWPSPEQL | A*02:01 | | |
| | GBM14R | SLWPSPEQL | A*02:01 | | |
| | GBM20R | AEAQELLGY | B*44:02; B*44:03 | | |
| | ZH617 | SLWPSPEQL | A*02:01 | | |
| | ZH681 | SLWPSPEQL | A*02:01 | | |
| | ZH757 | SLWPSPEQL | A*02:01 | | |
| T-box transcription factor TBX15/18 (TBX15/18) | 11%/8% P | | | Q96SF7 ■ | 18 |
| 1 peptide multi-maps to non-GBM-exclusive TBX20 (Q9UMR3) | 13%/8% R | | | O95935 ■ | 11 |
| | GBM3P | GLDPHQQYY (TBX15/18) | A*01:01 | | |
| | GBM6P | YQNQQITRL (TBX15/18) | A*02:05; C*12:03 | | |
| | GBM9P | DIVPVDNKRYR (TBX15/18/20) | A*33:05 | | |
| | | GLDPHQQYY (TBX15/18) | A*01:01 | | |
| | GBM9R | GLDPHQQYY (TBX15/18) | A*01:01 | | |
| | GBM11R | YQNQQITRL (TBX15/18) | A*02:05; B*41:01 | | |
| | GBM12R | KTFNFPETVF (TBX15) | B*15:01 | | |
| | ZH654 | KTFNFPETVF (TBX15) | B*57:01 | | |
| Exostosin-like 3 (EXTL3) | 11% P / 13% R | | | O43909 ■ | 12 |
| | GBM5P | KSDTQNLLY | A*01:01 | | |
| | GBM13P | KSDTQNLLY | A*01:01 | | |
| | GBM13R | KSDTQNLLY | A*01:01 | | |
| | GBM16P | KSDTQNLLY | A*01:01 | | |
| | GBM16R | KSDTQNLLY | A*01:01 | | |
| | ZH645 | KSDTQNLLY | A*01:01 | | |
| | ZH753 | LPYLNKVVV | B*51:01 | | |
| Uncharacterized protein C7orf50 (C7orf50) | 11% P / 13% R | | | Q9BRJ6 ■ | 12 |
| | GBM10R | VPDEHFSTL | B*07:02 | | |
| | GBM16P | VPDEHFSTL | B*07:02 | | |

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| | GBM16R | VPDEHFSTL | B*07:02 | | |
| | GBM21P | VPDEHFSTL | B*35:03 | | |
| | GBM23P | VPDEHFSTL | B*35:02; C*04:01 | | |
| | GBM23R | VPDEHFSTL | B*35:03; C*04:01 | | |
| | ZH802 | AYLEGLQGR | A*31:01 | | |
| Uncharacterized protein C21orf62 (C21orf62) | 8% P / 17% R | | | Q9NYP8 ■ | 6 |
| | GBM6P | LPLAVERTSY | B*35:01 | | |
| | GBM6R | LPLAVERTSY | B*35:01 | | |
| | GBM10R | RTFPMPSNK | A*03:01 | | |
| | GBM16P | RTFPMPSNK | A*03:01 | | |
| | GBM22R | SYNGHLTIWF | A*24:02 | | |
| | GBM23R | RTFPMPSNK | A*03:01 | | |
| | ZH810 | SYNGHLTIWF | A*24:02 | | |
| BTB/POZ domain-containing protein 17 (BTBD17) | 13% P / 4% R | | | A6NE02 ■ | 1 |
| | GBM3P | DELELFHAL | B*18:01 | | |
| | GBM4P | AVFDKFIRY | A*03:01 | | |
| | GBM7R | AVFDKFIRY | A*11:01; C*02:02 | | |
| | ZH616 | AVFDKFIRY | A*29:02 | | |
| | ZH631 | AVFDKFIRY | A*11:01; A*32:01 | | |
| | | NASDVVLRV | B*51:01 | | |
| | ZH654 | RARPPPAVAER | A*31:01 | | |
| Peroxisome biogenesis factor 10 (PEX10) | 13% P / 4% R | | | O60683 ■ | 7 |
| | GBM3P | QRARKEWRL | B*27:05; C*07:01 | | |
| | GBM6P | AKRLTGITY | B*15:03 | | |
| | GBM18R | AVLPYLLDK | A*11:01 | | |
| | ZH613 | RSLPGEDLRAR | A*31:01 | | |
| | ZH654 | RSLPGEDLRAR | A*31:01 | | |
| | ZH802 | RSLPGEDLRAR | A*31:01 | | |
| Sodium- and chloride-dependent creatine transporter 1 (SLC6A8) | 13% P / 4% R | | | P48029 ■ | 20 |
| | GBM5P | LLDLLPASY | A*01:01; B*15:01 | | |
| 1 peptide multi-maps to non-GBM-exclusive SLC6A6 (P31641) | GBM16P | LLDLLPASY | A*01:01 | | |
| | GBM16R | LLDLLPASY | A*01:01 | | |
| | ZH616 | VVYYEPLVY | A*29:02 | | |
| | ZH631 | IAYPRAVTL | C*03:03 | | |
| | ZH810 | VWIDAGTQIFF | A*24:02 | | |
| 5'-AMP-activated protein kinase subunit beta-2 (PRKAB2) | 11% P / 8% R | | | O43741 ■ | 9 |
| | GBM6P | GTINNLIHV | A*02:05; C*12:03 | | |
| 1 peptide multi-maps to non-GBM-exclusive PRKAB1 | GBM11P | GTINNLIHV | A*02:05 | | |
| | GBM14R | LPEPNHVML | B*35:03; C*04:01 | | |
| | GBM23P | LPEPNHVML | B*35:02; C*04:01 | | |
| | GBM23R | LPEPNHVML | B*35:02; C*04:01 | | |
| | ZH613 | GTINNLIHV | A*02:05 | | |
| Transcription factor E2F1 (E2F1) | 11% P / 8% R | | | Q01094 ♦ | 12 |
| | GBM3P | DTDSQRLAY | A*01:01 | | |
| | GBM13P | DTDSQRLAY | A*01:01 | | |
| | GBM16P | DTDSQRLAY | A*01:01 | | |
| | GBM16R | DTDSQRLAY | A*01:01 | | |
| | GBM17R | DTDSQRLAY | A*01:01 | | |
| | ZH617 | DTDSQRLAY | A*01:01 | | |
| Solute carrier family 15 member 2 (SLC15A2) | 11% P / 8% R | | | Q16348 ■ | 17 |
| | GBM4P | APSSMKSVL | B*07:02 | | |
| | GBM10R | APSSMKSVL | B*07:02 | | |
| | GBM16P | APSSMKSVL | B*07:02 | | |
| | GBM23P | VMFSVTGLEF | A*23:01 | | |
| | GBM23R | VMFSVTGLEF | A*23:01 | | |
| | ZH681 | APSSMKSVL | B*07:02 | | |
| Sugar phosphate exchanger 3 (SLC37A3) | 11% P / 8% R | | | Q8NCC5 ■ | 22 |
| | GBM9P | DRLNLRWVL | B*14:02 | | |
| | GBM10R | SPNDKSINAL | B*07:02 | | |
| | GBM16P | SPNDKSINAL | B*07:02 | | |
| | GBM16R | SPNDKSINAL | B*07:02 | | |
| | ZH616 | SVLQYGYEY | A*29:02 | | |
| | ZH761 | ALTEWLRFY | A*03:01 | | |
| | | VAQVKAISF | C*16:01 | | |
| Frizzled-3 (FZD3) | 11% P / 8% R | | | Q9NPG1 ■ | 8 |
| | GBM5P | SRPDLILFL | C*06:02 | | |
| | GBM13P | SRPDLILFL | C*06:02 | | |
| | GBM16P | SRPDLILFL | C*07:02 | | |
| | GBM16R | SRPDLILFL | C*07:02 | | |

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|---|---|---|---|---|---|
| | GBM23R | SRPDLILFL | C*06:02 | | |
| | ZH654 | SRPDLILFL | C*06:02 | | |
| Protein Dos (DOS) | 11% P / 4% R | | | Q8N350 ■ | 0 |
| | ZH613 | LPPLKIVTI | B*51:01 | | |
| | ZH631 | LPPLKIVTI | B*51:01 | | |
| | ZH654 | LPPLKIVTI | B*51:01 | | |
| | ZH678 | LPPLKIVTI | B*51:01 | | |
| | ZH753 | LPPLKIVTI | B*51:01 | | |
| Embryonal Fyn-associated substance (EFS) | 11% P / 4% R | | | O43281 ■ | 16 |
| | GBM7P | LPALPVPEA | B*07:02; B*56:01 | | |
| | GBM18R | IPRASGTQL | B*07:02 | | |
| | ZH616 | IPRASGTQL | B*07:02 | | |
| | | STGDLQLLY | A*29:02 | | |
| | ZH678 | AESPQELSF | B*44:03 | | |
| | ZH761 | AESPQELSF | B*44:02; B*44:03 | | |
| Transmembrane protein 17 (TMEM17) | 11% P / 4% R | | | Q86X19 ■ | 3 |
| | GBM11P | LLFNEGLTNL | A*02:01 | | |
| | GBM14R | NLQEKVPEL | A*02:01 | | |
| | ZH617 | NLQEKVPEL | A*02:01; B*08:01 | | |
| | ZH681 | NLQEKVPEL | A*02:01 | | |
| | ZH761 | NLQEKVPEL | A*02:01 | | |
| Transcription factor E2F7 (E2F7) | 11% P / 4% R | | | Q96AV8 ■ | 8 |
| | GBM7P | ISLDEVAVSL | A*02:01 | | |
| | GBM11P | ISLDEVAVSL | A*02:01 | | |
| | ZH617 | ISLDEVAVSL | A*02:01; C*05:01 | | |
| | ZH681 | | | | |
| | ZH753 | ISLDEVAVSL | A*02:01 | | |
| | | ISLDEVAVSL | A*02:01; C*15:02 | | |
| Transmembrane protein 237 (TMEM237) | 11% P / 4% R | | | Q96Q45 ■ | 7 |
| | GBM5P | LAYPFQSLL | C*03:03 | | |
| | GBM6P | FQAADRSEL | A*02:05 | | |
| | | LQPWIVVNL | A*02:05 | | |
| | GBM9P | DVKPSWTTR | A*33:05 | | |
| | GBM14R | DVKPSWTTR | A*33:01 | | |
| | ZH802 | DVKPSWTTR | A*33:01 | | |
| Lysyl oxidase homolog 2 (LOXL2) | 11% P / 4% R | | | Q9Y4K0 ■ | 26 |
| | GBM6P | FGFPGERTY | C*12:03 | | |
| | GBM15P | AEMVQQTTY | B*44:02 | | |
| | GBM18R | RTYNTKVYKM | *A*03:01* | | |
| | GBM21P | FGFPGERTY | C*12:03 | | |
| | ZH613 | ASNAFQETW | B*58:01 | | |
| Heat shock factor 2-binding protein (HSF2BP) | 8% P / 8% R | | | O75031 ♦ | 13 |
| | GBM3P | VLLDTILQL | A*02:01 | | |
| | GBM7P | VLLDTILQL | A*02:01 | | |
| | GBM12R | VLLDTILQL | A*02:01 | | |
| | GBM13R | VLLDTILQL | A*02:01 | | |
| | ZH750 | VLLDTILQL | A*02:01 | | |
| Transcription factor HES-4 (HES4) | 8% P / 8% R | | | Q9HCC6 ■ | 13 |
| | GBM4P | RINESLAQLK | A*03:01 | | |
| | GBM10R | RINESLAQLK | A*03:01 | | |
| | GBM16R | RINESLAQLK | A*03:01 | | |
| | GBM18P | RINESLAQLK | A*03:01 | | |
| | GBM23P | RINESLAQLK | A*03:01 | | |
| Uroporphyrinogen-III synthase (UROS) | 8% P / 8% R | | | P10746 ■ | 18 |
| | GBM12R | IGPTTARAL | C*01:02 | | |
| | GBM17R | AESPTPQAL | B*44:03 | | |
| | ZH613 | SLPSFSEKL | A*02:05 | | |
| | ZH645 | IGPTTARAL | C*01:02 | | |
| | ZH761 | IGPTTARAL | C*01:02 | | |
| Blood vessel epicardial substance (BVES) | 5% P / 13% R | | | Q8NE79 ■ | 5 |
| | GBM5P | TAIGFTPEL | C*03:03 | | |
| | GBM16R | SPEFRSTQM | B*07:02 | | |
| | GBM18R | SPEFRSTQM | B*07:02 | | |
| | GBM22R | TAIGFTPEL | C*03:04; C*12:03 | | |
| | ZH616 | | | | |
| | | SPEFRSTQM | B*07:02 | | |
| Olfactory receptor 51A7 (OR51A7) | 5% P / 13% R | | | Q8NH64 ■ | 13 |
| | GBM2P | GLILAIRSI | A*02:01 | | |
| | GBM13R | ILAIRSILL | A*02:01; B*08:01 | | |
| | GBM16P | ILAIRSILL | B*08:01 | | |
| | GBM18R | ILAIRSILL | B*08:01 | | |
| | ZH753 | ILAIRSILL | A*02:01; B*08:01 | | |

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|---|---|---|---|---|---|
| Zinc finger and BTB domain-containing protein 47 (ZBTB47) | <u>5% P / 13% R</u> | | | Q9UFB7 ■ | 10 |
| | GBM4P | RPKPPPGVA | B*07:02 | | |
| | GBM12R | FLLESELLL | A*02:01 | | |
| | GBM13R | FLLESELLL | A*02:01; *B*13:02* | | |
| | GBM14R | FLLESELLL | A*02:01 | | |
| | GBM16P | RPKPPPGVA | B*07:02 | | |

**Glioblastoma-associated HLA class II antigens**

| | | | | | |
|---|---|---|---|---|---|
| Glypican-5 (GPC5) | <u>32% P / 46% R</u> | | | P78333 ■ | 1 |
| | GBM2P | LFQWRLLGAVRGLPD | | | |
| | GBM2R | KLFQWRLLGAVRGLPD | | | |
| | | LFQWRLLGAVRGLPD | | | |
| | | LFQWRLLGAVRGLPDSP | | | |
| | GBM6P | LFQWRLLGAVRGLPD | | | |
| | GBM6R | LFQWRLLGAVRGLPD | | | |
| | GBM7P | INSLRLYRSFYGGL | | | |
| | | LFQWRLLGAVRGLPD | | | |
| | GBM7R | LFQWRLLGAVRGLPD | | | |
| | GBM9R | INSLRLYRSFYGGL | | | |
| | GBM10R | FINSLRLYRSFYGGLA | | | |
| | | INSLRLYRSFYGG | | | |
| | | INSLRLYRSFYGGL | | | |
| | | INSLRLYRSFYGGLA | | | |
| | | VNRFFDSLFPLVYN | | | |
| | | VNRFFDSLFPLVYNH | | | |
| | GBM11P | INSLRLYRSFYGG | | | |
| | | INSLRLYRSFYGGL | | | |
| | GBM11R | INSLRLYRSFYGGL | | | |
| | GBM12P | GIDPVINQIIDKLKH | | | |
| | | KLFQWRLLGAVRGLPD | | | |
| | | LFQWRLLGAVRGLPD | | | |
| | GBM12R | LFQWRLLGAVRGLPD | | | |
| | GBM13P | GIDPVINQIIDKLKH | | | |
| | GBM16P | FINSLRLYRSFYGGLA | | | |
| | | INSLRLYRSFYGGL | | | |
| | | VRKLFQWRLLGAVRGLPD | | | |
| | GBM18P | IDPVINQIIDKLKH | | | |
| | GBM19R | LFQWRLLGAVRGLPD | | | |
| | GBM22P | GIDPVINQIIDKLKH | | | |
| | | IDPVINQIIDKLKH | | | |
| | GBM22R | GIDPVINQIIDKLKH | | | |
| | GBM23R | GIDPVINQIIDKLKH | | | |
| | ZH616 | FINSLRLYRSFYGGLA | | | |
| | | INSLRLYRSFYGGL | | | |
| | | INSLRLYRSFYGGLA | | | |
| | ZH654 | GIDPVINQIIDKLKH | | | |
| | | IDPVINQIIDKLKH | | | |
| | ZH753 | LFQWRLLGAVRGLPD | | | |
| | ZH761 | LFQWRLLGAVRGLPD | | | |
| Procollagen galactosyltransferase 2 (COLGALT2) | <u>24% P / 13% R</u> | | | Q8IYK4 ■ | 4 |
| | GBM5P | DLKAFSAEPLLIYPT | | | |
| | | DLKAFSAEPLLIYPTHY | | | |
| | GBM6P | DLKAFSAEPLLIYPTH | | | |
| | GBM7P | DLKAFSAEPLLIYPT | | | |
| | | DLKAFSAEPLLIYPTHY | | | |
| | GBM9R | DLKAFSAEPLLIYP | | | |
| | | DLKAFSAEPLLIYPT | | | |
| | | DLKAFSAEPLLIYPTH | | | |
| | | DLKAFSAEPLLIYPTHY | | | |
| | GBM10R | DLKAFSAEPLLIYP | | | |
| | | DLKAFSAEPLLIYPT | | | |
| | | DLKAFSAEPLLIYPTH | | | |
| | | DLKAFSAEPLLIYPTHY | | | |
| | GBM16P | DLKAFSAEPLLIYPT | | | |
| | | DLKAFSAEPLLIYPTH | | | |
| | | DLKAFSAEPLLIYPTHY | | | |
| | | LKAFSAEPLLIYPTHY | | | |
| | GBM20R | DLKAFSAEPLLIYPT | | | |
| | ZH645 | DLKAFSAEPLLIYPT | | | |
| | ZH678 | DLKAFSAEPLLIYPT | | | |
| | ZH681 | DLKAFSAEPLLIYPT | | | |
| | ZH757 | DLKAFSAEPLLIYPT | | | |
| | | DLKAFSAEPLLIYPTH | | | |
| | ZH761 | DLKAFSAEPLLIYPTHY | | | |
| Nucleolar protein 16 (NOP16) | <u>29% P / 4% R</u> | | | Q9Y3C1 ■ | 10 |
| | GBM5P | PKAKGKTRRQKF | | | |
| | GBM6P | PKAKGKTRRQKF | | | |
| | GBM13P | PKAKGKTRRQKF | | | |
| | GBM16R | PKAKGKTRRQKF | | | |

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|---|---|---|---|---|
|  | GBM18P | PKAKGKTRRQKF |  |  |
|  | ZH613 | PKAKGKTRRQKF |  |  |
|  | ZH617 | PKAKGKTRRQKF |  |  |
|  | ZH631 | PKAKGKTRRQKF |  |  |
|  | ZH645 | PKAKGKTRRQKF |  |  |
|  | ZH678 | PKAKGKTRRQKF |  |  |
|  | ZH681 | PKAKGKTRRQKF |  |  |
|  | ZH802 | PKAKGKTRRQKF |  |  |
| N-acetyltransferase ESCO1 (ESCO1) | <u>18% P / 17% R</u> |  | Q5FWF5 ■ | 12 |
|  | GBM11R | KVLEVKSDSKEDENLVINEVINSPK |  |  |
|  | GBM12R | KVLEVKSDSKEDENLVINEVINSPK |  |  |
|  | GBM14P | KVLEVKSDSKEDENLVINEVINSPK |  |  |
|  | GBM15R | KVLEVKSDSKEDENLVINEVINSPK |  |  |
|  | GBM18P | KVLEVKSDSKEDENLVINEVINSPK |  |  |
|  | GBM18R | KVLEVKSDSKEDENLVINEVINSPK |  |  |
|  | GBM19P | KVLEVKSDSKEDENLVINEVINSPK |  |  |
|  | GBM22P | KVLEVKSDSKEDENLVINEVINSPK |  |  |
|  | ZH617 | KVLEVKSDSKEDENLVINEVINSPK |  |  |
|  | ZH645 | KVLEVKSDSKEDENLVINEVINSPK |  |  |
|  | ZH791 | KVLEVKSDSKEDENLVINEVINSPK |  |  |
| Gamma-butyrobetaine dioxygenase (BBOX1) | <u>16% P / 21% R</u> |  | O75936 ■ | 10 |
|  | GBM5P | KLGKRMGFLYLTFYGHTWQVQDK |  |  |
|  | GBM9P | KAEALDGAHLMQIL |  |  |
|  | GBM9R | KAEALDGAHLMQIL |  |  |
|  | GBM10R | HKIIELDDKGQVVR |  |  |
|  |  | KAEALDGAHLMQIL |  |  |
|  |  | KHKIIELDDKGQVVR |  |  |
|  | GBM11P | KAEALDGAHLMQIL |  |  |
|  | GBM11R | KAEALDGAHLMQIL |  |  |
|  | GBM13P | HKIIELDDKGQVVR |  |  |
|  |  | KAEALDGAHLMQIL |  |  |
|  |  | KHKIIELDDKGQVVR |  |  |
|  |  | SKHKIIELDDKGQVVR |  |  |
|  | GBM13R | SKHKIIELDDKGQVVR |  |  |
|  | GBM14R | KAEALDGAHLMQIL |  |  |
|  | GBM16P | HKIIELDDKGQVVR |  |  |
|  |  | KAEALDGAHLMQIL |  |  |
|  |  | KHKIIELDDKGQVVR |  |  |
|  |  | SKHKIIELDDKGQVVR |  |  |
|  | GBM18P | HKIIELDDKGQVVR |  |  |
|  |  | KHKIIELDDKGQVVR |  |  |
| Malignant fibrous histiocytoma-amplified sequence 1 (MFHAS1) | <u>13% P / 21% R</u> |  | Q9Y4C4 ■ | 4 |
|  | GBM2P | KLNLSHNQLPALPAQLGALAHL |  |  |
|  | GBM7P | KLNLSHNQLPALPAQLGALAHL |  |  |
|  | GBM7R | KLNLSHNQLPALPAQLGALAHL |  |  |
|  | GBM11R | KLNLSHNQLPALPAQLGALAHL |  |  |
|  | GBM13P | KLNLSHNQLPALPAQLGALAHL |  |  |
|  | GBM20P | KLNLSHNQLPALPAQLGALAHL |  |  |
|  | GBM20R | KLNLSHNQLPALPAQLGALAHL |  |  |
|  | GBM21R | KLNLSHNQLPALPAQLGALAHL |  |  |
|  | ZH753 | KLNLSHNQLPALPAQLGALAHL |  |  |
|  | ZH761 | KLNLSHNQLPALPAQLGALAHL |  |  |
| Polypeptide N-acetylgalactosaminyl-transferase 15 (GALNT15) | <u>13% P / 17% R</u> |  | Q8N3T1 ■ | 2 |
|  | GBM4R | GGSYRLIKQPRRQD |  |  |
|  | GBM6P | GGSYRLIKQPRRQD |  |  |
|  |  | GGSYRLIKQPRRQDK |  |  |
|  |  | GSYRLIKQPRRQDK |  |  |
|  |  | RGGSYRLIKQPRRQD |  |  |
|  |  | RGGSYRLIKQPRRQDK |  |  |
|  | GBM10R | GGSYRLIKQPRRQD |  |  |
|  |  | RGGSYRLIKQPRRQD |  |  |
|  | GBM16P | GGSYRLIKQPRRQD |  |  |
|  | GBM16R | GGSYRLIKQPRRQD |  |  |
|  | GBM17R | GGSYRLIKQPRRQDK |  |  |
|  |  | GGSYRLIKQPRRQDKE |  |  |
|  | GBM18P | LPEVRHPLCLQQHPQ |  |  |
|  |  | RNRVRIAETWLGSFK |  |  |
|  | ZH616 | GGSYRLIKQPRRQD |  |  |
|  |  | GGSYRLIKQPRRQDK |  |  |
|  | ZH757 | GGSYRLIKQPRRQD |  |  |
| Kynurenine-oxoglutarate transaminase 1 (CCBL1) | <u>16% P / 13% R</u> |  | Q16773 ■ | 11 |
|  | GBM4R | FPPPDFAVEAFQHAVSG |  |  |
|  | GBM5P | FPPPDFAVEAFQHAVSG |  |  |
|  | GBM6P | FPPPDFAVEAFQHAVSG |  |  |
|  | GBM7P | RDHMIRSLQSVGLKPIIP |  |  |
|  | GBM12R | GLVAIPVSIFYSVPHQKHFDHYIRF |  |  |
|  | GBM18P | LDPMELAGKFTSRTKALVLNTPNNP |  |  |
|  | GBM18R | EQLLFRQPSSYFVQ |  |  |
|  | ZH645 | FPPPDFAVEAFQHAVSG |  |  |
|  | ZH654 | FPPPDFAVEAFQHAVSG |  |  |

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|---|---|---|---|---|
| Ras-specific guanine nucleotide-releasing factor RalGPS1 (RALGPS1) | <u>13% P / 13% R</u> | | Q5JS13 ■ | 1 |
| | GBM11R | PTPPVPRHRKSHS | | |
| | GBM17R | PTPPVPRHRKSHS | | |
| | GBM19P | KRTREYIRSLKMVP | | |
| | ZH654 | PTPPVPRHRKSH | | |
| | | PTPPVPRHRKSHS | | |
| | ZH750 | PTPPVPRHRKSH | | |
| | | PTPPVPRHRKSHS | | |
| | ZH753 | PTPPVPRHRKSH | | |
| | | PTPPVPRHRKSHS | | |
| | ZH802 | PTPPVPRHRKSH | | |
| | | PTPPVPRHRKSHS | | |
| | ZH829 | PTPPVPRHRKSHS | | |
| Tripartite motif-containing protein 58 (TRIM58) | <u>16% P / 8% R</u> | | Q8NG06 ■ | 3 |
| | GBM12R | KKTVIWKEKVEMQRQRFRLEFEKHR | | |
| | GBM13P | KKTVIWKEKVEMQRQRFRLEFEKHR | | |
| | GBM16P | KKTVIWKEKVEMQRQRFRLEFEKHR | | |
| | GBM22P | KKTVIWKEKVEMQRQRFRLEFEKHR | | |
| | ZH645 | KKTVIWKEKVEMQRQRFRLEFEKHR | | |
| | ZH757 | KKTVIWKEKVEMQRQRFRLEFEKHR | | |
| | ZH761 | KKTVIWKEKVEMQRQRFRLEFEKHR | | |
| | ZH784 | KKTVIWKEKVEMQRQRFRLEFEKHR | | |
| Anaphase-promoting complex subunit CDC26 (CDC26) | <u>5% P / 25% R</u> | | Q8NHZ8 ■ | 1 |
| | GBM8P | AIGLSSDPKSRE | | |
| | GBM8R | AIGLSSDPKSRE | | |
| | GBM12P | AIGLSSDPKSRE | | |
| | GBM14R | AIGLSSDPKSRE | | |
| | GBM15R | AIGLSSDPKSRE | | |
| | GBM17R | AIGLSSDPKSRE | | |
| | GBM19R | AIGLSSDPKSRE | | |
| | GBM21R | AIGLSSDPKSRE | | |
| NKG2D ligand 2 (ULBP2) / retinoic acid early transcript 1G protein (RAET1G) | <u>8%/11% P</u> | | Q9BZM5 ■ | 2 |
| | <u>17%/13% R</u> | | Q6H3X3 ■ | 3 |
| | GBM4P | KAQNPVLREVVDILTEQLL (RAET1G/RAET1L) | | |
| 2 peptides multi-map to GBM-exclusive retinoic acid early transcript 1L protein (RAET1L; Q5VY80) | GBM4R | LILCCLLIILPCFILP (ULBP2/RAET1L) | | |
| | GBM7R | KLNVTTAWKAQNPVLREVVDILT (ULBP2/RAET1G) | | |
| | GBM8R | KLNVTTAWKAQNPVLREVVDILT (ULBP2/RAET1G) | | |
| | GBM10R | KLNVTTAWKAQNPVLREVVDILT (ULBP2/RAET1G) | | |
| | GBM13P | KLNVTTAWKAQNPVLREVVDILT (ULBP2/RAET1G) | | |
| | ZH791 | KLNVTTAWKAQNPVLREVVDILT (ULBP2/RAET1G) | | |
| | ZH829 | KLNVTTAWKAQNPVLREVVDILT (ULBP2/RAET1G) | | |
| Hepatocyte nuclear factor 6 (ONECUT1) | <u>13% P / 13% R</u> | | Q9UBC0 ■ | 2 |
| | GBM9R | KRKEQEHGKDRGNTPKKP | | |
| | GBM11P | KRKEQEHGKDRGNTPKKP | | |
| | GBM11R | KRKEQEHGKDRGNTPKKP | | |
| | GBM13P | KRKEQEHGKDRGNTPKKP | | |
| | GBM14P | KRKEQEHGKDRGNTPKKP | | |
| | GBM16R | KRKEQEHGKDRGNTPKKP | | |
| | GBM21P | KRKEQEHGKDRGNTPKKP | | |
| | ZH802 | KRKEQEHGKDRGNTPKKP | | |
| Sushi repeat-containing protein SRPX2 (SRPX2) | <u>11% P / 21% R</u> | | O60687 ■ | 7 |
| | GBM2P | IDRDRYMEPVTPEE | | |
| | GBM9R | APDPSNRYYKMQISMLQQST | | |
| | GBM14P | GEHVIRYTAYDR | | |
| | | GEHVIRYTAYDRAYN | | |
| | GBM14R | GEHVIRYTAYDR | | |
| | | GEHVIRYTAYDRAY | | |
| | | GEHVIRYTAYDRAYN | | |
| | GBM18R | IDRDRYMEPVTPEE | | |
| | GBM20P | APDPSNRYYKMQISMLQQST | | |
| | GBM20R | APDPSNRYYKMQISMLQQST | | |
| | GBM21P | EGEHVIRYTAYDRAY | | |
| | | GEHVIRYTAYDR | | |
| | | GEHVIRYTAYDRAY | | |
| | | GEHVIRYTAYDRAYN | | |
| | GBM21R | GEHVIRYTAYDRAYN | | |
| | | GPEPGSHFPEGEHVIRYTAYDRAYN | | |
| Complement C1q-like protein 3 (C1QL3) | <u>8% P / 21% R</u> | | Q5VWW1 ■ | 1 |
| | GBM7P | EPGDEVYIKLDGGKAHG | | |
| | | EPGDEVYIKLDGGKAHGGN | | |
| | GBM7R | EPGDEVYIKLDGGKAHGGN | | |
| | GBM12R | EPGDEVYIKLDGGKAHGGN | | |
| | GBM14R | TVPKIAFYAGLKRQHEG | | |
| | | VPKIAFYAGLKRQHEG | | |
| | GBM21P | TVPKIAFYAGLKRQHEG | | |
| | | VPKIAFYAGLKRQH | | |

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|---|---|---|---|
|  | GBM21R | TVPKIAFYAGLKRQH | |
|  |  | TVPKIAFYAGLKRQHEG | |
|  |  | VPKIAFYAGLKRQH | |
|  |  | VPKIAFYAGLKRQHE | |
|  |  | VPKIAFYAGLKRQHEG | |
|  | ZH616 | KGEPGRQGLPGPPGAP | |
|  | ZH753 | EPGDEVYIKLDGGKAHG | |
|  |  | EPGDEVYIKLDGGKAHGGN | |
| Zinc finger protein GLIS1 (GLIS1) | 5% P / 21% R | | Q8NBF1 ▪ 6 |
|  | GBM4 | LPSKPSYPPFQSPPPPPLP | |
|  | GBM9R | LPSKPSYPPFQSPPPPPL | |
|  | GBM10R | LPSKPSYPPFQSPPPPPLP | |
|  | GBM11R | LPSKPSYPPFQSPPPPPLP | |
|  | GBM16P | LPSKPSYPPFQSPPPPPLP | |
|  | GBM16R | LPSKPSYPPFQSPPPPPLP | |
|  | ZH616 | LPSKPSYPPFQSPPPPPL | |
| Roundabout homolog 2 (ROBO2) | 13% P / 13% R | | Q9HCK4 ▪ 3 |
|  | GBM10R | KKDKVRIDDKEER | |
|  |  | KKDKVRIDDKEERISIR | |
|  | GBM13P | GRYDIKDDYTLRIKK | |
|  |  | KKDKVRIDDKEERISIR | |
|  | GBM13R | KDKVRIDDKEERISIR | |
|  |  | KKDKVRIDDKEERIS | |
|  |  | KKDKVRIDDKEERISIR | |
|  |  | WKKDKVRIDDKEERISIR | |
|  | GBM16P | KDKVRIDDKEERIS | |
|  |  | KKDKVRIDDKEER | |
|  |  | KKDKVRIDDKEERIS | |
|  |  | KKDKVRIDDKEERISIR | |
|  |  | WKKDKVRIDDKEERIS | |
|  |  | WKKDKVRIDDKEERISIR | |
|  | GBM16R | KKDKVRIDDKEERISIR | |
|  | GBM18P | KKDKVRIDDKEERISIR | |
|  | ZH613 | LTDRPPPIILQGPANQTL | |
|  | ZH654 | RNEVVITENNNSIT | |
| Striatin-4 (STRN4) | 13% P / 8% R | | Q9NRL3 ▪ 4 |
|  | GBM11P | SGSHDCSLRLWSLDNK | |
|  | GBM14R | KGQENLKTD | |
|  | GBM18P | DDRGIRFLDNRTGKPV | |
|  | GBM18R | DDRGIRFLDNRTGKPV | |
|  | ZH613 | NSPLVWKEGRQLLR | |
|  | ZH654 | KGQENLKTD | |
|  | ZH802 | DDRGIRFLDNRTGKPV | |
| Beta-crystallin S (CRYGS) | 13% P / 4% R | | P22914 ▪ 1 |
|  | GBM16P | RGRQYLLDKKEYRKPID | |
|  | GBM18P | GRQYLLDKKEYRKPID | |
|  | GBM20R | KITFYEDKNFQGRR | |
|  | ZH645 | GRQYLLDKKEYRKPID | |
|  |  | RGRQYLLDKKEYRKPID | |
|  | ZH681 | GRQYLLDKKEYRKPID | |
|  | ZH802 | GRQYLLDKKEYRKPID | |
|  |  | KGDFSGQMYETTED | |
| Aftiphilin (AFTPH) | 8% P / 13% R | | Q6ULP2 ▪ 1 |
|  | GBM9P | LYELTTSKLEISTSSL | |
|  | GBM10R | LYELTTSKLEISTSSL | |
|  | GBM17R | LYELTTSKLEISTSSL | |
|  | GBM18P | LYELTTSKLEISTSSL | |
|  | GBM18R | LYELTTSKLEISTSSL | |
|  | ZH750 | LYELTTSKLEISTSSL | |
| Latent-transforming growth factor beta-binding protein (LTBP3) | 13% P / 8% R | | Q9NS15 ▪ 3 |
|  | GBM5P | ESNSFWDTSPLLLGKPP | |
|  | GBM13P | ESNSFWDTSPLLLGKPP | |
|  |  | SNSFWDTSPLLLGKPP | |
|  | GBM13R | ESNSFWDTSPLLLGKPP | |
|  |  | SNSFWDTSPLLLGKPP | |
|  | GBM14P | VQVHRIESSNAESAAPS | |
|  | GBM14R | ASVQVHRIESSNAESAAPS | |
|  |  | QVHRIESSNAESAAPS | |
|  |  | SVQVHRIESSNAESAAPS | |
|  |  | VQVHRIESSNAESAAPS | |
|  | ZH654 | ESNSFWDTSPLLLGKPP | |
|  |  | SNSFWDTSPLLLGKPP | |
|  | ZH681 | ESNSFWDTSPLLLGKPP | |
|  |  | SNSFWDTSPLLLGKPP | |
| DNA repair protein RAD51 homolog 1 (RAD51) | 8% P / 8% R | | Q06609 ♦ 2 |
|  | GBM4P | KLVPMGFTTATEFHQRRSEII | |
|  | GBM17P | KLVPMGFTTATEFHQRRSEII | |
|  | GBM18R | NDVKKLEEAGFHTV | |
|  | GBM20P | KLVPMGFTTATEFHQRRSEII | |

| | GBM22R | KTQICHTLAVTCQLPI | | |
|---|---|---|---|---|
| Protein Dos (DOS) | <u>8% P / 8% R</u> | | Q8N350 ■ | 1 |
| | GBM6P | KVKKWKLEPSQRAAS | | |
| | | KVKKWKLEPSQRAASL | | |
| | GBM7P | KVKKWKLEPSQRAAS | | |
| | | KVKKWKLEPSQRAASL | | |
| | GBM10R | RRDYSIDEKTDALFHEFLRHDP | | |
| | GBM12P | KVKKWKLEPSQRAAS | | |
| | | KVKKWKLEPSQRAASL | | |
| | GBM20R | KVKKWKLEPSQRAAS | | |
| | | KVKKWKLEPSQRAASL | | |
| | | VKKWKLEPSQRAAS | | |
| Copper-transporting ATPase 2 (ATP7B) | <u>8% P / 8% R</u> | | P35670 ▢ | 4 |
| | GBM9P | LMAGKAEIKYDPEVIQP | | |
| | GBM10R | KSIEDRISNLKGIIS | | |
| | GBM13P | KSIEDRISNLKGIIS | | |
| | GBM18R | GEDNLIIREEQVPME | | |
| | ZH617 | KGGKPLEMAHKIKTVMFDKTGTITH | | |
| Putative mitochondrial ribosome-binding factor A (RBFA) | <u>8% P / 8% R</u> | | Q8N0V3 ■ | 11 |
| | GBM2P | KNWLKKFASKTKK | | |
| | GBM3R | KNWLKKFASKTKK | | |
| | GBM5P | KNWLKKFASKTKK | | |
| | GBM6P | KNWLKKFASKTKK | | |
| | GBM7R | KNWLKKFASKTKK | | |

**Supplementary Table 3. Glioblastoma-associated HLA class I ligands presented on at least six primary and four recurrent tumors of a minimum of eight different patients.** Peptides already reported to derive from glioblastoma-associated antigens were excluded from this listing. In turn, some of those antigens not designated as glioblastoma-associated due to a CNS-associated expression profile are listed herein with glioblastoma-associated peptides. The number of positive tumors other than glioblastoma was based on n=824 HLA class I peptidome datasets. HLA restrictions not passing manual assessment as quality control are indicated in italic. These combinations of sequence and HLA restriction were excluded from downstream analyses such as calculation of peptides matching per patient worldwide. All peptides were restricted to at least one HLA allotype covered by the CNS-related subset (brain, cerebellum, and spinal cord) of the benign database. Frequencies of positive tumors are given separately for n=38 primary (P) and n=24 recurrent (R) glioblastomas.

| Peptide sequence | HLA restriction | Frequency of positive tumors | | Antigen (UniProt accession) | Protein frequency on glioblastomas | Peptide-positive non-GBM tumors / Protein frequency on non-GBM tumors | Protein frequency on benign samples (n=418) |
|---|---|---|---|---|---|---|---|
| ALAAELNQL | A*02:01 A*02:05 | <u>55% P</u> GBM2P GBM2R GBM3P GBM6P GBM6R GBM7P GBM7R GBM10P GBM10R GBM11P GBM11R GBM12P GBM13R GBM14P GBM14R GBM15P | <u>42% R</u> GBM20P GBM20R GBM21 ZH613 ZH616 ZH617 ZH645 ZH678 ZH681 ZH720 ZH750 ZH757 ZH761 ZH784 ZH829 | Glial fibrillary acidic protein (GFAP; P14136) | 100% P 88% R | 6 12% | 16% |
| FLHDISDVQL | A*02:01 A*02:05 | <u>47% P</u> GBM2P GBM2R GBM3P GBM6P GBM6R GBM7P GBM7R GBM10R GBM11P GBM11R GBM13P GBM13R GBM14P GBM14R | <u>42% R</u> GBM19P GBM20R GBM21P ZH613 ZH616 ZH617 ZH645 ZH678 ZH681 ZH720 ZH753 ZH757 ZH761 ZH784 | Ceramide synthase 1 (CERS1; P27544) | 68% P 71% R | 3 2% | 2% |

| YVSSGEMMV | A*02:01 | 47% P | 42% R | Glial fibrillary acidic | 100% P | 2 | 16% |
|---|---|---|---|---|---|---|---|
| | A*02:05 | GBM3P | GBM21P | protein (GFAP; P14136) | 88% R | 12% | |
| | C*02:02 | GBM6P | GBM23R | | | | |
| | *C*04:01* | GBM6R | ZH613 | | | | |
| | *C*05:01* | GBM7P | ZH616 | | | | |
| | C*15:02 | GBM7R | ZH617 | | | | |
| | C*16:01 | GBM10R | ZH645 | | | | |
| | | GBM11P | ZH678 | | | | |
| | | GBM12R | ZH681 | | | | |
| | | GBM13P | ZH753 | | | | |
| | | GBM13R | ZH757 | | | | |
| | | GBM14P | ZH761 | | | | |
| | | GBM14R | ZH802 | | | | |
| | | GBM15P | ZH829 | | | | |
| GLLDGVFNV | A*02:01 | 45% | 46% | Carnosine synthase 1 | 63% P | 13 | 0% |
| | *B*13:02* | GBM2P | GBM19R | (CARNS1; A5YM72) | 71% R | 2% | |
| | | GBM2R | GBM21P | | | | |
| | | GBM3P | GBM21R | | | | |
| | | GBM5P | ZH617 | | | | |
| | | GBM7P | ZH645 | | | | |
| | | GBM7R | ZH678 | | | | |
| | | GBM10R | ZH681 | | | | |
| | | GBM11R | ZH720 | | | | |
| | | GBM12R | ZH750 | | | | |
| | | GBM13P | ZH753 | | | | |
| | | GBM13R | ZH757 | | | | |
| | | GBM14P | ZH761 | | | | |
| | | GBM14R | ZH784 | | | | |
| | | GBM19P | ZH829 | | | | |
| YQDLLNVKL | *A*02:01* | 47% P | 38% R | Glial fibrillary acidic | 100% P | 7 | 16% |
| | *A*02:05* | GBM3P | GBM20P | protein (GFAP; P14136) | 88% R | 12% | |
| | B*13:02 | GBM7P | GBM20R | | | | |
| | C*02:02 | GBM7R | ZH613 | | | | |
| | C*04:01 | GBM9P | ZH616 | | | | |
| | C*05:01 | GBM9R | ZH617 | | | | |
| | C*08:02 | GBM11P | ZH678 | | | | |
| | C*15:02 | GBM11R | ZH681 | | | | |
| | | GBM12R | ZH720 | | | | |
| | | GBM13P | ZH753 | | | | |
| | | GBM14P | ZH757 | | | | |
| | | GBM14R | ZH761 | | | | |
| | | GBM15P | ZH784 | | | | |
| | | GBM19P | ZH802 | | | | |
| | | GBM19R | | | | | |
| SLWAGVVVL | A*02:01 | 42% P | 42% R | Chitinase-3-like protein | 63% P | 2 | 1% |
| | | GBM2P | GBM19R | 2 (CHI3L2; Q15782) | 67% R | 2% | |
| | | GBM2R | GBM20P | | | | |
| | | GBM3P | GBM21P | | | | |
| | | GBM7P | GBM21R | | | | |
| | | GBM7R | ZH617 | | | | |
| | | GBM10R | ZH645 | | | | |
| | | GBM12P | ZH681 | | | | |
| | | GBM12R | ZH750 | | | | |
| | | GBM13P | ZH753 | | | | |
| | | GBM13R | ZH757 | | | | |
| | | GBM14P | ZH761 | | | | |
| | | GBM14R | ZH784 | | | | |
| | | GBM19P | ZH829 | | | | |
| ALVSNLYVI | A*02:01 | 42% P | 17% R | Protein transport protein | 97% / 97% P | 31 | 63% / 61% |
| | | GBM2P | GBM21P | Sec61 subunit alpha | 100% / 100% R | 60% / 51% | |
| | | GBM3P | ZH617 | isoform 1/2 | | | |
| | | GBM7P | ZH645 | (SEC61A1/2; P61619 / | | | |
| | | GBM11P | ZH678 | Q9H9S3) | | | |
| | | GBM13P | ZH681 | | | | |
| | | GBM13R | ZH720 | | | | |
| | | GBM14R | ZH753 | | | | |
| | | GBM15P | ZH757 | | | | |
| | | GBM20P | ZH761 | | | | |
| | | GBM20R | ZH829 | | | | |
| ALIEVGEGVNL | A*02:01 | 37% P | 29% R | PNMA-like protein 1 | 45% P | 8 | 0% |
| | | GBM2P | GBM21P | (PNMAL1; Q86V59) | 42% R | 2% | |
| | | GBM2R | ZH617 | | | | |
| | | GBM7P | ZH645 | | | | |
| | | GBM10R | ZH681 | | | | |
| | | GBM11P | ZH720 | | | | |
| | | GBM13P | ZH753 | | | | |
| | | GBM13R | ZH757 | | | | |
| | | GBM14R | ZH761 | | | | |
| | | GBM19P | ZH784 | | | | |

| Peptide | HLA | Samples | | Protein | | | |
|---|---|---|---|---|---|---|---|
| | | GBM20P GBM20R | ZH829 | | | | |
| ILDHNTMQV | A*02:01 C*05:01 | <u>32% P</u> GBM3P GBM7P GBM7R GBM10R GBM11R GBM12R GBM13P GBM13R GBM9R GBM21P | <u>33% R</u> ZH617 ZH645 ZH678 ZH681 ZH720 ZH753 ZH757 ZH761 ZH784 ZH829 | Pleckstrin homology domain-containing family H member 1 (PLEKHH1; Q9ULM0) | 63% P 79% R | 0 4% | 2% |
| ALAQYLITA | A*02:01 | <u>34% P</u> GBM2R GBM3P GBM7P GBM7R GBM10R GBM11P GBM11R GBM12R GBM13P GBM13R | <u>29% R</u> GBM20P ZH617 ZH645 ZH678 ZH681 ZH720 ZH753 ZH757 ZH761 ZH829 | Poly(rC)-binding protein 4 (PCBP4; P57723) | 61% P 59% R | 5 4% | 11% |
| GLLDQIQAL | A*02:01 | <u>32% P</u> GBM3P GBM7P GBM7R GBM10R GBM12R GBM13R GBM14P GBM14R GBM20R GBM21P | <u>29% R</u> ZH617 ZH678 ZH681 ZH720 ZH750 ZH753 ZH757 ZH761 ZH829 | Neuroligin-2/3 / X-/Y-linked neuroligin-4 (NLGN2/3/4X/4Y; Q8NFZ4 / Q9NZ94 / Q8N0W4 / Q8NFZ3) | 34% / 61% / 50% / 50% P 29% / 54% / 42% / 42% R | 7 2% / 3% / 2% / 2% | 1% / 4% / 2% / 2% |
| VLDSHIHAY | A*01:01 A*03:01 B*15:01 *C*01:02* C*04:01 *C*07:01* | <u>37% P</u> GBM3P GBM4P GBM4R GBM5P GBM5R GBM6P GBM9P GBM9R GBM10R GBM13P GBM13R GBM16P | <u>38% R</u> GBM16R GBM17P GBM17R GBM18P GBM22R GBM23P GBM23R ZH617 ZH645 ZH654 ZH761 | Receptor-type tyrosine-protein phosphatase zeta (PTPRZ1; P23471) | 97% P 83% R | 6 6% | 5% |
| KLTEENTTL | A*02:01 A*02:05 *B*13:02* | <u>37% P</u> GBM3P GBM7P GBM11P GBM12R GBM13P GBM14P GBM14R GBM15P GBM20P | <u>17% R</u> GBM20R GBM21P ZH616 ZH617 ZH678 ZH681 ZH753 ZH757 ZH761 | Retrotransposon-derived protein PEG10 (PEG10; Q86TG7) | 66% P 46% R | 18 10% | 6% |
| YYTVRNFTL | A*23:01 A*24:02 C*06:02 C*07:02 *C*07:04* | <u>34% P</u> GBM2P GBM4P GBM4R GBM8P GBM10R GBM11P GBM11R GBM15P GBM16P GBM16R GBM18R | <u>33% R</u> GBM19P GBM19R GBM22P GBM22R GBM23P ZH616 ZH681 ZH757 ZH784 ZH791 | Receptor-type tyrosine-protein phosphatase zeta (PTPRZ1; P23471) | 97% P 83% R | 0 6% | 5% |
| GLVEKVQAA | A*02:01 A*02:05 | <u>34% P</u> GBM2R GBM3P GBM6P GBM7P GBM7R GBM10R GBM11P GBM11R | <u>29% R</u> GBM13R GBM15P GBM20P GBM20R GBM21P ZH613 ZH617 ZH645 | Apolipoprotein E (APOE; P02649) | 76% P 67% R | 25 16% | 12% |

| Peptide | HLA | % P / samples (P) | % R / samples (R) | Protein | %P / %R | | |
|---|---|---|---|---|---|---|---|
| | | GBM12R<br>GBM13P | ZH678<br>ZH681 | | | | |
| AIIDGVESV | A*02:01<br>A*02:05 | 34% P<br>GBM7P<br>GBM10R<br>GBM11P<br>GBM12P<br>GBM12R<br>GBM13P<br>GBM14P<br>GBM14R<br>GBM15P | 21% R<br>GBM20R<br>GBM21P<br>ZH617<br>ZH645<br>ZH678<br>ZH681<br>ZH753<br>ZH757<br>ZH761 | Receptor-type tyrosine-protein phosphatase zeta (PTPRZ1; P23471) | 97% P<br>83% R | 0<br>6% | 5% |
| SLPELVHAV | A*02:01<br>A*02:05 | 32% P<br>GBM3P<br>GBM6P<br>GBM7P<br>GBM7R<br>GBM10R<br>GBM11P<br>GBM11R<br>GBM12R<br>GBM13P<br>GBM13R | 29% R<br>GBM14R<br>GBM21P<br>ZH613<br>ZH617<br>ZH678<br>ZH681<br>ZH753<br>ZH757<br>ZH761 | Sestrin-3 (SESN3; P58005) | 42% P<br>42% R | 19<br>12% | 4% |
| LLWGNAIFL | A*02:01 | 34% P<br>GBM2P<br>GBM2R<br>GBM3P<br>GBM7P<br>GBM7R<br>GBM12R<br>GBM13R<br>GBM14P<br>GBM19P | 17% R<br>GBM21P<br>ZH616<br>ZH617<br>ZH678<br>ZH681<br>ZH750<br>ZH761<br>ZH829 | Kinesin-like protein KIF1A (KIF1A; Q12756) | 87% P<br>79% R | 9<br>14% | 15% |
| AIIGGMFTV | A*02:01<br>A*02:05 | 32% P<br>GBM6P<br>GBM10R<br>GBM11P<br>GBM11R<br>GBM12R<br>GBM13P<br>GBM13R<br>GBM14P<br>GBM14R<br>GBM20P | 29% R<br>GBM20R<br>GBM21P<br>ZH613<br>ZH617<br>ZH681<br>ZH720<br>ZH753<br>ZH757<br>ZH761 | Endoplasmic reticulum-Golgi intermediate compartment protein 3 (ERGIC3; Q9Y282) | 71% P<br>63% R | 22<br>39% | 26% |
| KIGPVGAVV | A*02:01<br>A*02:05<br>*C*01:02*<br>C*15:02 | 18% P<br>GBM6R<br>GBM7R<br>GBM10R<br>GBM11R<br>GBM12R<br>GBM13R<br>GBM19R<br>GBM21P | 33% R<br>ZH613<br>ZH678<br>ZH681<br>ZH720<br>ZH753<br>ZH757<br>ZH829 | Myelin-associated glycoprotein (MAG; P20916) | 50% P<br>63% R | 0<br>0% | 1% |
| ALQTIQLFL | A*02:01<br>*B*13:02* | 32% P<br>GBM2P<br>GBM2R<br>GBM3P<br>GBM3R<br>GBM7P<br>GBM7R<br>GBM13P<br>GBM13R<br>GBM14P<br>GBM14R | 29% R<br>GBM15P<br>GBM21P<br>ZH617<br>ZH645<br>ZH681<br>ZH720<br>ZH753<br>ZH761<br>ZH784 | Dystonin (DST; Q03001) | 97% P<br>92% R | 9<br>41% | 55% |
| QLNEQVHSL | A*02:01<br>A*02:05 | 32% P<br>GBM3P<br>GBM10R<br>GBM11P<br>GBM13P<br>GBM13R<br>GBM14P<br>GBM14R<br>GBM20P<br>GBM20R | 21% R<br>GBM21P<br>ZH617<br>ZH678<br>ZH681<br>ZH753<br>ZH757<br>ZH761<br>ZH829 | Microtubule-associated protein RP/EB family member 2 (MAPRE2; Q15555) | 39% P<br>21% R | 7<br>6% | 9% |
| SLGLFLAQV | A*02:01 | 29% P<br>GBM3P<br>GBM3R<br>GBM7P | 25% R<br>GBM20R<br>GBM21P<br>ZH617 | Scavenger receptor class A member 3 (SCARA3; Q6AZY7) | 53% P<br>38% R | 10<br>4% | 5% |

|  |  | | | | | | |
|---|---|---|---|---|---|---|---|
|  |  | GBM7R | ZH645 |  | | | |
|  |  | GBM12R | ZH678 |  | | | |
|  |  | GBM13R | ZH750 |  | | | |
|  |  | GBM14R | ZH757 |  | | | |
|  |  | GBM15P | ZH761 |  | | | |
|  |  | GBM20P |  |  | | | |
| ALLDGTVFEI | A*02:01 | <u>29% P</u> | <u>25% R</u> | Protein DGCR6 / DGCR6L (DGCR6/6L; Q9BY27 / Q14129 | 34% / 34% P 33% / 33% R | 24 7% / 6% | 3% / 2% |
|  |  | GBM3P | GBM20P |  | | | |
|  |  | GBM3R | GBM20R |  | | | |
|  |  | GBM7P | GBM21P |  | | | |
|  |  | GBM10R | ZH645 |  | | | |
|  |  | GBM11P | ZH681 |  | | | |
|  |  | GBM13P | ZH720 |  | | | |
|  |  | GBM13R | ZH757 |  | | | |
|  |  | GBM14R | ZH784 |  | | | |
|  |  | GBM19P |  |  | | | |
| ALSPNNHEV | A*02:01 | <u>29% P</u> | <u>21% R</u> | Actin-related protein 2/3 complex subunit 1A (ARPC1A; Q92747) | 29% P 21% R | 9 7% | 10% |
|  |  | GBM3P | GBM21P |  | | | |
|  |  | GBM7P | ZH617 |  | | | |
|  |  | GBM7R | ZH678 |  | | | |
|  |  | GBM12R | ZH681 |  | | | |
|  |  | GBM13P | ZH753 |  | | | |
|  |  | GBM14R | ZH757 |  | | | |
|  |  | GBM20P | ZH761 |  | | | |
|  |  | GBM20R | ZH829 |  | | | |
| KIPPVTPSI | A*02:01 A*02:05 C*01:02 C*02:02 *C*03:03* C*12:03 C*15:02 | <u>29% P</u> | <u>21% R</u> | Arrestin domain-containing protein 4 (ARRDC4; Q8NCT1) | 63% P / 50% R | 12 | 12% |
|  |  | GBM3P | GBM14R |  | | | |
|  |  | GBM5P | GBM21P |  | | | |
|  |  | GBM10R | ZH617 |  | | | |
|  |  | GBM11P | ZH645 |  | | | |
|  |  | GBM12R | ZH678 |  | | | |
|  |  | GBM13P | ZH681 |  | | | |
|  |  | GBM13R | ZH753 |  | | | |
|  |  | GBM14P | ZH757 |  | | | |
| LLLDTVTSI | A*02:01 | <u>26% P</u> | <u>25% R</u> | Microtubule-associated protein 1A (MAP1A; P78559) | 76% P 67% R | 2 | 6% |
|  |  | GBM3P | GBM21P |  | | | |
|  |  | GBM7P | ZH681 |  | | | |
|  |  | GBM10R | ZH720 |  | | | |
|  |  | GBM13P | ZH753 |  | | | |
|  |  | GBM13R | ZH757 |  | | | |
|  |  | GBM14R | ZH761 |  | | | |
|  |  | GBM20P | ZH784 |  | | | |
|  |  | GBM20R | ZH829 |  | | | |
| GLLPSPLAV | A*02:01 *B*13:02* | <u>26% P</u> | <u>25% R</u> | Zinc finger protein 385A (ZNF385A; Q96PM9) | 71% P 67% R | 25 | 15% |
|  |  | GBM3P | GBM14R |  | | | |
|  |  | GBM5P | GBM21P |  | | | |
|  |  | GBM5R | ZH617 |  | | | |
|  |  | GBM7P | ZH645 |  | | | |
|  |  | GBM10R | ZH678 |  | | | |
|  |  | GBM12R | ZH753 |  | | | |
|  |  | GBM13P | ZH761 |  | | | |
|  |  | GBM13R | ZH829 |  | | | |
| TLQEQLEKA | A*02:01 A*02:05 | <u>26% P</u> | <u>25% R</u> | Pinin (PNN; Q9H307) | 63% P 42% R | 21 | 21% |
|  |  | GBM3P | GBM15P |  | | | |
|  |  | GBM7P | GBM20P |  | | | |
|  |  | GBM10R | GBM20R |  | | | |
|  |  | GBM11P | GBM21P |  | | | |
|  |  | GBM12R | ZH616 |  | | | |
|  |  | GBM13R | ZH617 |  | | | |
|  |  | GBM14P | ZH681 |  | | | |
|  |  | GBM14R | ZH753 |  | | | |
| TLYEQEIEV | A*02:01 | <u>24% P</u> | <u>38% R</u> | Procollagen galactosyltransferase 2 (COLGALT2; Q8IYK4) | 39% P 54% R | 3 | 1% |
|  |  | GBM2P | GBM13P |  | | | |
|  |  | GBM2R | GBM13R |  | | | |
|  |  | GBM3P | GBM14R |  | | | |
|  |  | GBM7P | GBM20R |  | | | |
|  |  | GBM7R | ZH617 |  | | | |
|  |  | GBM10R | ZH678 |  | | | |
|  |  | GBM11P | ZH681 |  | | | |
|  |  | GBM11R | ZH753 |  | | | |
|  |  | GBM12R | ZH757 |  | | | |
| GTLEIPVAQK | A*03:01 A*11:01 | <u>29% P</u> | <u>25% R</u> | Neuronal cell adhesion molecule (NRCAM; Q92823) | 47% P 29% R | 0 | 1% |
|  |  | GBM4P | GBM16P |  | | | |
|  |  | GBM4R | GBM17R |  | | | |
|  |  | GBM6P | GBM18P |  | | | |
|  |  | GBM6R | GBM22P |  | | | |
|  |  | GBM7P | GBM23P |  | | | |
|  |  | GBM7R | ZH631 |  | | | |
|  |  | GBM10R | ZH678 |  | | | |

|  |  | GBM12P<br>GBM12R | ZH791 |  |  |  |  |
|---|---|---|---|---|---|---|---|
| RVSLPSYPR | A*03:01<br>A*11:01<br>A*31:01 | 29% P<br>GBM4P<br>GBM6P<br>GBM7P<br>GBM7R<br>GBM10R<br>GBM16P<br>GBM16R<br>GBM17R | 21% R<br>GBM18P<br>GBM22P<br>GBM23P<br>GBM23R<br>ZH631<br>ZH654<br>ZH678<br>ZH802 | Neurocan core protein (NCAN; O14594) | 34% P<br>21% R | 2 | 1% |
| ILNVDGLIGV | A*02:01 | 29% P<br>GBM7P<br>GBM10R<br>GBM11P<br>GBM13P<br>GBM13R<br>GBM14R<br>GBM19P<br>GBM20P | 17% R<br>GBM20R<br>GBM21P<br>ZH645<br>ZH681<br>ZH720<br>ZH757<br>ZH829 | ATP-citrate synthase (ACLY; P53396) | 89% P<br>63% R | 11 | 30% |
| ILKDGIHNV | A*02:01<br>A*02:05 | 29% P<br>GBM3P<br>GBM11P<br>GBM13P<br>GBM13R<br>GBM14R<br>GBM20P<br>GBM20R<br>GBM21P | 17% R<br>ZH616<br>ZH617<br>ZH645<br>ZH678<br>ZH681<br>ZH753<br>ZH761 | Prolow-density lipoprotein receptor-related protein 1 (LRP1; Q07954) | 74% P<br>71% R | 14 | 38% |
| KLQEANAQL | A*02:01 | 26% P<br>GBM3P<br>GBM7P<br>GBM7R<br>GBM10R<br>GBM11P<br>GBM12R<br>GBM14P<br>GBM14R | 25% R<br>GBM20P<br>GBM20R<br>GBM21P<br>ZH617<br>ZH678<br>ZH681<br>ZH753<br>ZH757 | Lethal(2) giant larvae protein homolog 1 (LLGL1; Q15334) | 47% P<br>54% R | 10 | 6% |
| QLNEKVAQL | A*02:01<br>B*08:01 | 26% P<br>GBM9P<br>GBM12R<br>GBM13P<br>GBM13R<br>GBM14R<br>GBM16P<br>GBM20P<br>GBM20R | 21% R<br>GBM21P<br>ZH617<br>ZH678<br>ZH681<br>ZH753<br>ZH761<br>ZH829 | Lanosterol 14-alpha demethylase (CYP51A1; Q16850) | 82% P<br>58% R | 32 | 31% |
| NIIPRFVQV | A*02:01<br>A*02:05<br>B*08:01<br>C*16:01 | 26% P<br>GBM7P<br>GBM11P<br>GBM13R<br>GBM14R<br>GBM19P<br>GBM20P<br>GBM20R | 17% R<br>GBM21P<br>ZH617<br>ZH678<br>ZH681<br>ZH753<br>ZH757<br>ZH761 | SLIT-ROBO Rho GTPase-activating protein 2 / 2C (SRGAP2 / 2C; O75044 / P0DJJ0) | 66% / 66% P<br>54% / 54% R | 14<br>14% / 11% | 15% / 14% |
| SLNGTIFTV | A*02:01 | 26% P<br>GBM3P<br>GBM7P<br>GBM12R<br>GBM13P<br>GBM13R<br>GBM14R<br>GBM20P | 17% R<br>GBM21P<br>ZH617<br>ZH678<br>ZH681<br>ZH753<br>ZH757<br>ZH761 | Bromodomain and PHD finger-containing protein 3 (BRPF3; Q9ULD4) | 29% P<br>21% R | 11<br>3% | 1% |
| VIFDLPTTV | A*02:01<br>A*02:05<br>C*12:03<br>C*16:01 | 26% P<br>GBM3P<br>GBM7P<br>GBM10R<br>GBM11P<br>GBM13P<br>GBM13R<br>GBM14R | 17% R<br>GBM21P<br>ZH617<br>ZH645<br>ZH678<br>ZH753<br>ZH757<br>ZH761 | Uncharacterized protein KIAA0556 (KIAA0556; O60303) | 39% P<br>29% R | 17<br>8% | 5% |
| ALDGKIYEL | A*02:01 | 26% P<br>GBM3P<br>GBM7P<br>GBM10R<br>GBM11P<br>GBM13P<br>GBM13R<br>GBM14R | 17% R<br>GBM20R<br>GBM21P<br>ZH617<br>ZH681<br>ZH720<br>ZH757<br>ZH761 | Rho GTPase-activating protein 5 (ARHGAP5; Q13017) | 42% P<br>42% R | 11<br>11% | 13% |

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| GLVAGGIIGA | A*02:01 | <u>26% P</u><br>GBM3P<br>GBM7P<br>GBM10R<br>GBM12R<br>GBM13P<br>GBM13R<br>GBM14R | <u>17% R</u><br>GBM20P<br>GBM21P<br>ZH617<br>ZH678<br>ZH681<br>ZH757<br>ZH761 | Translocase of inner mitochondrial membrane domain-containing protein 1 (TIMMDC; Q9NPL8) | 39% P<br>21% R | 38<br>15% | 10% |
| AVATFLQSV | A*02:01<br>A*02:05<br>C*15:02 | <u>24% P</u><br>GBM6P<br>GBM6R<br>GBM10R<br>GBM11P<br>GBM11R<br>GBM13R<br>GBM14R<br>GBM20P | <u>29% R</u><br>GBM20R<br>GBM21P<br>ZH613<br>ZH617<br>ZH678<br>ZH681<br>ZH753<br>ZH757 | Ubiquitin-like modifier-activating enzyme 1 (UBA1; P22314) | 87% P<br>83% R | 8<br>48% | 54% |
| YLWTDVYSA | A*02:01 | <u>24% P</u><br>GBM2R<br>GBM3P<br>GBM7P<br>GBM7R<br>GBM10R<br>GBM12R<br>GBM13P<br>GBM13R | <u>29% R</u><br>GBM19P<br>GBM19R<br>ZH617<br>ZH678<br>ZH681<br>ZH753<br>ZH757<br>ZH829 | Protein-arginine deiminase type-2 (PADI2; P22314) | 87% P<br>83% R | 2<br>13% | 6% |
| SLMGTVFLL | A*02:01 | <u>24% P</u><br>GBM3P<br>GBM7P<br>GBM10R<br>GBM11P<br>GBM11R<br>GBM12R<br>GBM13R<br>GBM14P | <u>25% R</u><br>GBM14R<br>ZH645<br>ZH681<br>ZH720<br>ZH750<br>ZH753<br>ZH757 | Sphingomyelin synthase-related protein 1 (SAMD8; Q96LT4) | 55% P<br>50% R | 21<br>8% | 7% |
| GLIEIISNA | A*02:01<br>A*02:05 | <u>24% P</u><br>GBM3P<br>GBM6P<br>GBM6R<br>GBM7P<br>GBM10R<br>GBM11P<br>GBM12R | <u>21% R</u><br>GBM13R<br>GBM14R<br>ZH613<br>ZH645<br>ZH678<br>ZH681<br>ZH757 | U5 small nuclear ribonucleoprotein 200 kDa helicase (SNRNP200; O75643) | 100% P<br>88% R | 14<br>72% | 74% |
| NLLYPVPLV | A*02:01 | <u>21% P</u><br>GBM3P<br>GBM7P<br>GBM7R<br>GBM10R<br>GBM12R<br>GBM13R<br>GBM21P | <u>25% R</u><br>ZH645<br>ZH678<br>ZH681<br>ZH750<br>ZH753<br>ZH757<br>ZH784 | Kinesin-like protein KIF1A (KIF1A; Q12756) | 87% P<br>79% R | 2<br>14% | 15% |
| TLSTVIATV | A*02:01<br>A*02:05 | <u>21% P</u><br>GBM3P<br>GBM6P<br>GBM7P<br>GBM10R<br>GBM12R<br>GBM13R<br>GBM14R | <u>21% R</u><br>GBM21P<br>ZH617<br>ZH681<br>ZH720<br>ZH753<br>ZH761 | Atrophin-1 (ATN1; P54259) | 68% P<br>71% R | 10<br>12% | 10% |
| SLLEQAIAL | A*02:01 | <u>18% P</u><br>GBM2R<br>GBM3P<br>GBM7P<br>GBM7R<br>GBM10R<br>GBM12R<br>GBM13R | <u>29% R</u><br>ZH678<br>ZH681<br>ZH753<br>ZH757<br>ZH761<br>ZH784<br>ZH829 | Suppression of tumorigenicity 18 protein (ST18; O60284) | 21% P<br>29% R | 0<br>0% | 0% |
| APSGTRVVQV | B*07:02 | <u>29% P</u><br>GBM2P<br>GBM4P<br>GBM4R<br>GBM7P<br>GBM7R<br>GBM8P<br>GBM10R<br>GBM11P<br>GBM11R | <u>25% R</u><br>GBM16P<br>GBM16R<br>GBM18P<br>GBM18R<br>ZH616<br>ZH681<br>ZH757<br>ZH791 | Protocadherin gamma-C3 (PCDHGC3; Q9UN70) | 66% P<br>63% R | 2<br>8% | 11% |

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| ALADLQEAV | A*02:01<br>A*02:05<br>*B*13:02* | 29% P<br>GBM3P<br>GBM7P<br>GBM7R<br>GBM10R<br>GBM11P<br>GBM11R<br>GBM13P<br>GBM13R | 21% R<br>GBM14P<br>GBM14R<br>GBM21P<br>ZH616<br>ZH617<br>ZH645<br>ZH681<br>ZH757 | Protein TANC1<br>(TANC1; Q9C0D5) | 63% P<br>54% R | 12<br>7% | 10% |
| KLAPPPKKA | A*02:01 | 29% P<br>GBM3P<br>GBM7P<br>GBM7R<br>GBM14P<br>GBM14R<br>GBM20P<br>GBM20R<br>GBM21P | 17% R<br>ZH616<br>ZH617<br>ZH645<br>ZH678<br>ZH681<br>ZH753<br>ZH829 | Astrocytic<br>phosphoprotein PEA-15<br>(PEA15; Q15121) | 76% P<br>58% R | 22<br>28% | 36% |
| SPYSKTLVL | B*07:02<br>B*14:02<br>B*35:03 | 26% P<br>GBM4P<br>GBM4R<br>GBM7P<br>GBM10R<br>GBM11P<br>GBM11R<br>GBM14R<br>GBM16P<br>GBM16R | 25% R<br>GBM18P<br>GBM18R<br>GBM22P<br>GBM22R<br>ZH616<br>ZH681<br>ZH757<br>ZH791 | E3 ubiquitin-protein<br>ligase TRIM9 (TRIM9;<br>Q9C026) | 53% P<br>42% R | 1<br>0% | 0% |
| SPSGLRDSTV | B*07:02 | 26% P<br>GBM2P<br>GBM2R<br>GBM4P<br>GBM4R<br>GBM7P<br>GBM8P<br>GBM10R<br>GBM16P | 21% R<br>GBM16R<br>GBM18P<br>ZH616<br>ZH681<br>ZH757<br>ZH784<br>ZH791 | ATP-sensitive inward<br>rectifier potassium<br>channel 10 (KCNJ10;<br>P78508) | 92% P<br>79% R | 0<br>7% | 12% |
| GVIESVVTI | A*02:01<br>A*02:05<br>B*13:02<br>C*02:02 | 26% P<br>GBM3P<br>GBM6P<br>GBM6R<br>GBM7P<br>GBM11P<br>GBM11R<br>GBM12R<br>GBM13P | 17% R<br>GBM13R<br>GBM14R<br>ZH613<br>ZH617<br>ZH681<br>ZH757<br>ZH761 | Microtubule-associated<br>protein 2 (MAP2;<br>P11137) | 66% P<br>50% R | 1<br>4% | 7% |
| GLLGGLPRL | A*02:01 | 26% P<br>GBM7P<br>GBM7R<br>GBM12R<br>GBM13P<br>GBM13R<br>GBM21P<br>ZH678 | 29% R<br>ZH681<br>ZH720<br>ZH750<br>ZH753<br>ZH757<br>ZH761<br>ZH829 | Homeobox protein<br>Nkx-6.2 (NKX6-2;<br>Q9C056) | 32% P<br>33% R | 0<br>0% | 1% |
| GLAPSIRTK | A*03:01<br>A*11:01 | 24% P<br>GBM4P<br>GBM4R<br>GBM6P<br>GBM6R<br>GBM8P<br>GBM10R<br>GBM16P<br>GBM16R<br>GBM17R | 33% R<br>GBM18P<br>GBM18R<br>GBM22R<br>GBM23P<br>GBM23R<br>ZH678<br>ZH761<br>ZH791 | Tenascin (TNC;<br>P24821) | 87% P<br>83% R | 11<br>14% | 13% |
| RLFDEPQLA | A*02:01<br>*B*13:02* | 24% P<br>GBM3P<br>GBM5P<br>GBM5R<br>GBM7P<br>GBM10R<br>GBM13R<br>GBM14P | 21% R<br>GBM14R<br>GBM21P<br>ZH617<br>ZH681<br>ZH753<br>ZH757<br>ZH761 | BTB/POZ domain-<br>containing protein 1/2<br>(BTBD1/2; Q9H0C5 /<br>Q9BX70) | 74% / 74% P<br>58% / 67% R | 8<br>34% / 38% | 33% / 36% |
| SILDDVAMV | A*02:01 | 24% P<br>GBM10R<br>GBM12R<br>GBM13P<br>GBM13R<br>GBM14P<br>GBM20P | 17% R<br>ZH617<br>ZH678<br>ZH720<br>ZH753<br>ZH757<br>ZH761 | RNA-binding protein 25<br>(RBM25; P49756) | 50% P<br>33% R | 31<br>17% | 10% |

| | | GBM21P | | | | | |
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| IPKAKPLTL | B*07:02<br>B*08:01 | 21% P<br>GBM4P<br>GBM4R<br>GBM7P<br>GBM9P<br>GBM9R<br>GBM10R<br>GBM13P<br>GBM13R | 33% R<br>GBM16P<br>GBM16R<br>GBM18R<br>ZH617<br>ZH681<br>ZH753<br>ZH757<br>ZH784 | Stearoyl-CoA desaturase 5 (SCD5; Q86SK9) | 68% P<br>58% R | 4<br>6% | 4% |
| GLDPQGDRSFL | A*02:01<br>*B*07:02*<br>C*05:01 | 21% P<br>GBM7P<br>GBM7R<br>GBM10R<br>GBM13R<br>GBM14R<br>GBM20P<br>GBM20R | 25% R<br>GBM21P<br>ZH617<br>ZH678<br>ZH681<br>ZH753<br>ZH757<br>ZH761 | Protein LCHN (LCHN; A4D1U4) | 29% P<br>29% R | 24<br>5% | 2% |
| GVIDFSMYL | A*02:01<br>C*02:02<br>C*15:02<br>C*16:01 | 21% P<br>GBM7P<br>GBM10R<br>GBM12R<br>GBM13P<br>GBM13R<br>ZH617<br>ZH681 | 21% R<br>ZH720<br>ZH750<br>ZH753<br>ZH757<br>ZH761<br>ZH784 | RUN and FYVE domain-containing protein 2/3 (RUFY2/3; Q8WXA3 / Q7L099) | 42% / 34%<br>29% / 38% R | 6<br>4% / 3% | 2% / 0% |
| KMDENQFVAV | A*02:01 | 18% P<br>GBM2P<br>GBM10R<br>GBM11P<br>GBM13R<br>GBM14R<br>GBM20P<br>GBM20R | 25% R<br>ZH617<br>ZH681<br>ZH720<br>ZH753<br>ZH757<br>ZH784 | Dihydropyrimidinase-related protein 1/2/3 (CRMP1 / DPYSL2/3; Q14194 / Q16555 / Q14195 | 71% / 79% / 68% P<br>67% / 79% / 67% R | 11<br>19% / 25% / 29% | 30% / 41% / 33% |
| NLYEGQITV | A*02:01 | 18% P<br>GBM10R<br>GBM11P<br>GBM11R<br>GBM12R<br>GBM13R<br>GBM14R<br>GBM21P | 25% R<br>ZH645<br>ZH678<br>ZH681<br>ZH753<br>ZH757<br>ZH761 | ATP-binding cassette sub-family A member 3 (ABCA3; Q99758) | 37% P<br>29% R | 3<br>5% | 4% |
| ALTPVVVTL | A*02:01 | 18% P<br>GBM3P<br>GBM7P<br>GBM10R<br>GBM12R<br>GBM13R<br>GBM14R | 21% R<br>GBM21P<br>ZH681<br>ZH720<br>ZH753<br>ZH757<br>ZH761 | Cyclin-dependent kinase 4 (CDK4; P11802) | 50% P<br>54% R | 28<br>13% | 8% |
| VLYEDSLSSQV | A*02:01 | 29% P<br>GBM2P<br>GBM3P<br>GBM7P<br>GBM7R<br>GBM10P<br>GBM10R<br>GBM11P<br>GBM11R | 21% R<br>GBM13P<br>GBM13R<br>GBM20P<br>GBM20R<br>ZH617<br>ZH678<br>ZH720<br>ZH757 | Integral membrane protein 2C (ITM2C; Q9NQX7) | 55% P<br>46% R | 10<br>16% | 18% |
| IAFPGDILM | B*35:02<br>*C*01:02*<br>C*02:02<br>C*03:03<br>C*03:04<br>*C*05:01*<br>*C*08:02*<br>C*12:03<br>C*15:06 | 26% P<br>GBM3P<br>GBM6P<br>GBM6R<br>GBM7P<br>GBM7R<br>GBM12R<br>GBM14P<br>GBM14R | 25% R<br>GBM21P<br>GBM22P<br>GBM22R<br>GBM23P<br>GBM23R<br>ZH613<br>ZH631<br>ZH654 | Excitatory amino acid transporter 2 (SLC1A2; P43004) | 66% P<br>58% R | 0<br>3% | 4% |
| KPKPTPDYL | B*07:02 | 24% P<br>GBM2R<br>GBM4P<br>GBM4R<br>GBM7P<br>GBM7R<br>GBM10R<br>GBM11P<br>GBM11R | 29% R<br>GBM16P<br>GBM16R<br>GBM18P<br>GBM18R<br>ZH616<br>ZH681<br>ZH757<br>ZH791 | Protein quaking (QKI; Q96PU8) | 92% P<br>88% R | 8<br>52% | 58% |

| Peptide | HLA | % P (samples) | % R (samples) | Protein | | | |
|---|---|---|---|---|---|---|---|
| SPSEARQDVDL | B*07:02 | 24% P<br>GBM2R<br>GBM4P<br>GBM4R<br>GBM7P<br>GBM7R<br>GBM10R<br>GBM11P<br>GBM16P | 25% R<br>GBM16R<br>GBM18P<br>GBM18R<br>ZH616<br>ZH681<br>ZH757<br>ZH791 | Microtubule-associated protein 1B (MAP1B; P46821) | 100% P<br>88% R | 9<br>24% | 29% |
| SLSSGPLTQK | A*03:01<br>A*11:01 | 21% P<br>GBM2R<br>GBM4P<br>GBM4R<br>GBM6P<br>GBM6R<br>GBM10R<br>GBM16P<br>GBM16R | 29% R<br>GBM17R<br>GBM18P<br>GBM23P<br>GBM23R<br>ZH631<br>ZH761<br>ZH791 | Brain and acute leukemia cytoplasmic protein (BAALC; Q8WXS3) | 45% P<br>54% R | 2<br>2% | 3% |
| SLDSTLHAV | A*02:01 | 21% P<br>GBM3P<br>GBM10R<br>GBM11P<br>GBM13P<br>GBM13R<br>GBM14R<br>GBM20P | 21% R<br>GBM20R<br>ZH616<br>ZH617<br>ZH645<br>ZH681<br>ZH753 | Leucine-rich repeat and coiled-coil domain-containing protein 1 (LRRCC1; Q9C099) | 39% P<br>21% R | 11<br>4% | 3% |
| ATYYGAFIK | A*03:01<br>A*11:01 | 21% P<br>GBM4P<br>GBM4R<br>GBM7P<br>GBM10R<br>GBM16P<br>GBM16R<br>GBM17R | 21% R<br>GBM18R<br>GBM23P<br>ZH631<br>ZH678<br>ZH761<br>ZH791 | Mitogen-activated protein kinase kinase kinase kinase 4 / misshapen-like kinase 1 / TRAF2 and NCK-interacting protein kinase (MAP4K4 / MINK1 / TNIK; O95819 / Q8N4C8 / Q9UKE5) | 61% / 63% / 66% P<br>58% / 54% / 58% R | 2<br>20% / 22% / 19% | 22% / 23% / 23% |
| FLQGTIIAL | A*02:01 | 21% P<br>GBM2P<br>GBM2R<br>GBM3P<br>GBM7P<br>GBM10R<br>GBM12R | 17% R<br>ZH678<br>ZH681<br>ZH753<br>ZH757<br>ZH761<br>ZH829 | Myelin regulatory factor (MYRF; Q9Y2G1) | 47% P<br>63% R | 1<br>1% | 1% |
| KMLDEAVFQV | A*02:01 | 18% P<br>GBM3P<br>GBM7P<br>GBM10R<br>GBM11P<br>GBM13R<br>GBM14R | 17% R<br>ZH645<br>ZH681<br>ZH720<br>ZH757<br>ZH784 | Neuron navigator 1 (NAV1; Q8NEY1) | 53% P<br>38% R | 12<br>10% | 11% |
| VPRGFPSDTQL | B*07:02 | 18% P<br>GBM2P<br>GBM2R<br>GBM4P<br>GBM4R<br>GBM7P<br>GBM7R<br>GBM10R<br>GBM11R | 33% R<br>GBM16P<br>GBM16R<br>GBM18R<br>ZH681<br>ZH757<br>ZH784<br>ZH791 | Chondroadherin-like protein (CHADL; Q6NUI6) | 24% P<br>46% R | 0<br>2% | 0% |
| SVMSILPKI | A*02:01<br>C*15:02 | 18% P<br>GBM3P<br>GBM7P<br>GBM7R<br>GBM13R<br>GBM14R<br>ZH645 | 21% R<br>ZH678<br>ZH681<br>ZH720<br>ZH753<br>ZH757<br>ZH784 | Serine incorporator 1 (SERINC1; Q9NRX5) | 39% P<br>59% R | 29<br>7% | 10% |
| HLDFISIMTY | A*01:01 | 24% P<br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13P<br>GBM13R<br>GBM16P | 25% R<br>GBM16R<br>GBM17P<br>GBM17R<br>ZH617<br>ZH645<br>ZH654<br>ZH784 | Chitinase-3-like protein 1 (CHI3L1; P36222) | 68% P<br>63% R | 8<br>4% | 3% |
| SPFLQGQAL | B*07:02 | 26% P<br>GBM2P<br>GBM2R | 25% R<br>GBM11P<br>GBM11R | Adenomatous polyposis coli protein 2 (APC2; O95996) | 58% P<br>54% R | 0<br>2% | 1% |

| Peptide | HLA | Samples | Samples | Protein | P/R | n/% | % |
|---|---|---|---|---|---|---|---|
| | | GBM4P<br>GBM4R<br>GBM7P<br>GBM7R<br>GBM10P<br>GBM10R | GBM16P<br>GBM16R<br>GBM18P<br>ZH616<br>ZH681<br>ZH791 | | | | |
| FLDYGALSLY | A*01:01 | <u>24% P</u><br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13P<br>GBM13R<br>GBM16P | <u>25% R</u><br>GBM16R<br>GBM17P<br>GBM17R<br>ZH617<br>ZH645<br>ZH654<br>ZH784 | Progestin and adipoQ receptor family member 6 (PAQR6; Q6TCH4) | 32% P<br>29% R | 1<br>1% | 0% |
| GTEKLIETY | A*01:01 | <u>24% P</u><br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13P<br>GBM13R<br>GBM16P | <u>25% R</u><br>GBM16R<br>GBM17P<br>GBM17R<br>ZH617<br>ZH645<br>ZH654<br>ZH784 | Myelin proteolipid protein (PLP1; P60201) | 71% P<br>71% R | 1<br>1% | 10% |
| KPHSTPATL | B*07:02 | <u>24% P</u><br>GBM2P<br>GBM2R<br>GBM4P<br>GBM4R<br>GBM7P<br>GBM7R<br>GBM8P<br>GBM10R | <u>25% R</u><br>GBM16P<br>GBM16R<br>GBM18P<br>GBM18R<br>ZH616<br>ZH681<br>ZH791 | Transcription factor RFX4 (RFX4; Q33E94) | 55% P<br>46% R | 0<br>0% | 0% |
| QLDHLSLYY | A*01:01 | <u>24% P</u><br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13P<br>GBM13R<br>GBM16P | <u>25% R</u><br>GBM16R<br>GBM17P<br>GBM17R<br>ZH617<br>ZH645<br>ZH654<br>ZH784 | Electroneutral sodium bicarbonate exchanger 1 (SLC4A8; Q2Y0W8) | 26% P<br>29% R | 6<br>5% | 3% |
| FPDNQRPLL | B*07:02<br>B*35:02<br>C*04:01 | <u>24% P</u><br>GBM4P<br>GBM4R<br>GBM7P<br>GBM8P<br>GBM10R<br>GBM11P<br>GBM16P | <u>21% R</u><br>GBM16R<br>GBM18P<br>GBM18R<br>GBM23P<br>GBM23R<br>ZH681<br>ZH757 | ETS translocation variant 1 (ETV1; P50549) | 47% P<br>33% R | 7<br>5% | 3% |
| MTDPSKNLGY | A*01:01 | <u>21% P</u><br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM13P<br>GBM13R<br>GBM16P | <u>21% R</u><br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH654<br>ZH784 | Protein FAM5B (FAM5B; Q9C0B6) | 24% P<br>25% R | 1<br>1% | 1% |
| TMMSRPPVL | A*02:01<br>B*08:01<br>B*14:02<br>*C*01:02*<br>*C*07:02*<br>*C*12:03* | <u>24% P</u><br>GBM6P<br>GBM10P<br>GBM10R<br>GBM11P<br>GBM11R<br>GBM14P<br>GBM14R | <u>17% R</u><br>ZH616<br>ZH617<br>ZH678<br>ZH720<br>ZH753<br>ZH757 | Protein wntless homolog OS=Homo sapiens (WLS; Q5T9L3) | 76% P<br>58% R | 9<br>12% | 15% |
| VSDPKATMY | A*01:01 | <u>21% P</u><br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13P<br>GBM13R | <u>25% R</u><br>GBM16P<br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH654<br>ZH784 | Gamma-aminobutyric acid receptor subunit beta-1 (GABRB1; P18505) | 42% P<br>42% R | 1<br>2% | 4% |
| SYIGLGHIY | C*07:02 | <u>21% P</u><br>GBM2P<br>GBM2R<br>GBM4P<br>GBM4R | <u>25% R</u><br>GBM16P<br>GBM16R<br>GBM18P<br>GBM18R | Chloride transport protein 6 (CLCN6; P51797) | 26% P<br>33% R | 14<br>3% | 1% |

|  |  | GBM8P<br>GBM10R<br>GBM11P | ZH681<br>ZH757<br>ZH784 |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| VVDPSSNLYY | A*01:01 | 21% P<br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13P<br>GBM13R | 25% R<br>GBM16P<br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH654<br>ZH784 | Cyclic nucleotide-gated cation channel alpha-3 (CNGA3; Q16281) | 24% P<br>25% R | 4<br>1% | 1% |
| SSDKVTVNY | A*01:01 | 21% P<br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13P<br>GBM16P | 21% R<br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH654<br>ZH784 | Protein-arginine deiminase type-2 (PADI2; Q9Y2J8) | 87% P<br>83% R | 22<br>13% | 6% |
| YPHSGGSEL | B*07:02<br>B*35:01<br>B*35:02<br>B*35:03 | 18% P<br>GBM2P<br>GBM2R<br>GBM4P<br>GBM7P<br>GBM12R<br>GBM14P<br>GBM14R | 29% R<br>GBM16R<br>GBM18R<br>GBM21P<br>GBM22P<br>GBM22R<br>GBM23P<br>GBM23R | T-box brain protein 1 (TBR1; Q16650) | 18% P<br>33% R | 0<br>2% | 3% |
| SPVPATPIL | B*07:02<br>B*35:01<br>B*35:03 | 18% P<br>GBM4P<br>GBM4R<br>GBM6P<br>GBM7P<br>GBM10R<br>GBM14R<br>GBM16P | 25% R<br>GBM16R<br>GBM18P<br>GBM18R<br>GBM21P<br>GBM22R<br>ZH757 | E3 ubiquitin-protein ligase TRIM9 (TRIM9; Q9C026) | 53% P<br>42% R | 0<br>0% | 0% |
| ELDVVREIY | A*01:01<br>B*15:01 | 18% P<br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13R<br>GBM16P | 25% R<br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH654<br>ZH784 | Formin-like protein 2 (FMNL2; Q96PY5) | 58% P<br>71% R | 18<br>17% | 14% |
| FMVDKAIYL | A*02:01 | 16% P<br>GBM10R<br>GBM11P<br>GBM11R<br>GBM13R<br>ZH645<br>ZH678 | 21% R<br>ZH681<br>ZH720<br>ZH753<br>ZH757<br>ZH784 | Ecto-NOX disulfide-thiol exchanger 1 (ENOX1; Q8TC92) | 26% P<br>21% R | 18 | 0% |
| LLQDRLVSV | A*02:01 | 18% P<br>GBM3P<br>GBM10R<br>GBM11P<br>GBM11R<br>GBM13P<br>GBM14R | 21% R<br>GBM20P<br>GBM20R<br>GBM21P<br>ZH617<br>ZH681<br>ZH753 | Pleckstrin homology domain-containing family A member 4 (PLEKHA4; Q9H4M7) | 18% P<br>21% R | 7 | 1% |
| ALYGKTEVV | A*02:01 | 18% P<br>GBM3P<br>GBM10R<br>GBM13P<br>GBM13R<br>GBM20R<br>GBM21P | 21% R<br>ZH617<br>ZH645<br>ZH681<br>ZH753<br>ZH757 | Caskin-2 (CASKIN2; Q8WXE0) | 32% P<br>46% R | 8 | 16% |
| LLYDQPLQV | A*02:01<br>*B*13:02* | 18% P<br>GBM10R<br>GBM11P<br>GBM13R<br>GBM20P<br>GBM20R<br>GBM21P | 17% R<br>ZH645<br>ZH678<br>ZH681<br>ZH753<br>ZH757 | Protein LAP2 (ERBB2IP; Q96RT1) | 76% P<br>75% R | 10<br>36% | 35% |
| LLYEHQNNL | A*02:01 | 18% P<br>GBM10R<br>GBM13P<br>GBM13R<br>GBM14R<br>GBM20P<br>GBM21P | 17% R<br>ZH617<br>ZH678<br>ZH681<br>ZH753<br>ZH757 | Growth arrest-specific protein 8 (GAS8; O95995) | 21% P<br>17% R | 14<br>4% | 3% |

| Peptide | HLA | P samples | R samples | Protein | | | |
|---|---|---|---|---|---|---|---|
| LMYPYIYHV | A*02:01 | 18% P<br>GBM10R<br>GBM11P<br>GBM13P<br>GBM13R<br>GBM19P<br>GBM20R | 17% R<br>GBM21P<br>ZH617<br>ZH681<br>ZH753<br>ZH757 | Constitutive coactivator of PPAR-gamma-like protein 2 (FAM120C; Q9NX05) | 50% P<br>50% R | 3<br>8% | 8% |
| YLDPRITVA | A*02:01 | 18% P<br>GBM3P<br>GBM7R<br>GBM10R<br>GBM20P<br>GBM20R<br>GBM21P | 17% R<br>ZH616<br>ZH617<br>ZH678<br>ZH681<br>ZH753 | DNA topoisomerase 1 (TOP1; P11387) | 53% P<br>50% R | 12<br>32% | 19% |
| FLDEKSGSFV | A*02:01<br>C*04:01<br>C*05:01 | 18% P<br>GBM2P<br>GBM10R<br>GBM11P<br>GBM14R<br>GBM20P<br>GBM20R | 17% R<br>ZH616<br>ZH617<br>ZH681<br>ZH753<br>ZH757 | F-box only protein 32 (FBXO32; Q969P5) | 63% P<br>63% R | 12<br>16% | 12% |
| YITEGQIYV | A*02:01<br>A*02:05 | 18% P<br>GBM2R<br>GBM7P<br>GBM10R<br>GBM11P<br>GBM11R<br>GBM20P | 17% R<br>ZH617<br>ZH645<br>ZH681<br>ZH753<br>ZH757 | V-type proton ATPase subunit B, brain isoform (ATP6V1B2; P21281) | 71% P<br>71% R | 17<br>35% | 29% |
| YLEDFYTRM | A*02:01<br>A*02:05<br>*C*01:02*<br>C*02:02<br>C*05:01<br>C*08:02 | 18% P<br>GBM3R<br>GBM11P<br>GBM13R<br>GBM14R<br>GBM19P<br>GBM20P | 17% R<br>GBM20R<br>GBM21P<br>ZH617<br>ZH681<br>ZH757 | Peroxisomal sarcosine oxidase (PIPOX; Q9P0Z9) | 29% P<br>17% R | 2<br>1% | 3% |
| LLYPVPLVH | A*03:01 | 16% P<br>GBM2R<br>GBM4P<br>GBM4R<br>GBM6P<br>GBM6R<br>GBM8P<br>GBM10R | 33% R<br>GBM16P<br>GBM16R<br>GBM17R<br>GBM18P<br>GBM18R<br>GBM22R<br>GBM23P | Kinesin-like protein KIF1A (KIF1A; Q12756) | 87% P<br>79% R | 11<br>14% | 15% |
| LPASPSVSL | B*07:02<br>B*35:01<br>B*35:02<br>B*35:03 | 16% P<br>GBM4P<br>GBM7P<br>GBM10R<br>GBM12R<br>GBM14R<br>GBM16P<br>GBM16R | 29% R<br>GBM18R<br>GBM21P<br>GBM22P<br>GBM22R<br>GBM23P<br>GBM23R | Ectoderm-neural cortex protein 1 (ENC1; O14682) | 89% P<br>75% R | 15<br>23% | 15% |
| VLLEGELIDV | A*02:01 | 16% P<br>GBM2R<br>GBM10R<br>GBM11P<br>GBM13P<br>GBM13R<br>GBM20R | 21% R<br>ZH645<br>ZH720<br>ZH753<br>ZH757<br>ZH829 | UPF0552 protein C15orf38 (C15orf38; Q7Z6K5) | 21% P<br>25% R | 4<br>2% | 2% |
| ALDASILNV | A*02:01<br>*B*13:02* | 16% P<br>GBM3P<br>GBM5P<br>GBM10R<br>GBM12R<br>GBM13R | 17% R<br>GBM14R<br>GBM21P<br>ZH645<br>ZH678<br>ZH757 | Regulator of G-protein signaling 12 (RGS12; O14924) | 26% P<br>17% R | 11<br>8% | 5% |
| GLLPLLREA | A*02:01 | 16% P<br>GBM2R<br>GBM3P<br>GBM12R<br>GBM14R<br>GBM15P | 17% R<br>GBM20R<br>GBM21P<br>ZH617<br>ZH645<br>ZH678 | Stabilin-1 (STAB1; Q9NY15) | 82% P<br>63% R | 34<br>34% | 31% |
| VPWQGTMTL | B*07:02<br>B*35:01<br>B*35:03<br>B*39:10<br>B*56:01 | 16% P<br>GBM4P<br>GBM6P<br>GBM7P<br>GBM10R<br>GBM14R | 17% R<br>GBM16P<br>GBM18R<br>GBM22P<br>ZH720<br>ZH784 | Cystatin-C (CST3; P01034) | 29% P<br>38% R | 10<br>5% | 7% |

| Peptide | HLA | Samples (P) | Samples (R) | Protein | Frequency | Count | % |
|---|---|---|---|---|---|---|---|
| AVASVIIYR | A*03:01<br>A*11:01<br>A*31:01<br>A*68:01 | 21% P<br>GBM4P<br>GBM4R<br>GBM6P<br>GBM6R<br>GBM8P<br>GBM10R<br>GBM12P | 25% R<br>GBM12R<br>GBM16P<br>GBM16R<br>GBM18P<br>GBM18R<br>ZH631<br>ZH802 | Cadherin EGF LAG seven-pass G-type receptor 2 (CELSR2; Q9HCU4) | 50% P<br>63% R | 2<br>10% | 11% |
| ISDHGTVTY | A*01:01 | 21% P<br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13P<br>GBM13R | 21% R<br>GBM16P<br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH654 | Lysosome-associated membrane glyco-protein 2 (LAMP2; P13473) | 39% P<br>46% R | 27<br>16% | 12% |
| VSESHGQLSY | A*01:01 | 21% P<br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13P<br>GBM13R | 21% R<br>GBM16P<br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH654 | OTU domain-containing protein 4 (OTUD4; Q01804) | 26% P<br>21% R | 35<br>6% | 3% |
| AVFPEGALTK | A*03:01<br>A*11:01 | 21% P<br>GBM4P<br>GBM4R<br>GBM7P<br>GBM7R<br>GBM10R<br>GBM16P<br>GBM16R | 21% R<br>GBM18P<br>GBM18R<br>GBM23P<br>ZH631<br>ZH678<br>ZH791 | Ankyrin-2 OS=Homo sapiens (ANK2; Q01484) | 87% P<br>75% R | 12<br>19% | 33% |
| STEERTFQY | A*01:01 | 21% P<br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13P<br>GBM13R | 21% R<br>GBM16P<br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH654 | Peroxisomal carnitine O-octanoyltransferase (CROT; Q9UKG9) | 37% P<br>29% R | 13<br>7% | 3% |
| LDSHIHAY | A*01:01 | 21% P<br>GBM3P<br>GBM5P<br>GBM9P<br>GBM9R<br>GBM13P<br>GBM13R | 17% R<br>GBM16P<br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH654 | Receptor-type tyrosine-protein phosphatase zeta (PTPRZ1; P23471) | 97% P<br>83% R | 6<br>6% | 5% |
| SVPYFVTAL | A*02:05<br>C*01:02<br>*C*12:03* | 21% P<br>GBM6P<br>GBM6R<br>GBM7P<br>GBM7R<br>GBM11P<br>GBM11R | 17% R<br>GBM12R<br>ZH613<br>ZH645<br>ZH757<br>ZH761<br>ZH829 | Transmembrane 6 superfamily member 1 (TM6SF1; Q9BZW5) | 55% P<br>58% R | 3<br>18% | 23% |
| APSGLRSQVQF | B*07:02 | 18% P<br>GBM2R<br>GBM4P<br>GBM4R<br>GBM7P<br>GBM7R<br>GBM10R<br>GBM11P | 29% R<br>GBM11R<br>GBM16P<br>GBM16R<br>GBM18P<br>GBM18R<br>ZH681<br>ZH757 | GRAM domain-containing protein 3 (GRAMD3; Q96HH9) | 47% P<br>54% R | 9<br>11% | 13% |
| HTDMADIEQY | A*01:01 | 16% P<br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13R | 25% R<br>GBM16P<br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH784 | N-acetylaspartate synthetase (NAT8L; Q8N9F0) | 53% P<br>50% R | 6<br>5% | 5% |
| LLDPAQRTLY | A*01:01 | 18% P<br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13P<br>GBM13R | 25% R<br>GBM16P<br>GBM16R<br>GBM17R<br>ZH617<br>ZH654<br>ZH784 | Zinc finger protein 557/558 (ZNF557/558; Q8N988 / Q96NG5) | 21% / 26% P<br>33% / 38% R | 33<br>6% / 6% | 2% / 1% |

| Peptide | HLA | % P | % R | Protein | % P / % R | | |
|---|---|---|---|---|---|---|---|
| SPIERNEQL | B*07:02<br>B*08:01<br>B*35:02<br>B*35:03 | 18% P<br>GBM4P<br>GBM4R<br>GBM10R<br>GBM14P<br>GBM14R<br>GBM16P<br>GBM18P | 25% R<br>GBM18R<br>GBM21P<br>GBM22R<br>GBM23P<br>GBM23R<br>ZH681 | Zinc finger protein 226 (ZNF226; Q9NYT6) | 29% P<br>33% R | 7<br>3% | 2% |
| ISEELVQKY | A*01:01 | 18% P<br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13R | 21% R<br>GBM16P<br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH654 | Reticulon-4 (RTN4; Q9NQC3) | 79% P<br>75% R | 34<br>32% | 38% |
| KVQAGNSSL | B*07:02<br>*C*01:02*<br>C*02:02<br>C*16:01 | 18% P<br>GBM2R<br>GBM4P<br>GBM4R<br>GBM7P<br>GBM7R<br>GBM10R | 21% R<br>GBM16P<br>GBM16R<br>GBM18P<br>ZH616<br>ZH681<br>ZH761 | Phosphoserine aminotransferase (PSAT1; Q9Y617) | 50% P<br>54% R | 5<br>8% | 9% |
| AVYDTNPAK | A*03:01<br>A*11:01 | 18% P<br>GBM4P<br>GBM4R<br>GBM7P<br>GBM16P<br>GBM16R<br>GBM17R | 17% R<br>GBM18P<br>GBM23R<br>ZH631<br>ZH761<br>ZH791 | Glycolipid transfer protein (GLTP; Q9NZD2) | 39% P<br>46% R | 0<br>9% | 8% |
| KIIDEDGLLNL | A*02:01<br>A*02:05 | 18% P<br>GBM6P<br>GBM6R<br>GBM11P<br>GBM11R<br>GBM20R<br>ZH613 | 17% R<br>ZH617<br>ZH681<br>ZH720<br>ZH753<br>ZH757 | Replication factor C subunit 1 (RFC1; P35251) | 63% P<br>63% R | 2<br>30% | 17% |
| SRAPSTYTY | C*07:01<br>C*07:02 | 18% P<br>GBM4P<br>GBM4R<br>GBM8P<br>GBM10R<br>GBM11P<br>GBM16P | 17% R<br>GBM16R<br>GBM18R<br>ZH616<br>ZH681<br>ZH757 | Radial spoke head protein 3 homolog (RSPH3; Q86UC2) | 26% P<br>21% R | 4<br>6% | 4% |
| VLFDDELLM | A*02:01 | 18% P<br>GBM10R<br>GBM13P<br>GBM13R<br>GBM20P<br>GBM20R<br>ZH616 | 17% R<br>ZH617<br>ZH681<br>ZH753<br>ZH757<br>ZH761 | Non-syndromic hearing impairment protein 5 (DFNA5; O60443) | 55% P<br>38% R | 7<br>4% | 7% |
| VVAEELENV | A*02:01<br>A*02:05 | 18% P<br>GBM6P<br>GBM6R<br>GBM10R<br>GBM11P<br>GBM11R<br>GBM14R | 17% R<br>GBM21P<br>ZH613<br>ZH617<br>ZH681<br>ZH761 | F-box only protein 22 (FBXO22; Q8NEZ5) | 24% P<br>21% R | 8<br>12% | 6% |
| TLLLWPINK | A*03:01<br>A*11:01 | 18% P<br>GBM4P<br>GBM4R<br>GBM6R<br>GBM7P<br>GBM16P<br>GBM16R | 17% R<br>GBM17P<br>GBM18R<br>ZH631<br>ZH678<br>ZH761 | 1-acyl-sn-glycerol-3-phosphate acyltransferase delta (AGPAT4; Q9NRZ5) | 34% P<br>33% R | 3<br>4% | 3% |
| ESDLYSLAHSY | A*01:01 | 16% P<br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9R<br>GBM13R<br>GBM16P | 21% R<br>GBM16R<br>ZH617<br>ZH645<br>ZH654<br>ZH784 | Nuclear protein 1 (NUPR1; O60356) | 18% P<br>21% R | 32<br>5% | 1% |
| GTDYINASY | A*01:01 | 16% P<br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9R<br>GBM13R<br>GBM16P | 21% R<br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH654 | Receptor-type tyrosine-protein phosphatase gamma/zeta (PTPRG / PTPRZ; P23470 / P23471) | 61% / 97% P<br>46% / 83% R | 0<br>7% / 6% | 12% / 5% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| KTIEDTLMTV | A*02:01<br>A*02:05<br>C*16:01 | <u>16% P</u><br>GBM6P<br>GBM6R<br>GBM10R<br>GBM11P<br>GBM11R<br>GBM13R | <u>21% R</u><br>GBM20R<br>ZH613<br>ZH617<br>ZH720<br>ZH757 | Arfaptin-1 (ARFIP1;<br>P53367) | 47% P<br>38% R | 15<br>14% | 7% |
| RTDTALTNTY | A*01:01 | <u>16% P</u><br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9R<br>GBM13R<br>GBM16P | <u>21% R</u><br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH654 | Paired box protein<br>Pax-6 (PAX6; P26367) | 24% P<br>21% R | 2<br>1% | 0% |
| IPDRSGPEL | B*07:02<br>B*35:02<br>B*35:03 | <u>16% P</u><br>GBM4R<br>GBM7P<br>GBM10R<br>GBM16P<br>GBM16R<br>GBM18R | <u>21% R</u><br>GBM21P<br>GBM23P<br>GBM23R<br>ZH616<br>ZH681 | Progressive ankylosis<br>protein homolog<br>(ANKH; Q9HCJ1) | 76% P<br>79% R | 13<br>16% | 12% |
| GLIDEQIL | A*02:01 | <u>16% P</u><br>GBM7P<br>GBM10R<br>GBM13P<br>GBM13R<br>GBM14R | <u>17% R</u><br>ZH645<br>ZH678<br>ZH681<br>ZH753<br>ZH757 | Dystonin (DST;<br>Q03001) | 97% P<br>92% R | 8<br>41% | 55% |
| KILDTSVAYV | A*02:01 | <u>16% P</u><br>GBM10R<br>GBM11P<br>GBM13R<br>GBM20P<br>GBM20R | <u>17% R</u><br>GBM21P<br>ZH617<br>ZH720<br>ZH753<br>ZH757 | Kinesin-like protein<br>KIF1B (KIF1B; O60333) | 95% P<br>79% R | 0<br>27% | 26% |
| TAISRIYTV | A*02:01<br>A*02:05<br>B*08:01<br>B*51:01<br>C*12:03 | <u>21% P</u><br>GBM6P<br>GBM6R<br>GBM9P<br>GBM11P<br>GBM11R<br>GBM13P | <u>17% R</u><br>GBM16P<br>GBM16R<br>GBM18P<br>GBM18R<br>ZH613<br>ZH654 | Carboxypeptidase E<br>(CPE; P16870) | 87% P<br>71% R | 0<br>10% | 14% |
| VTAQVVGTERY | A*01:01 | <u>18% P</u><br>GBM5P<br>GBM5R<br>GBM9P<br>GBM9R<br>GBM13P<br>GBM13R | <u>21% R</u><br>GBM16P<br>GBM16R<br>GBM17R<br>ZH617<br>ZH645<br>ZH654 | Phospholipase D2<br>(PLD2; O149399) | 32% P<br>29% R | 23<br>9% | 8% |
| FPAGPPSHSL | B*07:02<br>B*35:02<br>B*35:03 | <u>18% P</u><br>GBM4P<br>GBM10R<br>GBM16P<br>GBM16R<br>GBM18P<br>GBM18R | <u>21% R</u><br>GBM21P<br>GBM22P<br>GBM22R<br>GBM23P<br>GBM23R<br>ZH681 | Peptidyl-prolyl cis-trans<br>isomerase FKBP10<br>(FKBP10; Q96AY3) | 42% P<br>25% R | 11<br>9% | 5% |
| RLKEGANINK | A*03:01 | <u>18% P</u><br>GBM4P<br>GBM4R<br>GBM6P<br>GBM6R<br>GBM16P<br>GBM16R | <u>21% R</u><br>GBM17R<br>GBM18P<br>GBM23P<br>GBM23R<br>ZH761<br>ZH791 | Kinesin-like protein<br>KIF1A/B/C (KIF1A/B/C;<br>Q12756 / O60333 /<br>O43896) | 87% / 95% /<br>37% P<br>79% / 79% /<br>29% R | 1<br>14% / 27% / 11% | 15% / 26%<br>/ 9% |
| AVDPGLLGY | A*01:01<br>B*15:01 | <u>18% P</u><br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM13P<br>GBM13R | <u>17% R</u><br>GBM16P<br>GBM16R<br>GBM17R<br>ZH617<br>ZH645 | Atrophin-1 (ATN1;<br>P54259) | 68% P<br>71% R | 17<br>12% | 10% |
| FSSSHEGFSY | A*01:01<br>B*57:01<br>C*02:02 | <u>18% P</u><br>GBM3P<br>GBM5P<br>GBM5R<br>GBM9P<br>GBM13P<br>GBM13R | <u>17% R</u><br>GBM16P<br>GBM16R<br>GBM17R<br>ZH617<br>ZH654 | ETS translocation<br>variant 5 (ETV5;<br>P41161) | 63% P<br>54% R | 7<br>11% | 8% |

| Peptide | HLA | Samples | Samples | Protein | % P/R | Count | % |
|---|---|---|---|---|---|---|---|
| GQDGSVVQF | B*15:01 *C*04:01* C*05:01 | 18% P GBM2P GBM2R GBM5P GBM5R GBM12R GBM15P | 17% R GBM20P GBM20R ZH616 ZH617 ZH802 | Small ubiquitin-related modifier 2/3/4 (SUMO2/3/4; P61956 / P55854 / Q6EEV6) | 26% / 34% / 18% P 29% / 38% / 17% R | 27 24% / 21% / 7% | 13% / 12% / 2% |
| LSSGPLTQK | A*03:01 A*11:01 | 18% P GBM4P GBM4R GBM6P GBM7P GBM7R GBM16P | 17% R GBM16R GBM17R ZH631 ZH678 ZH791 | Brain and acute leukemia cytoplasmic protein (BAALC; Q8WXS3) | 45% P 54% R | 2 2% | 3% |
| VTDESIPSY | A*01:01 | 18% P GBM3P GBM5P GBM9P GBM9R GBM13P GBM13R | 17% R GBM16P GBM16R GBM17R ZH617 ZH654 | A-kinase anchor protein 9 OS=Homo sapiens GN=AKAP9; Q99996) | 61% P 50% R | 11 34% | 32% |
| YRPLTVLTF | C*07:02 | 16% P GBM2P GBM4P GBM4R GBM10R GBM11P GBM11R | 25% R GBM16P GBM16R GBM18P GBM18R ZH757 ZH784 | Transmembrane and TPR repeat-containing protein 3/4 (TMTC3/4; Q6ZXV5 / Q5T4D3) | 53% / 39% P 63% / 46% R | 15 18% / 7% | 18% / 7% |
| FLDPAQRDLY | A*01:01 | 16% P GBM5P GBM5R GBM9P GBM9R GBM13R GBM16P | 21% R GBM16R GBM17R ZH617 ZH645 ZH654 | Zinc finger protein 14 homolog (ZFP14; Q9HCL3) | 18% P 25% R | 13 4% | 3% |
| TPASAGHVW | B*07:02 B*35:01 | 16% P GBM4P GBM4R GBM6P GBM7P GBM10R GBM12R | 21% R GBM16R GBM18P GBM18R ZH681 | Transcription factor SOX-9 (SOX9; P48436) | 82% P 79% R | 11 15% | 7% |
| SVDSNLLSDY | A*01:01 | 16% P GBM3P GBM5P GBM5R GBM9P GBM9R GBM13R | 21% R GBM16P GBM16R GBM17R ZH617 ZH645 | Oxidative stress-induced growth inhibitor 2 (OSGIN2; Q9Y236) | 26% P 33% R | 39 13% | 7% |
| YADGESFLGY | A*01:01 C*02:02 | 16% P GBM3P GBM5P GBM5R GBM9P GBM9R GBM16P | 21% R GBM16R GBM17R ZH617 ZH645 ZH784 | Proteasome subunit beta type-4 (PSMB4; P28070) | 58% P 42% R | 19 28% | 20% |
| AVAPQVPAL | A*02:01 A*02:05 *C*01:02* C*12:03 C*15:02 | 16% P GBM6P GBM6R GBM7P GBM10R GBM11P | 17% R GBM11R ZH613 ZH681 ZH753 ZH757 | Protein FAM118B (FAM118B; Q9BPY3) | 18% P 21% R | 2 2% | 0% |
| MTEVLPNQRY | A*01:01 | 16% P GBM3P GBM5P GBM9P GBM9R GBM13R | 17% R GBM16P GBM16R GBM17R ZH617 ZH645 | SEC14-like protein 2 (SEC14L2; O76054) | 32% P 25% R | 2 1% | 1% |
| NRYINIVAY | B*27:05 C*07:01 C*07:02 | 16% P GBM4P GBM4R GBM10R GBM11P GBM16P | 17% R GBM16R GBM18R ZH616 ZH681 ZH757 | Receptor-type tyrosine-protein phosphatase zeta (PTPRZ1; P23471) | 97% P 83% R | 0 6% | 5% |
| FVDPYPVNKY | A*01:01 C*02:02 | 16% P GBM3P GBM5P GBM5R | 17% R GBM16R GBM17R ZH617 | Kelch-like protein 15 (KLHL15; Q96M94) | 24% P 29% R | 18 12% | 15% |

| | | GBM9P | ZH645 | | | | |
|---|---|---|---|---|---|---|---|
| | | GBM9R | ZH654 | | | | |
| RPLVGPLAL | B*07:02 | 16% P | 17% R | Potassium/sodium | 16% P | 2 | 0% |
| | | GBM2P | GBM16P | hyperpolarization- | 21% R | 1% | |
| | | GBM4P | GBM16R | activated cyclic | | | |
| | | GBM4R | GBM18R | nucleotide-gated | | | |
| | | GBM7P | ZH681 | channel 2 (HCN2; | | | |
| | | GBM10R | ZH757 | Q9UL51) | | | |
| RVFSTSPAK | A*03:01 | 16% P | 17% R | Calcium and integrin- | 76% P | 4 | 40% |
| | | GBM4R | GBM18P | binding protein 1 (CIB1; | 71% R | 45% | |
| | | GBM6P | GBM23P | Q99828) | | | |
| | | GBM16P | GBM23R | | | | |
| | | GBM16R | ZH761 | | | | |
| | | GBM17R | ZH791 | | | | |
| TADGKTYYY | A*01:01 | 16% P | 17% R | Transcription elongation | 29% P | 21 | 4% |
| | C*02:02 | GBM3P | GBM16P | regulator 1 (TCERG1; | 38% R | 8% | |
| | | GBM5P | GBM16R | O14776) | | | |
| | | GBM5R | GBM17R | | | | |
| | | GBM9R | ZH617 | | | | |
| | | GBM13P | ZH645 | | | | |
| TVMQLRNEL | B*07:02 | 16% P | 17% R | Neuron navigator 2 | 63% P | 4 | 6% |
| | *C*01:02* | GBM4P | GBM16R | (NAV2; Q8IVL1) | 38% R | 12% | |
| | C*02:02 | GBM4R | GBM18R | | | | |
| | | GBM7P | ZH616 | | | | |
| | | GBM10R | ZH681 | | | | |
| | | GBM16P | ZH757 | | | | |



% of individuals — Cumulative % of population coverage
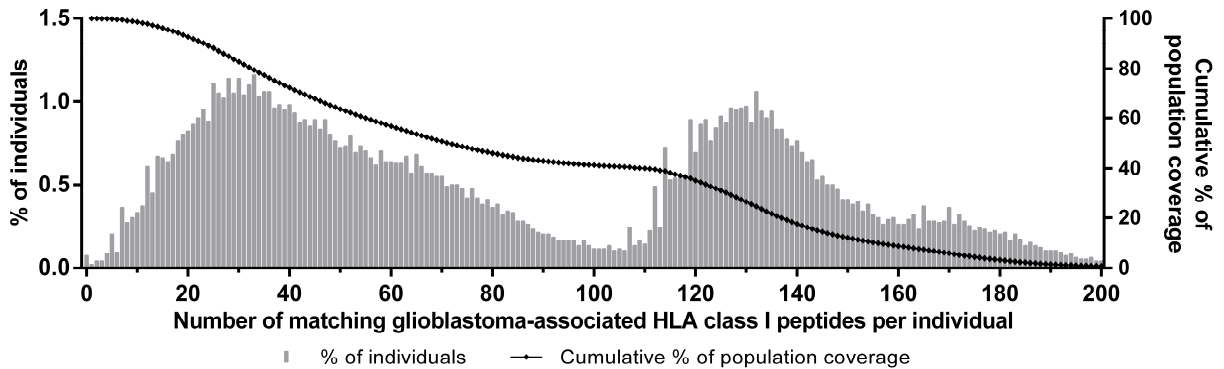
**Supplementary Figure 3. Population coverage of glioblastoma-associated HLA-A, -B, and -C ligands.** Using the population coverage tool provided by the IEDB Analysis Resource, the world population coverage of the 357 glioblastoma-associated peptides was calculated. The percentage of individuals with a specific number of matching peptides (max. of 235) is indicated by bar charts (associated with the left y-axis). The line diagram (associated with the right y-axis) shows the cumulative percentage of population coverage. The candidate target peptides cover 99.93% of the world population (first diamond on line diagram counted from the left) meaning that only 0.07% of all individuals are negative for all HLA-A, -B, and -C allotypes for which glioblastoma-associated peptides were defined. On average, 84 peptides are expected to match per patient worldwide.

**Supplementary Table 4. Glioblastoma-exclusive HLA class II-presented peptides derived from glioblastoma-associated HLA presentation hotspots.** Peptides already reported to derive from glioblastoma-associated antigens were excluded from this listing. In turn, some of those antigens not designated as glioblastoma-associated due to a CNS-associated expression profile are listed herein with glioblastoma-associated peptides. The number of positive tumors other than glioblastoma was based on n=585 HLA class II peptidome datasets. Frequencies of positive tumors are given separately for n=38 primary (P) and n=24 recurrent (R) glioblastomas.

| Antigen (UniProt accession) | Protein frequency on glioblasto-mas | Peptide sequence | Frequency of positive tumors | | Protein frequency on non-GBM tumors / Peptide-positive non-GBM tumors | Protein frequency on benign samples (n=364) |
|---|---|---|---|---|---|---|
| Fatty acid-binding protein, brain (FABP7; O15540) | 26% P 29% R | | | | 1% | 0% |
| | | DKVVIRTLSTFKN | 3% P / 0% R | ZH616 | 0 | |
| | | GDKVVIRTLSTFKN | 3% P / 0% R | ZH616 | 0 | |
| | | GDKVVIRTLSTFKNTE | 3% P / 0% R | ZH616 | 0 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | KPTVIISQEGDKVVI | 3% P / 5% R | GBM20P GBM20R | 1 | | |
| | | KPTVIISQEGDKVVIR | 24% P / 25% R | GBM5P GBM8P GBM12P GBM13R GBM15P GBM18R GBM20R GBM22R | GBM9R GBM13P GBM14R GBM18P GBM20P GBM22P ZH645 | 7 | |
| | | KPTVIISQEGDKVVIRT | 8% P / 4% R | GBM12P GBM13P GBM20R | GBM20P | 0 | |
| | | VIRTLSTFKNTEISF | 0% P / 4% R | GBM10R | 0 | | |
| Antithrombin-III (SERPINC1; P01008) | 89% P 83% R | | | | 50% | 48% |
| | | ASMMYQEGKFRYRR | 5% P / 4% R | GBM11P GBM17R | ZH681 | 0 | |
| | | SASMMYQEGKFRYR | 0% P / 4% R | GBM14R | 0 | | |
| | | SASMMYQEGKFRYRR | 18% P / 17% R | GBM4R GBM6P GBM11P GBM14P GBM21R ZH757 | GBM7P GBM11R GBM14R ZH616 ZH791 | 5 | |
| | | SASMMYQEGKFRYRRV | 3% P / 0% R | GBM6P | 0 | | |
| Fibronectin (FN1; P02751) | 95% P 88% R | | | | 54% | 65% |
| | | GQQMIFEEHGFRRTTPP | 8% P / 8% R | GBM6P GBM14P GBM21P | GBM14R GBM21R | 5 | |
| | | QMIFEEHGFRRTTPPT | 5% P / 0% R | GBM11P GBM14P | 0 | | |
| | | QQMIFEEHGFRRTTPP | 16% P / 21% R | GBM9R GBM10R GBM14R GBM16R GBM21R ZH791 | GBM14P GBM16P GBM21P ZH757 ZH829 | 11 | |
| | | VGQQMIFEEHGFRRTTPP | 3% P / 0% R | GBM6P | 0 | | |
| | | VGQQMIFEEHGFRRTTPPT | 3% P / 0% R | GBM16P | 3 | | |
| Superoxide dismutase [Mn], mitochondrial (SOD2; P04179) | 68% P 54% R | | | | 19% | 23% |
| | | EHAYYLQYKNVRPD | 3% P / 0% R | ZH645 | 0 | | |
| | | EHAYYLQYKNVRPDY | 3% P / 0% R | ZH645 | 0 | | |
| | | GTTGLIPLLGIDVWEH | 13% P / 13% R | GBM6P GBM7P GBM12R GBM14R ZH753 | GBM12P GBM14P GBM19P | 9 | |
| | | GTTGLIPLLGIDVWEHA | 11% P / 8% R | GBM6P GBM12P GBM14R ZH742 | GBM14P GBM19P | 6 | |
| | | TGLIPLLGIDVWEH | 0% P / 8% R | GBM14R ZH753 | 3 | | |
| | | TTGLIPLLGIDVWE | 3% P / 4% R | GBM19P ZH753 | 0 | | |
| | | TTGLIPLLGIDVWEH | 19% P / 21% R | GBM2P GBM2R GBM6R GBM12P GBM14P GBM15P ZH753 | GBM6P GBM7P GBM12R GBM14R GBM19P | 15 | |
| | | TTGLIPLLGIDVWEHA | 13% P / 8% R | GBM6P GBM7P GBM14P GBM19P | GBM12P GBM14R ZH753 | 4 | |
| | | WEHAYYLQYKNVRP | 3% P / 0% R | ZH645 | 0 | | |
| | | WEHAYYLQYKNVRPD | 5% P / 0% R | ZH645 ZH681 | 2 | | |
| | | WEHAYYLQYKNVRPDY | 3% P / 0% R | ZH645 | 2 | | |
| Collagen alpha-2(V) chain (COL5A2; P05997) | 34% P 29% R | | | | 12% | 3% |
| | | ARLPIIDLAPVDVGGTD | 11% P / 4% R | GBM5P GBM13P GBM18R | GBM18P ZH645 | 21 | |
| | | GNVGKTVFEYRTQNVAR | 0% P / 4% R | GBM14R | 5 | | |
| | | RLPIIDLAPVDVGG | 0% P / 4% R | GBM18R | 7 | | |
| | | RLPIIDLAPVDVGGTD | 13% P / 4% R | GBM5P GBM12P GBM18P ZH645 | GBM13P GBM18R | 26 | |

| | | VARLPIIDLAPVDVGGTD | 0% P / 4% R | GBM18R | 16 | |
|---|---|---|---|---|---|---|
| | | VGKTVFEYRTQNVAR | 8% P / 13% R | GBM11P | 14 | |
| | | | | GBM14P GBM14R | | |
| | | | | GBM16R GBM21P | | |
| | | | | GBM21R | | |
| Secretogranin-2 | 61% P | | | | 7% | 5% |
| (SCG2; P13521) | 58% R | DDVSKVIAYLKRL | 3% P / 4% R | GBM3P | 0 | |
| | | | | GBM23R | | |
| | | DDVSKVIAYLKRLVNA | 3% P / 4% R | GBM3P | 1 | |
| | | | | GBM22R | | |
| | | DDVSKVIAYLKRLVNAA | 3% P / 0% R | GBM3P | 1 | |
| | | DDVSKVIAYLKRLVNAAG | 3% P / 4% R | GBM3P | 5 | |
| | | | | GBM22R | | |
| | | DDVSKVIAYLKRLVNAAGS | 3% P / 0% R | GBM3P | 0 | |
| | | DDVSKVIAYLKRLVNAAGSG | 3% P / 4% R | GBM3P | 2 | |
| | | | | GBM22R | | |
| | | DVSKVIAYLKRL | 3% P / 0% R | GBM3P | 0 | |
| | | DVSKVIAYLKRLVNA | 3% P / 4% R | GBM3P | 0 | |
| | | | | GBM22R | | |
| | | DVSKVIAYLKRLVNAA | 3% P / 4% R | GBM3P | 5 | |
| | | | | GBM22R | | |
| | | DVSKVIAYLKRLVNAAG | 11% P / 13% R | GBM3P | 6 | |
| | | | | GBM3R GBM22P | | |
| | | | | GBM22R GBM23P | | |
| | | | | GBM23R ZH810 | | |
| | | DVSKVIAYLKRLVNAAGSG | 3% P / 4% R | GBM3P | 0 | |
| | | | | GBM22R | | |
| | | KVIAYLKRLVNA | 0% P / 4% R | GBM3R | 0 | |
| | | KVIAYLKRLVNAA | 3% P / 4% R | GBM3P | 0 | |
| | | | | GBM3R | | |
| | | KVIAYLKRLVNAAG | 3% P / 4% R | GBM3P | 0 | |
| | | | | GBM3R | | |
| | | LSDDVSKVIAYLKRLVNAAG | 0% P / 4% R | GBM22R | 0 | |
| | | LSDDVSKVIAYLKRLVNAAGSG | 3% P / 4% R | GBM3P | 1 | |
| | | | | GBM22R | | |
| | | SDDVSKVIAYLKRLVNAAG | 3% P / 4% R | GBM3P | 2 | |
| | | | | GBM22R | | |
| | | SDDVSKVIAYLKRLVNAAGSG | 3% P / 4% R | GBM3P | 1 | |
| | | | | GBM22R | | |
| | | SKVIAYLKRLVNA | 3% P / 4% R | GBM3P | 0 | |
| | | | | GBM3R | | |
| | | SKVIAYLKRLVNAA | 3% P / 8% R | GBM3P | 1 | |
| | | | | GBM3R GBM22R | | |
| | | SKVIAYLKRLVNAAG | 3% P / 8% R | GBM3P | 0 | |
| | | | | GBM22R GBM23R | | |
| | | VSKVIAYLKRLVNA | 5% P / 13% R | GBM3P | 3 | |
| | | | | GBM3R GBM22P | | |
| | | | | GBM22R GBM23R | | |
| | | VSKVIAYLKRLVNAA | 21% P / 21% R | GBM3P | 11 | |
| | | | | GBM3R GBM5P | | |
| | | | | GBM5R GBM19P | | |
| | | | | GBM22P GBM22R | | |
| | | | | GBM23R ZH678 | | |
| | | | | ZH750 ZH784 | | |
| | | | | ZH791 ZH810 | | |
| | | VSKVIAYLKRLVNAAG | 21% P / 21% R | GBM3P | 11 | |
| | | | | GBM3R GBM5P | | |
| | | | | GBM5R GBM22P | | |
| | | | | GBM22R GBM23P | | |
| | | | | GBM23R ZH678 | | |
| | | | | ZH750 ZH784 | | |
| | | | | ZH791 ZH810 | | |
| | | VSKVIAYLKRLVNAAGSG | 0% P / 4% R | GBM23R | 0 | |
| | | FPNPYNQEKVLPRLP | 3% P / 4% R | GBM10R | 0 | |
| | | | | GBM11P | | |
| | | FPNPYNQEKVLPRLPY | 21% P / 21% R | GBM4R | 4 | |
| | | | | GBM6P GBM7P | 0 | |
| | | | | GBM9P GBM9R | | |
| | | | | GBM10R GBM11P | | |
| | | | | GBM11R GBM16P | | |
| | | | | GBM16R ZH616 | | |
| | | | | ZH757 ZH791 | | |
| | | FPNPYNQEKVLPRLPYG | 21% P / 25% R | GBM4R | 4 | |
| | | | | GBM6P GBM7P | | |
| | | | | GBM9P GBM9R | | |
| | | | | GBM10R GBM11P | | |
| | | | | GBM11R GBM16P | | |
| | | | | GBM16R GBM17R | | |
| | | | | ZH616 ZH757 | | |
| | | | | ZH791 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | FPNPYNQEKVLPRLPYGA | 11% P / 13% R | GBM4R | 0 | |
| | | | | GBM6P GBM7P | | |
| | | | | GBM11P GBM11R | | |
| | | | | GBM16P GBM16R | | |
| | | FPNPYNQEKVLPRLPYGAG | 3% P / 8% R | GBM11P | 1 | |
| | | | | GBM11R GBM17R | | |
| | | NPYNQEKVLPRLPYGAG | 3% P / 4% R | GBM11P | 0 | |
| | | | | GBM11R | | |
| | | TSYFPNPYNQEKVLPRLPYG | 3% P / 0% R | GBM6P | 0 | |
| | | YFPNPYNQEKVLPRLPYG | 8% P / 4% R | GBM6P | 0 | |
| | | | | GBM7P GBM16P | | |
| | | | | GBM16R | | |
| | | YFPNPYNQEKVLPRLPYGA | 3% P / 0% R | GBM7P | 0 | |
| Neural cell adhesion molecule 1 (NCAM1; P13591) | 61% P 63% R | | | | 5% | 6% |
| | | DVRFIVLSNNYLQ | 8% P / 0% R | GBM6P | 0 | |
| | | | | GBM7P GBM12P | | |
| | | DVRFIVLSNNYLQIR | 8% P / 4% R | GBM6P | 1 | |
| | | | | GBM7P GBM19P | | |
| | | | | ZH753 | | |
| | | FIVLSNNYLQIRG | 3% P / 0% R | ZH802 | 0 | |
| | | KDVRFIVLSNNYLQ | 3% P / 4% R | GBM14R | 1 | |
| | | | | ZH761 | | |
| | | KDVRFIVLSNNYLQIR | 5% P / 4% R | GBM7R | 0 | |
| | | | | GBM19P ZH761 | | |
| | | KDVRFIVLSNNYLQIRG | 3% P / 0% R | GBM7P | 0 | |
| | | LSNNYLQIRGIKKTDE | 13% P / 17% R | GBM4R | 2 | |
| | | | | GBM6P GBM7P | | |
| | | | | GBM7R GBM10R | | |
| | | | | GBM16P GBM16R | | |
| | | | | ZH616 ZH757 | | |
| | | LSNNYLQIRGIKKTDEG | 5% P / 4% R | GBM4R | 0 | |
| | | | | GBM16P ZH616 | | |
| | | NNYLQIRGIKKTDE | 11% P / 8% R | GBM4R | 0 | |
| | | | | GBM6P GBM7P | | |
| | | | | GBM10R GBM16P | | |
| | | | | ZH616 | | |
| | | NYLQIRGIKKTDE | 3% P / 0% R | ZH616 | 0 | |
| | | SNNYLQIRGIKK | 5% P / 8% R | GBM4R | 0 | |
| | | | | GBM7P GBM10R | | |
| | | | | ZH616 | | |
| | | SNNYLQIRGIKKT | 11% P / 4% R | GBM4R | 1 | |
| | | | | GBM6P GBM7P | | |
| | | | | GBM16P ZH616 | | |
| | | SNNYLQIRGIKKTD | 8% P / 4% R | GBM7P | 0 | |
| | | | | GBM10R GBM16P | | |
| | | | | ZH616 | | |
| | | SNNYLQIRGIKKTDE | 13% P / 21% R | GBM4R | 0 | |
| | | | | GBM6P GBM7P | | |
| | | | | GBM9R GBM10R | | |
| | | | | GBM11P GBM11R | | |
| | | | | GBM16P GBM16R | | |
| | | | | ZH757 | | |
| | | SNNYLQIRGIKKTDEG | 8% P / 8% R | GBM4R | 0 | |
| | | | | GBM7P GBM10R | | |
| | | | | GBM16P ZH616 | | |
| | | SNNYLQIRGIKKTDEGT | 3% P / 0% R | ZH616 | 0 | |
| | | VRFIVLSNNYLQIR | 3% P / 0% R | ZH802 | 0 | |
| Receptor-type tyrosine-protein phosphatase zeta (PTPRZ1; P23471) | 8% P 4% R | | | | 2% | 4% |
| | | IDEDLTQVNVNLKKLKFQGW | 3% P / 0% R | GBM19P | 0 | |
| | | KQSPINIDEDLTQVN | 0% P / 4% R | GBM10R | 0 | |
| | | KQSPINIDEDLTQVNVN | 5% P / 8% R | GBM10R | 1 | |
| | | | | GBM16P GBM20P | | |
| | | | | GBM20R | | |
| | | NSPKQSPINIDEDLTQVNVN | 3% P / 0% R | GBM16P | 0 | |
| | | NSPKQSPINIDEDLTQVNVNL | 5% P / 0% R | GBM13P | 0 | |
| | | | | GBM16P | | |
| | | QSPINIDEDLTQVN | 21% P / 17% R | GBM9P | 2 | |
| | | | | GBM9R GBM10R | | |
| | | | | GBM13P GBM15P | | |
| | | | | GBM16P GBM16R | | |
| | | | | GBM18P GBM20R | | |
| | | | | ZH617 ZH654 | | |
| | | | | ZH802 | | |
| | | QSPINIDEDLTQVNV | 5% P / 4% R | GBM20R | 0 | |
| | | | | ZH654 ZH802 | | |

| Protein | | Peptide | % P / R | Samples | | # | | |
|---|---|---|---|---|---|---|---|---|
| | | QSPINIDEDLTQVNVN | 18% P / 25% R | GBM9P | | 2 | | |
| | | | | GBM9R | GBM10R | | | |
| | | | | GBM13P | GBM13R | | | |
| | | | | GBM16P | GBM16R | | | |
| | | | | GBM18P | GBM18R | | | |
| | | | | GBM20R | ZH617 | | | |
| | | | | ZH654 | ZH802 | | | |
| | | QSPINIDEDLTQVNVNL | 3% P / 4% R | GBM10R | | 0 | | |
| | | | | GBM16P | | | | |
| | | SPINIDEDLTQVN | 3% P / 4% R | GBM10R | | 0 | | |
| | | | | ZH802 | | | | |
| | | SPINIDEDLTQVNV | 3% P / 0% R | ZH802 | | 0 | | |
| | | SPINIDEDLTQVNVN | 16% P / 13% R | GBM10R | | 0 | | |
| | | | | GBM13P | GBM16P | | | |
| | | | | GBM16R | GBM18P | | | |
| | | | | GBM20R | ZH617 | | | |
| | | | | ZH654 | ZH802 | | | |
| | | SPINIDEDLTQVNVNL | 5% P / 8% R | GBM10R | | 0 | | |
| | | | | GBM16R | GBM18P | | | |
| | | | | ZH802 | | | | |
| | | SPKQSPINIDEDLTQVN | 16% P / 17% R | GBM9P | | 1 | | |
| | | | | GBM9R | GBM10R | | | |
| | | | | GBM16P | GBM18P | | | |
| | | | | GBM18R | GBM20R | | | |
| | | | | ZH617 | ZH654 | | | |
| | | | | ZH802 | | | | |
| | | SPKQSPINIDEDLTQVNV | 0% P / 4% R | GBM20R | | 1 | | |
| | | SPKQSPINIDEDLTQVNVN | 18% P / 17% R | GBM8P | | 1 | | |
| | | | | GBM9R | GBM10R | | | |
| | | | | GBM13P | GBM16P | | | |
| | | | | GBM16R | GBM18R | | | |
| | | | | GBM20P | GBM20R | | | |
| | | | | ZH617 | ZH802 | | | |
| | | SPKQSPINIDEDLTQVNVNL | 5% P / 13% R | GBM10R | | 1 | | |
| | | | | GBM13P | GBM16P | | | |
| | | | | GBM16R | GBM20R | | | |
| | | | | GBM20R | | | | |
| | | SPKQSPINIDEDLTQVNVNLK | 0% P / 4% R | GBM10R | | 0 | | |
| Tenascin (TNC; P24821) | 76% P 67% R | | | | | | 15% | 18% |
| | | ARVATYLPAPEGLKFKS | 3% P / 0% R | GBM16P | | 0 | | |
| | | IPVSARVATYLPAPEGLKFKS | 3% P / 0% R | GBM16P | | 0 | | |
| | | RVATYLPAPEGLKFKS | 8% P / 0% R | GBM6P | | 0 | | |
| | | | | GBM16P | ZH616 | | | |
| | | VATYLPAPEGLKFK | 8% P / 17% R | GBM4R | | 1 | | |
| | | | | GBM6P | GBM9R | | | |
| | | | | GBM10R | GBM16R | | | |
| | | | | GBM16R | ZH616 | | | |
| | | VATYLPAPEGLKFKS | 16% P / 13% R | GBM4R | | 2 | | |
| | | | | GBM6P | GBM7P | | | |
| | | | | GBM10R | GBM11P | | | |
| | | | | GBM16P | GBM16R | | | |
| | | | | ZH616 | ZH757 | | | |
| | | EPGQEYNVLLTAEKGRH | 11% P / 8% R | GBM21P | | 0 | | |
| | | | | GBM21R | GBM22P | | | |
| | | | | GBM22R | ZH654 | | | |
| | | | | ZH761 | | | | |
| | | EPGQEYNVLLTAEKGRHKS | 11% P / 8% R | GBM21P | | 1 | | |
| | | | | GBM21R | GBM22P | | | |
| | | | | GBM22R | ZH654 | | | |
| | | | | ZH761 | | | | |
| | | NVLLTAEKGRHKSKPA | 5% P / 8% R | GBM4R | | 0 | | |
| | | | | GBM6P | GBM10R | | | |
| | | | | GBM16P | | | | |
| | | QEYNVLLTAEKGRHK | 5% P / 4% R | GBM21P | | 0 | | |
| | | | | GBM21R | ZH654 | | | |
| | | YNVLLTAEKGRHKS | 3% P / 0% R | GBM3P | | 0 | | |
| Chitinase-3-like protein 1 (CHI3L1; P36222) | 89% P 96% R | | | | | | 8% | 12% |
| | | AEFIKEAQPGKK | 3% P / 13% R | GBM4R | | 0 | | |
| | | | | GBM10R | GBM16P | | | |
| | | | | GBM16R | | | | |
| | | AEFIKEAQPGKKQ | 8% P / 8% R | GBM4R | | 0 | | |
| | | | | GBM6P | GBM10R | | | |
| | | | | GBM16P | ZH616 | | | |
| | | AGKVTIDSSYDIA | 0% P / 4% R | GBM10R | | 0 | | |
| | | AGKVTIDSSYDIAK | 0% P / 4% R | GBM10R | | 0 | | |
| | | AQPGKKQLLLSAALSAGKV | 3% P / 0% R | ZH654 | | 0 | | |
| | | DKQHFTTLIKEMK | 3% P / 0% R | ZH791 | | 0 | | |
| | | DKQHFTTLIKEMKA | 3% P / 4% R | GBM11P | | 0 | | |
| | | | | GBM17R | | | | |

| | | | |
|---|---|---|---|
| DKQHFTTLIKEMKAE | 13% P / 8% R | GBM11P | 1 |
| | GBM11R | GBM17P | |
| | GBM17R | ZH645 | |
| | ZH681 | ZH802 | |
| DKQHFTTLIKEMKAEF | 3% P / 0% R | GBM11P | 0 |
| DKQHFTTLIKEMKAEFIKEAQPG | 11% P / 4% R | GBM3P | 1 |
| | GBM5P | GBM11P | |
| | GBM22P | GBM22R | |
| | ZH791 | | |
| EAQPGKKQLLLSAALSAGK | 3% P / 0% R | ZH654 | 0 |
| EAQPGKKQLLLSAALSAGKV | 3% P / 0% R | ZH654 | 0 |
| EMKAEFIKEAQPGKKQ | 3% P / 4% R | GBM10R | 0 |
| | GBM16P | | |
| FTTLIKEMKAEFIKE | 0% P / 4% R | GBM14R | 0 |
| FTTLIKEMKAEFIKEA | 0% P / 4% R | GBM14R | 0 |
| FTTLIKEMKAEFIKEAQPG | 5% P / 8% R | GBM2R | 1 |
| | GBM12P | GBM14R | |
| | GBM19P | | |
| GKKQLLLSAALSAGKV | 3% P / 0% R | ZH654 | 0 |
| IKEAQPGKKQLLLSAALSA | 3% P / 0% R | ZH654 | 0 |
| IKEAQPGKKQLLLSAALSAGKV | 5% P / 4% R | GBM20P | 0 |
| | GBM20R | ZH654 | |
| IKEMKAEFIKEA | 0% P / 4% R | GBM14R | 0 |
| IKEMKAEFIKEAQPG | 5% P / 4% R | GBM12P | 1 |
| | GBM14R | ZH654 | |
| KAEFIKEAQPGKK | 0% P / 8% R | GBM4R | 0 |
| | GBM10R | | |
| KAEFIKEAQPGKKQ | 11% P / 13% R | GBM4R | 0 |
| | GBM6P | GBM10R | |
| | GBM11P | GBM16P | |
| | GBM16R | ZH616 | |
| KEAQPGKKQLLLSAALSAGK | 3% P / 0% R | GBM12P | 0 |
| LIKEMKAEFIKE | 0% P / 4% R | GBM14R | 0 |
| LIKEMKAEFIKEA | 0% P / 4% R | GBM14R | 0 |
| LIKEMKAEFIKEAQPG | 3% P / 4% R | GBM12P | 1 |
| | GBM14R | | |
| MKAEFIKEAQPGKK | 5% P / 4% R | GBM6P | 0 |
| | GBM9P | GBM10R | |
| MKAEFIKEAQPGKKQ | 8% P / 8% R | GBM4R | 0 |
| | GBM6P | GBM9P | |
| | GBM10R | GBM16P | |
| QPGKKQLLLSAALSAGK | 3% P / 4% R | GBM20R | 0 |
| | ZH654 | | |
| QPGKKQLLLSAALSAGKV | 11% P / 8% R | GBM5P | 0 |
| | GBM20P | GBM20R | |
| | GBM23R | ZH645 | |
| | ZH654 | | |
| SAGKVTIDSSYDIAK | 0% P / 4% R | GBM10R | 0 |
| TLIKEMKAEFIK | 5% P / 4% R | GBM14R | 0 |
| | GBM21P | ZH616 | |
| TLIKEMKAEFIKE | 8% P / 4% R | GBM12P | 1 |
| | GBM14R | GBM21P | |
| | ZH616 | | |
| TLIKEMKAEFIKEA | 8% P / 8% R | GBM2R | 1 |
| | GBM12P | GBM14P | |
| | GBM14R | ZH616 | |
| TLIKEMKAEFIKEAQPG | 16% P / 25% R | GBM2R | 3 |
| | GBM6P | GBM7P | |
| | GBM12P | GBM12R | |
| | GBM14P | GBM14R | |
| | GBM15P | GBM15R | |
| | GBM21P | GBM21R | |
| | ZH753 | | |
| TTLIKEMKAEFIK | 0% P / 4% R | GBM14R | 0 |
| TTLIKEMKAEFIKE | 5% P / 4% R | GBM12P | 0 |
| | GBM14P | GBM14R | |
| TTLIKEMKAEFIKEA | 5% P / 13% R | GBM2R | 0 |
| | GBM12P | GBM14R | |
| | GBM15P | ZH753 | |
| TTLIKEMKAEFIKEAQPG | 3% P / 17% R | GBM2R | 1 |
| | GBM12P | GBM12R | |
| | GBM14R | ZH753 | |
| YPGRRDKQHFTTLIKEMKAE | 3% P / 0% R | GBM11P | 0 |
| ATVHRILGQQVPYATKG | 3% P / 0% R | ZH654 | 1 |
| DDQESVKSKVQYLKDRQLAG | 0% P / 4% R | GBM14R | 0 |
| DQESVKSKVQYLKDRQLAG | 0% P / 4% R | GBM14R | 0 |
| ESVKSKVQYLKDRQ | 3% P / 4% R | GBM18P | 1 |
| | GBM18R | | |
| ESVKSKVQYLKDRQLAG | 0% P / 4% R | GBM14R | 0 |
| GATVHRILGQQVPYATKG | 5% P / 4% R | GBM20P | 0 |
| | GBM20R | ZH654 | |

| Protein | Coverage | Peptide | P / R % | Samples | Count | | |
|---|---|---|---|---|---|---|---|
| | | GNQWVGYDDQESVKS | 0% P / 4% R | GBM20R | 0 | | |
| | | KSKVQYLKDRQLA | 3% P / 0% R | ZH654 | 0 | | |
| | | KSKVQYLKDRQLAG | 0% P / 4% R | GBM10R | 0 | | |
| | | SVKSKVQYLKDRQLAG | 0% P / 4% R | GBM14R | 0 | | |
| | | TVHRILGQQVPYA | 3% P / 0% R | ZH654 | 0 | | |
| | | TVHRILGQQVPYAT | 3% P / 0% R | ZH654 | 0 | | |
| | | TVHRILGQQVPYATK | 8% P / 4% R | GBM18P GBM20P ZH654 · GBM20R | 1 | | |
| | | TVHRILGQQVPYATKG | 11% P / 13% R | GBM9P GBM9R GBM18R GBM20R · GBM18P GBM20P ZH654 | 2 | | |
| | | TVHRILGQQVPYATKGN | 3% P / 0% R | ZH654 | 0 | | |
| | | TVHRILGQQVPYATKGNQ | 3% P / 0% R | ZH654 | 0 | | |
| | | VHRILGQQVPYA | 3% P / 0% R | ZH654 | 0 | | |
| | | VHRILGQQVPYAT | 3% P / 0% R | ZH654 | 0 | | |
| | | VHRILGQQVPYATK | 8% P / 0% R | GBM18P ZH654 · ZH802 | 0 | | |
| | | VHRILGQQVPYATKG | 11% P / 4% R | GBM18P GBM20P ZH654 · GBM20R ZH802 | 1 | | |
| | | VKSKVQYLKDRQLA | 3% P / 0% R | ZH654 | 0 | | |
| | | VKSKVQYLKDRQLAG | 16% P / 21% R | GBM4R GBM6P GBM9R GBM11P GBM14R GBM16R · GBM9P GBM10R GBM14P GBM16P ZH791 | 3 | | |
| | | VKSKVQYLKDRQLAGA | 0% P / 4% R | GBM10R | 0 | | |
| | | WVGYDDQESVKSK | 5% P / 4% R | GBM9P GBM20P · GBM20R | 0 | | |
| | | WVGYDDQESVKSKVQ | 5% P / 8% R | GBM9P GBM9R GBM20R · GBM20P | 1 | | |
| | | WVGYDDQESVKSKVQY | 3% P / 0% R | GBM20P | 0 | | |
| | | YDDQESVKSKVQYLKDRQLAG | 0% P / 4% R | GBM14R | 0 | | |
| Transforming growth factor beta-2 (TGFB2; P61812) | 37% P 38% R | | | | | 4% | 3% |
| | | AEFRVFRLQNPKAR | 8% P / 0% R | GBM18P ZH654 · ZH802 | 2 | | |
| | | AEFRVFRLQNPKARVP | 3% P / 0% R | ZH654 | 0 | | |
| | | AEFRVFRLQNPKARVPE | 16% P / 8% R | GBM6P GBM10R GBM18P ZH616 ZH802 · GBM16P GBM18R ZH654 | 4 | | |
| | | AEFRVFRLQNPKARVPEQ | 5% P / 0% R | ZH654 ZH802 | 0 | | |
| | | EFRVFRLQNPKAR | 5% P / 0% R | ZH654 ZH802 | 0 | | |
| | | EFRVFRLQNPKARVP | 3% P / 0% R | ZH654 | 0 | | |
| | | EFRVFRLQNPKARVPE | 21% P / 13% R | GBM4R GBM6P GBM9P GBM11P GBM16R ZH654 · GBM7P GBM10R GBM16P ZH616 ZH802 | 3 | | |
| | | FRVFRLQNPKARVP | 0% P / 4% R | GBM10R | 0 | | |
| | | FRVFRLQNPKARVPE | 16% P / 13% R | GBM4R GBM6P GBM10R GBM16P ZH616 · GBM7P GBM11P GBM16R ZH654 | 1 | | |
| Vasorin (VASN; Q6EMK4) | 39% P 38% R | | | | | 10% | 7% |
| | | DNELRALPPLRLPR | 8% P / 4% R | GBM4R GBM6P GBM16P · GBM12P | 7 | | |
| | | GKNRIRHIQPGAFD | 3% P / 0% R | ZH616 | 0 | | |
| | | GKNRIRHIQPGAFDT | 3% P / 0% R | ZH616 | 0 | | |
| | | IQPGAFDTLDRLLELK | 3% P / 0% R | GBM3P | 2 | | |
| | | KLQDNELRALPPLRLPR | 11% P / 0% R | GBM6P GBM12P ZH616 · GBM16P | 5 | | |
| | | KLQDNELRALPPLRLPRL | 5% P / 0% R | GBM6P GBM16P | 1 | | |
| | | LQDNELRALPPLRLPR | 8% P / 4% R | GBM4R GBM6P GBM16P · GBM12P | 7 | | |
| | | LQDNELRALPPLRLPRL | 5% P / 0% R | GBM6P GBM16P | 2 | | |

| | | Peptide | P / R | Samples | | Count | | |
|---|---|---|---|---|---|---|---|---|
| | | QDNELRALPPLRLPR | 3% P / 8% R | GBM6P<br>GBM10R | ZH753 | 13 | | |
| | | QDNELRALPPLRLPRL | 3% P / 4% R | GBM6P<br>GBM10R | | 7 | | |
| | | QPGAFDTLDRLLELK | 5% P / 4% R | GBM3P<br>GBM22P | GBM22R | 2 | | |
| Receptor expression-enhancing protein 4 (REEP4; Q9H6H4) | 24% P<br>25% R | | | | | | 1% | 1% |
| | | SLSRHEKEIDAYIVQAKERSYETV | 24% P / 21% R | GBM7R<br>GBM10R<br>GBM13P<br>GBM18P<br>GBM23P<br>ZH681<br>ZH761<br>ZH802 | GBM12R<br>GBM13R<br>GBM19R<br>ZH678<br>ZH750<br>ZH791 | 0 | | |
| Adseverin (SCIN; Q9Y6U3) | 32% P<br>42% R | | | | | | 4% | 2% |
| | | AGLQVWRIEKLELVPVPQ | 3% P / 4% R | GBM16R<br>ZH757 | | 0 | | |
| | | GDFYVGDAYLVLHTA | 3% P / 8% R | GBM4R<br>GBM20R | ZH791 | 0 | | |
| | | GLQVWRIEKLELVPVPQ | 13% P / 17% R | GBM6P<br>GBM9P<br>GBM10R<br>GBM16R<br>GBM21P | GBM9R<br>GBM16P<br>GBM20R<br>ZH757 | 1 | | |
| | | LQVWRIEKLELVPVP | 0% P / 4% R | GBM9R | | 0 | | |
| | | LQVWRIEKLELVPVPQ | 13% P / 17% R | GBM6P<br>GBM9P<br>GBM10R<br>GBM16R<br>ZH757 | GBM9R<br>GBM16P<br>GBM20R<br>ZH791 | 5 | | |
| | | VPVPQSAHGDFYVGDAYLVLHTA | 0% P / 4% R | GBM20R | | 0 | | |
| | | DKPLIIYKNGTSK | 8% P / 21% R | GBM4R<br>GBM9P<br>GBM10R<br>GBM11R<br>GBM16R | GBM9R<br>GBM11P<br>GBM16P | 1 | | |
| | | DKPLIIYKNGTSKK | 16% P / 25% R | GBM4R<br>GBM6P<br>GBM9P<br>GBM10R<br>GBM11R<br>GBM16R<br>ZH791 | GBM7R<br>GBM9R<br>GBM11P<br>GBM16P<br>ZH757 | 3 | | |
| | | DKPLIIYKNGTSKKG | 16% P / 21% R | GBM4R<br>GBM6P<br>GBM9R<br>GBM11P<br>GBM16P<br>ZH757 | GBM9P<br>GBM10R<br>GBM11R<br>GBM16R<br>ZH791 | 2 | | |
| | | DKPLIIYKNGTSKKGGQAPAPP | 5% P / 4% R | GBM6P<br>GBM10R | GBM16P | 0 | | |
| | | FKDKPLIIYKNGTSKKG | 3% P / 0% R | GBM6P | | 0 | | |
| | | KDKPLIIYKNGTSK | 3% P / 0% R | GBM6P | | 0 | | |
| | | KDKPLIIYKNGTSKK | 16% P / 21% R | GBM4R<br>GBM6P<br>GBM9R<br>GBM11P<br>GBM16P<br>ZH757 | GBM9P<br>GBM10R<br>GBM11R<br>GBM16R<br>ZH791 | 1 | | |
| | | KDKPLIIYKNGTSKKG | 5% P / 4% R | GBM6P<br>GBM10R | GBM11P | 0 | | |
| | | KEPVHLLSLFKDKPLIIYKNGTSK | 0% P / 4% R | GBM23R | | 0 | | |
| Scavenger receptor cysteine-rich type 1 protein M130 (CD163; Q86VB7) | 37% P<br>33% R | | | | | | 14% | 12% |
| | | AAELISVSKFLPIS | 8% P / 4% R | GBM9P<br>GBM9R<br>ZH616 | GBM16P | 11 | | |
| | | AAELISVSKFLPISG | 11% P / 4% R | GBM9P<br>GBM9R<br>ZH757 | ZH616<br>ZH791 | 5 | | |
| | | AELISVSKFLPIS | 5% P / 4% R | GBM9P<br>GBM9R | ZH791 | 10 | | |
| | | AELISVSKFLPISG | 18% P / 13% R | GBM4R<br>GBM9P<br>GBM12R<br>GBM16P<br>ZH681<br>ZH791 | GBM9R<br>GBM15P<br>ZH616<br>ZH757 | 10 | | |
| | | ELISVSKFLPIS | 5% P / 4% R | GBM9P<br>GBM9R | ZH791 | 1 | | |

| Protein | | Peptide | % P / % R | Samples | | Count | | |
|---|---|---|---|---|---|---|---|---|
| | | ELISVSKFLPISG | 5% P / 4% R | GBM9R | | 2 | | |
| | | | | GBM15P | ZH791 | | | |
| | | ENSHESADFSAAELISVSKFLPIS | 0% P / 4% R | GBM12R | | 2 | | |
| | | HESADFSAAELISVSKFLPIS | 3% P / 0% R | GBM16P | | 0 | | |
| | | SAAELISVSKFLPIS | 3% P / 4% R | GBM12R | | 4 | | |
| | | | | GBM15P | | | | |
| | | SENSHESADFSAAELISVSKFLPIS | 3% P / 4% R | GBM9R | | 0 | | |
| | | | | GBM16P | | | | |
| | | SHESADFSAAELISVSKFLPIS | 8% P / 13% R | GBM3P | | 24 | | |
| | | | | GBM5R | GBM9P | | | |
| | | | | GBM9R | GBM12R | | | |
| | | | | GBM16P | | | | |
| | | SHESADFSAAELISVSKFLPISG | 3% P / 4% R | GBM9R | | 4 | | |
| | | | | GBM16P | | | | |
| Brevican core protein (BCAN; Q96GW7) | 63% P 38% R | | | | | | 1% | 1% |
| | | AEDLNGELFLGD | 8% P / 4% R | GBM5P | | 0 | | |
| | | | | GBM10R | ZH757 | | | |
| | | | | ZH802 | | | | |
| | | AEDLNGELFLGDPP | 32% P / 25% R | GBM4R | | 1 | | |
| | | | | GBM5P | GBM5R | | | |
| | | | | GBM6P | GBM7P | | | |
| | | | | GBM10R | GBM16P | | | |
| | | | | GBM16R | GBM17R | | | |
| | | | | GBM23P | GBM23R | | | |
| | | | | ZH616 | ZH631 | | | |
| | | | | ZH645 | ZH654 | | | |
| | | | | ZH678 | ZH681 | | | |
| | | | | ZH802 | | | | |
| | | AEDLNGELFLGDPPE | 39% P / 25% R | GBM4R | | 1 | | |
| | | | | GBM5P | GBM5R | | | |
| | | | | GBM6P | GBM7P | | | |
| | | | | GBM10R | GBM16P | | | |
| | | | | GBM16R | GBM17R | | | |
| | | | | GBM18P | GBM19P | | | |
| | | | | GBM23P | GBM23R | | | |
| | | | | ZH616 | ZH631 | | | |
| | | | | ZH645 | ZH654 | | | |
| | | | | ZH681 | ZH757 | | | |
| | | | | ZH802 | ZH810 | | | |
| | | DLNGELFLGDPP | 3% P / 4% R | GBM5P | | 0 | | |
| | | | | GBM10R | | | | |
| | | DLNGELFLGDPPE | 13% P / 8% R | GBM5P | | 0 | | |
| | | | | GBM6P | GBM10R | | | |
| | | | | GBM18P | GBM23P | | | |
| | | | | GBM23R | ZH802 | | | |
| | | EDLNGELFLGDPP | 21% P / 17% R | GBM5P | | 0 | | |
| | | | | GBM6P | GBM10R | | | |
| | | | | GBM16R | GBM17R | | | |
| | | | | GBM23P | GBM23R | | | |
| | | | | ZH631 | ZH645 | | | |
| | | | | ZH681 | ZH757 | | | |
| | | | | ZH802 | | | | |
| | | EDLNGELFLGDPPE | 11% P / 17% R | GBM6P | | 0 | | |
| | | | | GBM10R | GBM16R | | | |
| | | | | GBM17R | GBM23P | | | |
| | | | | GBM23R | ZH757 | | | |
| | | | | ZH802 | | | | |
| | | LNGELFLGDPP | 3% P / 0% R | ZH802 | | 0 | | |
| | | LNGELFLGDPPE | 3% P / 0% R | ZH802 | | 0 | | |
| | | YAEDLNGELFLGD | 3% P / 0% R | GBM5P | | 0 | | |
| | | YAEDLNGELFLGDPP | 29% P / 21% R | GBM4R | | 1 | | |
| | | | | GBM5P | GBM5R | | | |
| | | | | GBM6P | GBM7P | | | |
| | | | | GBM10R | GBM17R | | | |
| | | | | GBM23P | GBM23R | | | |
| | | | | ZH631 | ZH645 | | | |
| | | | | ZH654 | ZH681 | | | |
| | | | | ZH757 | ZH802 | | | |
| | | | | ZH810 | | | | |
| | | YAEDLNGELFLGDPPE | 37% P / 25% R | GBM4R | | 0 | | |
| | | | | GBM5P | GBM5R | | | |
| | | | | GBM6P | GBM7P | | | |
| | | | | GBM10R | GBM16P | | | |
| | | | | GBM16R | GBM17R | | | |
| | | | | GBM19P | GBM23P | | | |
| | | | | GBM23R | ZH631 | | | |
| | | | | ZH645 | ZH654 | | | |
| | | | | ZH678 | ZH681 | | | |
| | | | | ZH757 | ZH802 | | | |
| | | | | ZH810 | | | | |

| Protein | Protein % | Peptide | Peptide % P / % R | Samples | Count | | |
|---|---|---|---|---|---|---|---|
| | | YAEDLNGELFLGDPPEK | 3% P / 0% R | GBM23P ZH757 | 0 | | |
| Prolow-density lipoprotein receptor-related protein 1 (LRP1; Q07954) | 95% P 96% R | | | | | 51% | 51% |
| | | DFHLSQSALYW | 3% P / 8% R | GBM12R GBM22P GBM22R | 0 | | |
| | | HKGDYSVLVPGLRN | 3% P / 4% R | GBM6P GBM10R | 0 | | |
| | | HKGDYSVLVPGLRNT | 5% P / 8% R | GBM4R GBM6P GBM10R ZH616 | 1 | | |
| | | HKGDYSVLVPGLRNTIA | 0% P / 4% R | GBM10R | 0 | | |
| | | IALDFHLSQSAL | 0% P / 4% R | GBM22R | 0 | | |
| | | IALDFHLSQSALY | 3% P / 0% R | GBM22P | 0 | | |
| | | IALDFHLSQSALYW | 5% P / 8% R | GBM12P GBM12R GBM22P GBM22R | 0 | | |
| | | IALDFHLSQSALYWT | 5% P / 4% R | GBM12P GBM22P GBM22R | 0 | | |
| | | IALDFHLSQSALYWTD | 3% P / 4% R | GBM22P GBM22R | 0 | | |
| | | KGDYSVLVPGLRN | 0% P / 8% R | GBM4R GBM10R | 0 | | |
| | | KGDYSVLVPGLRNT | 3% P / 8% R | GBM4R GBM6P GBM10R | 0 | | |
| | | LDFHLSQSALYW | 3% P / 4% R | GBM22P GBM22R | 1 | | |
| | | LDFHLSQSALYWT | 3% P / 0% R | GBM22P | 0 | | |
| | | LRNTIALDFHLSQS | 3% P / 0% R | GBM16P | 0 | | |
| | | LVPGLRNTIALDFHL | 5% P / 8% R | GBM5P GBM5R GBM13P GBM23R | 2 | | |
| | | VPGLRNTIALDFHL | 3% P / 0% R | GBM13P | 0 | | |
| Contactin-1 (CNTN1; Q12860) | 45% P 46% R | | | | | 3% | 4% |
| | | KPIPTIRWLKNGYAYH | 16% P / 21% R | GBM4R GBM6P GBM7P GBM10R GBM14P GBM14R GBM18P GBM18R ZH654 ZH753 ZH802 | 2 | | |
| | | KPIPTIRWLKNGYAYHK | 21% P / 29% R | GBM4R GBM6P GBM7P GBM7R GBM10R GBM14R GBM18P GBM18R GBM21R GBM23P ZH654 ZH753 ZH757 ZH761 ZH802 | 1 | | |
| | | KPIPTIRWLKNGYAYHKG | 11% P / 13% R | GBM4R GBM6P GBM7P GBM10R GBM14R GBM18P ZH761 | 2 | | |
| | | KPIPTIRWLKNGYAYHKGE | 3% P / 4% R | GBM4R GBM18P | 0 | | |
| Serine protease HTRA1 (HTRA1; Q92743) | 71% P 71% R | | | | | 29% | 17% |
| | | AYIIEVIPDTPAE | 5% P / 4% R | GBM18R ZH654 ZH802 | 3 | | |
| | | AYIIEVIPDTPAEA | 8% P / 4% R | GBM18P GBM18R ZH654 ZH802 | 4 | | |
| | | AYIIEVIPDTPAEAG | 8% P / 4% R | GBM18P GBM18R ZH654 ZH802 | 4 | | |
| | | DVIISINGQSVVS | 16% P / 4% R | GBM6P GBM7P GBM14P GBM14R GBM15P GBM19P ZH761 | 11 | | |
| | | DVIISINGQSVVSA | 8% P / 4% R | GBM6P GBM14P GBM14R GBM19P | 3 | | |
| | | DVIISINGQSVVSAN | 13% P / 8% R | GBM6P GBM7P GBM12P GBM14P GBM14R GBM19P ZH753 | 3 | | |
| | | DVIKRESTLNMVV | 0% P / 4% R | GBM18R | 1 | | |
| | | DVIKRESTLNMVVR | 8% P / 4% R | GBM18P GBM18R ZH654 ZH802 | 5 | | |
| | | DVIKRESTLNMVVRR | 0% P / 4% R | GBM18R | 1 | | |
| | | DVIKRESTLNMVVRRG | 0% P / 4% R | GBM13R | 0 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | DVSDVIKRESTLNMVVR | 8% P / 8% R | GBM18P | 11 | |
| | | | | GBM18R | GBM20R | |
| | | | | ZH654 | ZH802 | |
| | | DVSDVIKRESTLNMVVRR | 5% P / 4% R | GBM18P | 5 | |
| | | | | GBM18R | ZH802 | |
| | | ENDVIISINGQSVVS | 16% P / 4% R | GBM2P | 20 | |
| | | | | GBM6P | GBM7P | |
| | | | | GBM14P | GBM14R | |
| | | | | GBM15P | GBM19P | |
| | | ENDVIISINGQSVVSA | 11% P / 8% R | GBM6P | 5 | |
| | | | | GBM7P | GBM12P | |
| | | | | GBM14P | GBM14R | |
| | | | | ZH753 | | |
| | | ENDVIISINGQSVVSAN | 8% P / 13% R | GBM6P | 4 | |
| | | | | GBM7P | GBM12R | |
| | | | | GBM14R | GBM19P | |
| | | | | ZH753 | | |
| | | ENDVIISINGQSVVSAND | 3% P / 0% R | GBM6P | 1 | |
| | | GAYIIEVIPDTPAE | 0% P / 4% R | GBM18R | 1 | |
| | | GAYIIEVIPDTPAEA | 3% P / 4% R | GBM18R | 4 | |
| | | | | ZH654 | | |
| | | GAYIIEVIPDTPAEAG | 5% P / 4% R | GBM18P | 4 | |
| | | | | GBM18R | ZH654 | |
| | | GLKENDVIISINGQSVVSA | 3% P / 0% R | GBM6P | 0 | |
| | | IEVIPDTPAEAGGL | 0% P / 4% R | GBM10R | 0 | |
| | | IIEVIPDTPAEA | 3% P / 0% R | ZH654 | 3 | |
| | | IIEVIPDTPAEAG | 0% P / 4% R | GBM18R | 1 | |
| | | IIEVIPDTPAEAGGL | 3% P / 4% R | GBM16P | 3 | |
| | | | | GBM16R | | |
| | | IKRESTLNMVVRRGNE | 0% P / 4% R | GBM18R | 0 | |
| | | KENDVIISINGQSVVS | 3% P / 8% R | GBM14R | 3 | |
| | | | | GBM18R | ZH802 | |
| | | KENDVIISINGQSVVSA | 5% P / 4% R | GBM6P | 0 | |
| | | | | GBM14R | GBM19P | |
| | | SDVIKRESTLNM | 5% P / 4% R | GBM18R | 3 | |
| | | | | ZH654 | ZH802 | |
| | | SDVIKRESTLNMVV | 8% P / 4% R | GBM18P | 3 | |
| | | | | GBM18R | ZH654 | |
| | | | | ZH802 | | |
| | | SDVIKRESTLNMVVR | 8% P / 4% R | GBM18P | 4 | |
| | | | | GBM18R | ZH654 | |
| | | | | ZH802 | | |
| | | SDVIKRESTLNMVVRR | 0% P / 4% R | GBM13R | 0 | |
| | | VIISINGQSVV | 3% P / 4% R | GBM18R | 2 | |
| | | | | ZH802 | | |
| | | VIISINGQSVVS | 5% P / 0% R | ZH761 | 3 | |
| | | | | ZH802 | | |
| | | VIISINGQSVVSA | 0% P / 4% R | ZH753 | 0 | |
| | | VIKRESTLNMVV | 0% P / 4% R | GBM18R | 1 | |
| | | VIKRESTLNMVVR | 3% P / 0% R | ZH802 | 1 | |
| | | VIPDTPAEAG | 3% P / 0% R | ZH802 | 0 | |
| | | VSDVIKRESTLNM | 0% P / 4% R | GBM18R | 1 | |
| | | VSDVIKRESTLNMVV | 8% P / 8% R | GBM18P | 5 | |
| | | | | GBM18R | GBM20P | |
| | | | | GBM20R | ZH654 | |
| | | VSDVIKRESTLNMVVR | 11% P / 13% R | GBM9R | 16 | |
| | | | | GBM18P | GBM18R | |
| | | | | GBM20P | GBM20R | |
| | | | | ZH654 | ZH802 | |
| | | VSDVIKRESTLNMVVRR | 8% P / 0% R | GBM18P | 1 | |
| | | | | ZH654 | ZH802 | |
| | | YIIEVIPDTPAEA | 8% P / 4% R | GBM18P | 3 | |
| | | | | GBM18R | ZH654 | |
| | | | | ZH802 | | |
| | | YIIEVIPDTPAEAG | 8% P / 4% R | GBM18P | 1 | |
| | | | | GBM18R | ZH654 | |
| | | | | ZH802 | | |
| Neuronal cell adhesion molecule (NRCAM; Q92823) | 79% P 75% R | | | | 5% | 6% |
| | | EKKILTFQGSKTHG | 0% P / 4% R | GBM10R | 0 | |
| | | EKKILTFQGSKTHGMLPG | 3% P / 0% R | GBM12P | 0 | |
| | | HIEKKILTFQGSKTHG | 3% P / 0% R | ZH616 | 0 | |
| | | IEKKILTFQGSKTH | 16% P / 13% R | GBM4R | 0 | |
| | | | | GBM6P | GBM9P | |
| | | | | GBM10R | GBM16P | |
| | | | | GBM16R | ZH616 | |
| | | | | ZH757 | ZH791 | |

| | | Peptide | % P / % R | GBM samples | | Count | | |
|---|---|---|---|---|---|---|---|---|
| | | IEKKILTFQGSKTHG | 18% P / 17% R | GBM4R | | 1 | | |
| | | | | GBM6P | GBM7P | | | |
| | | | | GBM9P | GBM10R | | | |
| | | | | GBM14R | GBM16P | | | |
| | | | | GBM16R | ZH616 | | | |
| | | | | ZH757 | ZH791 | | | |
| | | IEKKILTFQGSKTHGM | 8% P / 8% R | GBM4R | | 0 | | |
| | | | | GBM6P | GBM10R | | | |
| | | | | ZH616 | ZH791 | | | |
| | | IEKKILTFQGSKTHGMLPG | 0% P / 8% R | GBM4R | | 0 | | |
| | | | | GBM10R | | | | |
| | | ILTFQGSKTHGMLPG | 3% P / 4% R | GBM12P | | 0 | | |
| | | | | GBM22R | | | | |
| | | KILTFQGSKTHGMLP | 3% P / 0% R | GBM12P | | 0 | | |
| | | KILTFQGSKTHGMLPG | 11% P / 8% R | GBM12P | | 0 | | |
| | | | | GBM12R | GBM22P | | | |
| | | | | GBM22R | ZH645 | | | |
| | | | | ZH681 | | | | |
| | | KKILTFQGSKTHG | 3% P / 8% R | GBM10R | | 0 | | |
| | | | | GBM14R | GBM16P | | | |
| | | KKILTFQGSKTHGMLPG | 3% P / 0% R | GBM12P | | 0 | | |
| Sodium-coupled neutral amino acid transporter 3 (SLC38A3; Q99624) | 18% P 17% R | | | | | | 2% | 7% |
| | | EGLLPVITPMAGNQRVEDPA | 3% P / 4% R | GBM14R | | 0 | | |
| | | | | GBM21P | | | | |
| | | GLLPVITPMAGNQRVEDPA | 13% P / 8% R | GBM6P | | 1 | | |
| | | | | GBM14P | GBM14R | | | |
| | | | | GBM16P | GBM21P | | | |
| | | | | GBM21R | ZH616 | | | |
| | | LLPVITPMAGNQRVE | 3% P / 4% R | GBM6P | | 0 | | |
| | | | | GBM14R | | | | |
| | | LLPVITPMAGNQRVED | 0% P / 4% R | GBM14R | | 0 | | |
| | | LLPVITPMAGNQRVEDP | 3% P / 4% R | GBM6P | | 0 | | |
| | | | | GBM14R | | | | |
| | | LLPVITPMAGNQRVEDPA | 13% P / 13% R | GBM4R | | 2 | | |
| | | | | GBM6P | GBM14P | | | |
| | | | | GBM14R | GBM16P | | | |
| | | | | GBM21P | GBM21R | | | |
| | | | | ZH616 | | | | |
| | | LPVITPMAGNQR | 0% P / 4% R | GBM14R | | 0 | | |
| | | LPVITPMAGNQRVE | 8% P / 8% R | GBM14P | | 1 | | |
| | | | | GBM14R | GBM21P | | | |
| | | | | GBM21R | ZH616 | | | |
| | | LPVITPMAGNQRVED | 0% P / 4% R | GBM14R | | 0 | | |
| | | LPVITPMAGNQRVEDP | 5% P / 13% R | GBM10R | | 2 | | |
| | | | | GBM14R | GBM21P | | | |
| | | | | GBM21R | ZH616 | | | |
| | | LPVITPMAGNQRVEDPA | 16% P / 17% R | GBM4R | | 3 | | |
| | | | | GBM6P | GBM10R | | | |
| | | | | GBM11P | GBM14P | | | |
| | | | | GBM14R | GBM16P | | | |
| | | | | GBM21P | GBM21R | | | |
| | | | | ZH616 | | | | |
| | | PVITPMAGNQRVE | 0% P / 4% R | GBM14R | | 0 | | |
| | | PVITPMAGNQRVED | 0% P / 4% R | GBM14R | | 0 | | |
| | | PVITPMAGNQRVEDP | 0% P / 4% R | GBM14R | | 0 | | |
| | | PVITPMAGNQRVEDPA | 5% P / 13% R | GBM6P | | 0 | | |
| | | | | GBM10R | GBM14P | | | |
| | | | | GBM14R | GBM21R | | | |
| | | SEGLLPVITPMAGNQRVEDPA | 3% P / 4% R | GBM6P | | 0 | | |
| | | | | GBM14R | | | | |

**Supplementary Table 5. Established TAAs and CTAs identified as glioblastoma-exclusive antigens on both primary and recurrent tumors or represented by glioblastoma-exclusive peptides on ≥ 2 primary as well as ≥ 2 recurrent neoplasms of a minimum of four different patients.** HLA restrictions not passing manual assessment as quality control are indicated in italic. The number of positive tumors other than glioblastoma was based on n=824 HLA class I and n=585 HLA class II peptidome datasets. The frequency of positive benign HLA peptidomes was calculated from n=418 (HLA class I) or n=364 (HLA class II) benign human specimens. Glioblastoma exclusivity of HLA class II-restricted peptides was evaluated for the exact sequence match. Frequencies of positive tumors are given separately for n=38 primary (P) and n=24 recurrent (R) glioblastomas.

| Peptide sequence | HLA restriction | Frequency of positive tumors | | Protein frequency on glioblastmas | Protein frequency on non-GBM tumors Peptide-positive non-GBM tumors | Protein frequency on benign samples |
|---|---|---|---|---|---|---|
| **Glioblastoma-exclusive HLA class I-presented antigens derived from established TAAs and CTAs** | | | | | | |
| Oligodendrocyte transcription factor (OLIG2; Q13516) | | | | 34% P / 33% R | 1% | 0% |
| Supplementary Table 2 | | | | | | |
| EMKRLVSEI | *B*08:01* | 0% P / 4% R | ZH753 | | 0 | |
| SIRPPHGLLK | A*03:01 | 3% P / 0% R | ZH761 | | 0 | |
| SLPGSGLPSV | A*02:01 | 3% P / 0% R | ZH757 | | 0 | |
| Transmembrane protein 255A (TMEM255A; Q5JRV8) | | | | 34% P / 17% R | 1% | 0% |
| Supplementary Table 2 | | | | | | |
| Paired box protein Pax-6 (PAX6; P26367) | | | | 24% P / 21% R | 1% | 0% |
| Supplementary Table 3 | | | | | | |
| FTQEQIEAL | A*02:05; C*12:03 | 3% P / 0% R | GBM6P | | 1 | |
| RYYETGSIRPR | A*31:01 | 8% P / 0% R | ZH613 ZH802 ZH654 | | 3 | |
| SALPPMPSF | C*12:03 | 3% P / 0% R | GBM6P | | 1 | |
| Immunoglobulin superfamily member 11 (IGSF11; Q5DX21) | | | | 18% P / 17% R | 0% | 0% |
| Supplementary Table 2 | | | | | | |
| Protein AF1q (MLLT11; Q13015) | | | | 16% P / 21% R | 2% | 0% |
| APIASIHSF | B*07:02; B*35:01 | 8% P / 21% R | GBM4P GBM4R GBM6P GBM6R GBM12R GBM16P GBM16R GBM18R | | 7 | |
| ATYKVKDSSVGK | A*03:01; A*11:01 | 5% P / 0% R | ZH631 ZH761 | | 0 | |
| NPEGDGLLEY | B*35:01 | 3% P / 0% R | GBM6P | | 1 | |
| QEKNPEGDGL | B*44:02 | 3% P / 0% R | GBM15P | | 0 | |
| CD276 antigen (CD276; Q5ZPR3) | | | | 5% P / 4% R | 4% | 0% |
| AQLNLIWQL | A*02:01; B*13:02 | 5% P / 4% R | GBM5P GBM13R GBM20P | | 6 | |
| Interleukin-13 receptor subunit alpha-2 (IL13RA2; Q14627) | | | | 5% P / 4% R | 0% | 0% |
| LLDTNYNLFY | A*01:01 | 3% P / 0% R | GBM3P | | 0 | |
| PLPPVYLTF | *A*23:01* | 0% P / 4% R | GBM23R | | 0 | |
| **Glioblastoma-exclusive HLA class I ligands derived from established TAAs and CTAs** | | | | | | |
| Ankyrin repeat domain-containing protein 40 (ANKRD40; Q6AI12) | | | | 55% P / 42% R | 10% | 6% |
| ELDRQELTY | A*01:01 | 18% P / 13% R | GBM3P GBM5P GBM9P GBM9R GBM13P GBM13R GBM16P GBM16R ZH617 ZH645 | | 12 | |
| Adenomatous polyposis coli protein (APC; P25054) | | | | 68% P / 58% R | 15% | 19% |
| LPSSSSSRGSL | B*07:02 | 16% P / 21% R | GBM4P GBM4R GBM7P GBM7R GBM10R GBM16P GBM16R GBM18P GBM18R ZH616 ZH681 | | 24 | |
| ELDTPINY | A*01:01 | 13% P / 17% R | GBM5P GBM9P GBM9R GBM13R GBM16P GBM16R GBM17R ZH645 ZH654 | | 3 | |
| KVMEEVSAI | A*02:01; C*02:02; C*15:02 | 11% P / 8% R | GBM3P GBM7R GBM14R ZH617 ZH678 ZH618 | | 4 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Brevican core protein (BCAN; Q96GW7)** | | | | 58% P / 21% R | 1% | 1% |
| YEVDTVLRY | B*18:01; B*44:03 | 8% P / 8% R | GBM3P GBM3R GBM17R ZH678 ZH720 | | 0 | |
| **Carbonic anhydrase 9 (CA9; Q16790)** | | | | 18% P / 8% R | 3% | 2% |
| SPRAAEPVQL | B*07:02 | 13% P / 8% R | GBM16P GBM16R GBM18P GBM18R ZH616 ZH681 ZH757 | | 11 | |
| **Cyclin-dependent kinase 4 (CDK4; P11802)** | | | | 50% P / 54% R | 13% | 8% |
| Supplementary Table 3 | | | | | | |
| **Ceramide synthase 1 (CERS1; P27544)** | | | | 68% P / 71% R | 2% | 2% |
| Supplementary Table 3 | | | | | | |
| VLTGQVHEL | A*02:01 | 16% P / 13% R | GBM10R GBM11P GBM14R ZH616 ZH678 ZH681 ZH753 ZH757 ZH761 | | 0 | |
| **Contactin-2 (CNTN2; Q02246)** | | | | 37% P / 58% R | 0% | 2% |
| IVRNGGTSM | B*07:02 | 11% P / 17% R | GBM4P GBM4R GBM7P GBM7R GBM16P GBM16R ZH681 | | 0 | |
| TEADIGSNLRW | B*44:02; B*44:03 | 11% P / 8% R | GBM17P GBM17R GBM19R ZH678 ZH761 ZH802 | | 0 | |
| **Cysteine and glycine-rich protein 2 (CSRP2; Q16527)** | | | | 26% P / 21% R | 2% | 3% |
| LTEKEGEIY | A*01:01 | 16% P / 17% R | GBM3P GBM5P GBM9P GBM9R GBM13P GBM13R GBM16P GBM16R GBM17R ZH654 | | 1 | |
| **Catenin beta-1 (CTNNB1; P35222)** | | | | 92% P / 96% R | 75% | 72% |
| ATVGLIRNL | *A*02:01*; *A*02:05*; *B*13:02*; B*57:01; C*03:03; *C*06:02*; *C*07:01*; C*12:03; C*15:06 | 21% P / 13% R | GBM5P GBM5R GBM6P GBM6R GBM11P GBM13P GBM17R GBM23P ZH654 ZH681 ZH791 | | 18 | |
| **Dedicator of cytokinesis protein 7 (DOCK7; Q96N67)** | | | | 95% P / 79% R | 29% | 27% |
| AMASIINRL | A*02:01; A*02:05 | 13% P / 8% R | GBM6P GBM6R GBM11P GBM11R ZH613 ZH617 ZH757 | | 3 | |
| ELKSSISAL | *B*08:01* | 11% P / 13% R | GBM13P GBM13R GBM16P GBM16R GBM18P ZH617 ZH753 | | 3 | |
| **Dihydropyrimidinase-related protein 4 (DPYSL4; O14531)** | | | | 47% P / 38% R | 6% | 12% |
| GLYDGPVHEV | A*02:01 | 13% P / 8% R | GBM7P GBM7R GBM12P GBM21P ZH616 ZH681 ZH753 | | 0 | |
| **Epidermal growth factor receptor (EGFR; P00533)** | | | | 58% P / 58% R | 11% | 6% |
| KITDFGLAK | A*03:01; A*11:01 | 11% P / 8% R | GBM6P GBM16P GBM16R GBM17R ZH631 ZH678 | | 1 | |
| SPSTSRTPLL | B*07:02 | 5% P / 17% R | GBM4R GBM10R GBM16P GBM16R ZH616 ZH784 | | 0 | |
| KLFGTSGQK | A*03:01 | 5% P / 13% R | GBM6P GBM16P GBM16R GBM17R GBM22R | | 2 | |
| QMDVNPEGKY | A*01:01 | 5% P / 8% R | GBM5P GBM16R GBM17R ZH654 | | 0 | |
| **Eukaryotic translation initiation factor 4E (EIF4E; P06730)** | | | | 11% P / 21% R | 8% | 4% |
| RLISKFDTV | A*02:01 | 5% P / 8% R | GBM3P GBM11P GBM13R ZH753 | | 2 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Elongation of very long chain fatty acids protein 2 (ELOVL2; Q9NXB9) | | | | | 39% P / 25% R  2% | 1% |
| YLPTFFLTV | A*02:01; A*02:05 | 26% P / 13% R | GBM3P  GBM6P<br>GBM7P  GBM7R<br>GBM11P  GBM13P<br>GBM13R  GBM19P<br>GBM20R  GBM21P<br>ZH720  ZH757<br>ZH829 | | 1 | |
| Fatty acid-binding protein, brain (FABP7; O15540) | | | | | 21% P / 13% R  1% | 0% |
| EYMKALGVGF | A*23:01; A*24:02 | 11% P / 8% R | GBM15P  GBM19P<br>GBM22P  GBM22R<br>GBM23P  GBM23R | | 1 | |
| Constitutive coactivator of PPAR-gamma-like protein 2 (FAM120C; Q9NX05) | | | | | 50% P / 50% R  8% | 8% |
| Supplementary Table 3 | | | | | | |
| HAFSEDPML | *B*35:02*; *B*35:03*;<br>C*03:04; C*12:03 | 8% P / 13% R | GBM14R  GBM21P<br>GBM22P  GBM22R<br>GBM23P  GBM23R | | 2 | |
| Neuronal membrane glycoprotein M6-b (GPM6G; Q13491) | | | | | 84% P / 79% R  5% | 14% |
| ATTYNYAVLK | A*11:01 | 11% P / 8% R | GBM7P  GBM7R<br>GBM18P  GBM18R<br>ZH631  ZH678 | | 0 | |
| Glutamate receptor ionotropic, kainate 3 (GRIK3; Q13003) | | | | | 39% P / 25% R  2% | 6% |
| LLYDAVHIV | A*02:01 | 11% P / 13% R | GBM10R  GBM11P<br>GBM13R  GBM14R<br>ZH617  ZH681<br>ZH757 | | 2 | |
| HEAT repeat-containing protein 1 (HEATR1; Q9H583) | | | | | 58% P / 42% R  28% | 24% |
| KMVEDLISV | A*02:01 | 5% P / 8% R | GBM3P  GBM10R<br>GBM14R  ZH617 | | 11 | |
| Hepatocyte cell adhesion molecule (HEPACAM; Q14CZ8) | | | | | 50% P / 33% R  0% | 1% |
| VPISRPQVL | B*07:02 | 11% P / 13% R | GBM4P  GBM4R<br>GBM7P  GBM7R<br>GBM10R  GBM16P<br>ZH681 | | 0 | |
| Integrin alpha-7 (ITGA7; Q13683) | | | | | 79% P / 63% R  10% | 16% |
| LLYPMQVEL | A*02:01; *C*01:02* | 24% P / 13% R | GBM10R  GBM11P<br>GBM13P  GBM13R<br>GBM20P  GBM20R<br>GBM21P  ZH617<br>ZH645  ZH678<br>ZH681  ZH757 | | 4 | |
| ATP-sensitive inward rectifier potassium channel 10 (KCNJ10; P78508) | | | | | 92% P / 79% R  7% | 12% |
| Supplementary Table 3 | | | | | | |
| RLNQVNVTF | A*32:01; B*15:01 | 8% P / 13% R | GBM2P  GBM2R<br>GBM5P  GBM5R<br>GBM12R  ZH631 | | 0 | |
| Melanoma-associated antigen F1 (MAGEF1; Q9HAY2) | | | | | 39% P / 33% R  12% | 22% |
| ALAAKALAR | A*03:01 | 5% P / 13% R | GBM4P  GBM4R<br>GBM8P  GBM17R<br>GBM23R | | 3 | |
| Membrane-associated guanylate kinase, WW and PDZ domain-containing protein 2 (MAGI2; Q86UL8) | | | | | 24% P / 21% R  1% | 2% |
| AVAPGPWKV | A*02:01; C*15:02 | 11% P / 13% R | GBM13R  GBM14R<br>ZH617  ZH678<br>ZH681  ZH753<br>ZH757 | | 2 | |
| C-1-tetrahydrofolate synthase, cytoplasmic (MTHFD1; P11586) | | | | | 47% P / 21% R  25% | 30% |
| TTESEVMKY | A*01:01 | 18% P / 8% R | GBM3P  GBM5P<br>GBM9P  GBM13R<br>GBM16P  GBM16R<br>ZH617  ZH645<br>ZH654 | | 17 | |
| Max-interacting protein 1 (MXI1; P50539) | | | | | 11% P / 21% R  3% | 3% |
| FPSMPSPRL | B*07:02; B*35:03;<br>B*56:01 | 8% P / 21% R | GBM7P  GBM7R<br>GBM10R  GBM14R<br>GBM16P  GBM16R<br>GBM18P  GBM18R | | 11 | |
| Neuroligin-4, X-linked (NLGN4X; Q8N0W4) | | | | | 50% P / 42% R  2% | 1% |
| NLDTLMTYV | A*02:01; C*05:01;<br>*C*08:02* | 21% P / 8% R | | | 1 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Neuronal cell adhesion molecule (NRCAM; Q92823) | | | | | 47% P / 29% R | 1% | 1% |
| Supplementary Table 3 | | | | | | | |
| SEPDNLVITW | B*44:02; B*44:03 | 16% P / 8% R | GBM17P<br>GBM20P<br>ZH617<br>ZH678 | GBM17R<br>GBM20R<br>ZH631<br>ZH761 | 0 | | |
| Poly(A) polymerase alpha (PAPOLA; P51003) | | | | | 53% P / 50% R | 25% | 24% |
| KIPTPIVGV | A*02:01 | 8% P / 8% R | GBM14R<br>ZH678<br>ZH753 | GBM21P<br>ZH681 | 3 | | |
| Protocadherin gamma-C5 (PCDHGC5; Q9Y5F6) | | | | | 68% P / 67% R | 5% | 7% |
| VSSDGTLKY | A*01:01; *B*57:01* | 16% P / 13% R | GBM5P<br>GBM9P<br>GBM13P<br>ZH617<br>ZH654 | GBM5R<br>GBM9R<br>GBM17R<br>ZH645 | 1 | | |
| Pecanex-like protein (PCNXL3; Q9H6A9) | | | | | 29% P / 12% R | 12% | 10% |
| GVLENIFGV | A*02:01; C*16:01 | 24% P / 13% R | GBM3P<br>GBM12R<br>GBM14P<br>GBM20P<br>ZH617<br>ZH681 | GBM7P<br>GBM13R<br>GBM14R<br>GBM21P<br>ZH678<br>ZH720 | 23 | | |
| E3 ubiquitin-protein ligase Praja-2 (PJA2; O43164) | | | | | 87% P / 79% R | 43% | 40% |
| GSSPEQVVRPK | A*11:01 | 8% P / 8% R | GBM12R<br>GBM18R<br>ZH678 | GBM18P<br>ZH631 | 5 | | |
| Pleckstrin homology domain-containing family A member 4 (PLEKHA4; Q9H4M7) | | | | | 18% P / 21% R | 2% | 1% |
| Supplementary Table 3 | | | | | | | |
| Receptor-type tyrosine-protein phosphatase zeta (PTPRZ1; P23471) | | | | | 97% P / 83% R | 6% | 5% |
| Supplementary Table 3 | | | | | | | |
| EEFETLKEF | B*18:01; B*44:02; B*44:03 | 32% P / 8% R | GBM3P<br>GBM17P<br>GBM20P<br>GBM21P<br>ZH617<br>ZH678<br>ZH761 | GBM8P<br>GBM19R<br>GBM20R<br>ZH616<br>ZH631<br>ZH720<br>ZH802 | 3 | | |
| KVFAGIPTV | A*02:01; A*02:05; A*32:01; *B*13:02*; C*15:02 | 29% P / 13% R | GBM5P<br>GBM10R<br>GBM13P<br>GBM14R<br>ZH617<br>ZH678<br>ZH757 | GBM7P<br>GBM11P<br>GBM13R<br>GBM21P<br>ZH631<br>ZH681<br>ZH761 | 2 | | |
| SENPETITY | B*18:01; B*44:02; B*44:03 | 26% P / 8% R | GBM3P<br>GBM17R<br>GBM21P<br>ZH617<br>ZH678<br>ZH761 | GBM8P<br>GBM20R<br>ZH616<br>ZH631<br>ZH750 | 1 | | |
| FLLPDTDGLTAL | A*02:01 | 26% P / 8% R | GBM3P<br>GBM10R<br>GBM21P<br>ZH645<br>ZH753<br>ZH761 | GBM7P<br>GBM19P<br>ZH617<br>ZH678<br>ZH757<br>ZH829 | 1 | | |
| VVYDTMIEK | A*03:01; A*11:01 | 18% P / 13% R | GBM7<br>GBM12P<br>GBM16R<br>ZH631<br>ZH761 | GBM10R<br>GBM16P<br>GBM17R<br>ZH678<br>ZH791 | 0 | | |
| FPTEVTPHAF | B*07:02; B*35:03 | 16% P / 21% R | GBM4P<br>GBM14R<br>GBM21P<br>GBM22R | GBM10R<br>GBM16P<br>GBM22P | 2 | | |
| SVDVYQVAK | A*11:01 | 13% P / 13% R | GBM7P<br>GBM12R<br>GBM18R<br>ZH678 | GBM7R<br>GBM18P<br>ZH631<br>ZH791 | 2 | | |
| FLYKVILSL | A*02:01; *B*13:02*; C*03:03 | 13% P / 8% R | GBM5P<br>GBM13R<br>ZH617<br>ZH829 | GBM13P<br>GBM14R<br>ZH750 | 1 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ALTTLMHQL | A*02:01; A*02:05 | 11% P / 17% R | GBM6P GBM11P GBM14R ZH613 | GBM6R GBM11R GBM20R ZH757 | | 0 | |
| FPSKATSEL | B*07:02; B*35:03 | 11% P / 13% R | GBM4P GBM14R GBM21P GBM22R | GBM10R GBM16P GBM22P | | 0 | |
| SPISKTFEL | B*07:02; B*08:01; B*35:01; B*35:03 | 8% P / 13% R | GBM6P GBM16R GBM22P | GBM10R GBM16R GBM22R | | 0 | |
| Cell differentiation protein RCD1 homolog (RQCD1; Q92600) | | | | | 55% P / 46% R | 17% | 13% |
| LLDDTGLAY | A*01:01; B*15:01 | 8% P / 8% R | GBM5P GBM13R GBM17R | GBM9P GBM16P | | 5 | |
| Structural maintenance of chromosomes protein 4 (SMC4; Q9NTJ3) | | | | | 71% P /46 % R | 38% | 24% |
| AVNPKEIASK | A*03:01 | 11% P / 17% R | GBM4P GBM6P GBM16R GBM23R | GBM4R GBM16P GBM17R ZH761 | | 31 | |
| Alpha-2,8-sialyltransferase 8E (ST8SIA5; O15466) | | | | | 11% P / 17% R | 1% | 1% |
| NTDVSIRVKY | A*01:01 | 5% P / 8% R | GBM13R ZH617 | GBM17R ZH654 | | | |
| Transforming growth factor beta-2 (TGFB2; P61812) | | | | | 18% P / 21% R | 3% | 1% |
| RPYFRIVRF | B*07:02; B*08:01 | 11% P / 13% R | GBM4R GBM9P GBM16R GBM18R | GBM7P GBM16P GBM18P | | 10 | |
| Tenascin (TNC; P24821) | | | | | 87% P / 83% R | 14% | 13% |
| Supplementary Table 3 | | | | | | | |
| GVLKKVIRH | A*03:01; A*11:01 | 13% P / 25% R | GBM4P GBM6P GBM16P GBM17R GBM18R ZH631 | GBM4R GBM6R GBM16R GBM18P GBM23R | | 10 | |
| ATEYEIELY | A*01:01 | 13% P / 17% R | GBM3P GBM5P GBM9R GBM16P ZH645 | GBM3R GBM9P GBM13R GBM16R | | 7 | |
| SPRDLTATEV | B*07:02 | 5% P / 13% R | GBM4R GBM16P GBM18P | GBM10R GBM16R | | 0 | |
| NRLLETVEY | C*07:01; C*07:02 | 5% P / 8% R | GBM4P GBM16P | GBM10R GBM18R | | 0 | |
| Vacuolar protein sorting-associated protein 13B (VPS13B; Q7Z7G8) | | | | | 74% P / 67% R | 30% | 25% |
| SLLQKQIML | A*02:01 | 11% P / 8% R | GBM10R GBM21P ZH753 | GBM11P ZH681 ZH757 | | 7 | |
| **Glioblastoma-exclusive HLA class II-presented antigens derived from established TAAs and CTAs** | | | | | | | |
| Fatty acid-binding protein, brain (FABP7; O15540) | | | | | 26% P / 29% R | 1% | 0% |
| Supplementary Table 4 | | | | | | | |
| Cyclin-dependent kinase 4 (CDK4; P11802) | | | | | 8% P / 8% R | 1% | 0% |
| DPHSGHFVALKSVRVPN | | 5% P / 4% R | GBM7P GBM14R | GBM14P | | 6 | |
| DPHSGHFVALKSVRVPNG | | 8% P / 8% R | GBM7P GBM12P GBM14R | GBM7R GBM14P | | 3 | |
| DPHSGHFVALKSVRVPNGG | | 3% P / 4% R | GBM7P | GBM7R | | 4 | |
| DPHSGHFVALKSVRVPNGGG | | 3% P / 0% R | GBM7P | | | 0 | |
| SGHFVALKSVRVPNGG | | 3% P / 0% R | GBM7P | | | 0 | |
| DNA repair protein RAD51 homolog 1 (RAD51; Q06609) | | | | | 8% P / 8% R | 0% | 0% |
| Supplementary Table 2 | | | | | | | |
| Ceramide synthase 1 (CERS1; P27544) | | | | | 5% P / 8% R | 0% | 0% |
| DLREYDTAEAQSLKPS | | 5% P / 4% R | GBM6P ZH753 | GBM12P | | 0 | |
| DLREYDTAEAQSLKPSK | | 5% P / 0% R | GBM6P | GBM12P | | 0 | |
| GGSYHRLHALAADLG | | 3% P / 0% R | GBM6P | | | 0 | |
| KVLYATSHCSLRTVPDI | | 0% P / 4% R | GBM2R | | | 0 | |
| LREYDTAEAQSLKPS | | 3% P / 0% R | GBM6P | | | 0 | |
| LREYDTAEAQSLKPSK | | 3% P / 0% R | GBM6P | | | 0 | |

| Lipase member I (LIPI; Q6XZB0) | | | | 3% P / 8% R | 0% | | 0% |
|---|---|---|---|---|---|---|---|
| FISFPCRSYKDYKT | 0% P / 4% R | GBM5R | | | 0 | | |
| KSIFSGIQFIKCNHQRAVHLFM | 0% P / 4% R | GBM21R | | | 0 | | |
| PDKTMMDGSFSFKLLNQLGMIEEP | 3% P / 0% R | GBM13P | | | 0 | | |
| Eukaryotic translation initiation factor 4E (EIF4E; P06730) | | | | 3% P / 4% R | 1% | | 0% |
| IEPMWEDEKNKRGGR | 0% P / 4% R | GBM10R | | | 1 | | |
| QEVANPEHYIKHPLQNRWALWFFKN | 3% P / 0% R | GBM16P | | | 0 | | |
| Protein AF1q (MLLT11; Q13015) | | | | 3% P / 4% R | 1% | | 0% |
| FWRAPIASIHSFEL | 0% P / 4% R | GBM22R | | | 1 | | |
| MRDPVSSQYSSFLF | 3% P / 0% R | ZH802 | | | 2 | | |
| WRAPIASIHSFEL | 0% P / 4% R | GBM22R | | | 4 | | |
| WRAPIASIHSFELDLL | 0% P / 4% R | GBM22R | | | 3 | | |
| Kinetochore protein Nuf2 (NUF2; Q9BZD4) | | | | 3% P / 4% R | 1% | | 0% |
| KTKIVDSPEKLKNYK | 3% P / 4% R | GBM13R | GBM16P | | 0 | | |
| Transcription factor ETV6 (ETV6; P41212) | | | | 3% P / 4% R | 1% | | 0% |
| MPSPIMHPLILNP | 0% P / 4% R | GBM5R | | | 0 | | |
| PGQRLLFRFMKTPDEI | 3% P / 0% R | GBM16P | | | 0 | | |
| Melanoma-associated antigen 1 (MAGEA1; P43355) | | | | 3% P / 4% R | 1% | | 0% |
| APEEEIWEELSVMEVYDGREHSAY | 3% P / 4% R | GBM10R | GBM16P | | 1 | | |
| Transcriptional repressor CTCFL (CTCFL; Q8NI51) | | | | 3% P / 4% R | 1% | | 0% |
| NQLLAERTKEQLF | 3% P / 4% R | GBM18R | ZH654 | | 1 | | |

**Glioblastoma-exclusive HLA class II-restricted peptides derived from established TAAs and CTAs**

| A-kinase anchor protein 3 (AKAP3; O75969) | | | | 5% P / 17% R | 1% | | 1% |
|---|---|---|---|---|---|---|---|
| EPKVPEQPVKEDRKLCERPLASSPP | 5% P / 13% R | GBM9R GBM16P ZH757 | GBM10R GBM16R | | 0 | | |
| Brevican core protein (BCAN; Q96GW7) | | | | 63% P / 38% R | 1% | | 1% |

Supplementary Table 4

| DAGWLSDQTVRYPIQ | 5% P / 13% R | GBM10R GBM16P ZH617 | GBM13R GBM16R | | 2 | | |
|---|---|---|---|---|---|---|---|
| EAYRFRVALPAYPA | 32% P / 13% R | GBM4R GBM5R GBM7P GBM11P GBM22P ZH616 ZH681 ZH802 | GBM5P GBM6P GBM10R GBM16P ZH613 ZH678 ZH757 | | 0 | | |
| RPRLRYEVDTVLRYR | 8% P / 13% R | GBM10R GBM16P GBM18P | GBM13R GBM16R ZH617 | | 0 | | |
| SPGWLADGSVRYPI | 11% P / 13% R | GBM10R GBM13R GBM16R ZH617 | GBM13P GBM16P GBM18P | | 0 | | |
| SPGWLADGSVRYPIV | 8% P / 13% R | GBM10R GBM16P GBM18P | GBM13R GBM16R ZH617 | | 1 | | |
| SPGWLADGSVRYPIVT | 8% P / 13% R | GBM10R GBM16P GBM18P | GBM13R GBM16R ZH617 | | 0 | | |
| SPGWLADGSVRYPIVTPS | 11% P / 13% R | GBM10R GBM13R GBM16R ZH617 | GBM13P GBM16P GBM18P | | 0 | | |
| SPRVKWTFLSRGREA | 13% P / 8% R | GBM5P GBM17R GBM22R ZH750 | GBM11P GBM22P ZH678 | | 0 | | |
| VNEAYRFRVALPAYPA | 37% P / 13% R | GBM3P GBM5P GBM6P GBM10R GBM16P ZH613 ZH645 ZH681 ZH802 | GBM4R GBM5R GBM7P GBM11P GBM22P ZH616 ZH678 ZH757 | | 0 | | |

| Clusterin (CLU; P10909) | | | | 87% P / 96% R | 53% | 59% |
|---|---|---|---|---|---|---|
| DNELQEMSNQGSK | 11% P / 13% R | GBM9P GBM18P GBM20R ZH802 | GBM9R GBM18R ZH654 | 24 | | |
| DNELQEMSNQGSKY | 13% P / 13% R | GBM9P GBM18P GBM20P ZH654 | GBM9R GBM18R GBM20R ZH802 | 23 | | |
| DQTVSDNELQEMSNQGSK | 8% P / 13% R | GBM9R GBM18R ZH654 | GBM18P GBM20R ZH802 | 8 | | |
| DQTVSDNELQEMSNQGSKY | 11% P / 13% R | GBM9P GBM18P GBM20R ZH802 | GBM9R GBM18R ZH654 | 14 | | |
| DQTVSDNELQEMSNQGSKYVN | 8% P / 13% R | GBM9R GBM18R ZH654 | GBM18P GBM20R ZH802 | 16 | | |
| DQTVSDNELQEMSNQGSKYVNK | 11% P / 8% R | GBM9P GBM18R ZH654 | GBM18P GBM20R ZH802 | 11 | | |
| DQTVSDNELQEMSNQGSKYVNKE | 11% P / 13% R | GBM9P GBM18P GBM20R ZH802 | GBM9R GBM18R ZH654 | 6 | | |
| DSDPITVTVPVEVSRKNPK | 5% P / 17% R | GBM5R GBM20R ZH645 | GBM13R GBM23R ZH654 | 9 | | |
| ERLTRKYNELLKSYQ | 11% P / 8% R | GBM21P GBM22P ZH654 | GBM21R GBM22R ZH761 | 1 | | |
| GVTEVVVKLFDSDPIT | 5% P / 13% R | GBM4R GBM14R GBM16R | GBM11P GBM16P | 6 | | |
| NELQEMSNQGSK | 8% P / 8% R | GBM18P GBM20R ZH802 | GBM18R ZH654 | 14 | | |
| NELQEMSNQGSKY | 11% P / 8% R | GBM9P GBM18R ZH654 | GBM18P GBM20R ZH802 | 14 | | |
| SDNELQEMSNQGSK | 16% P / 13% R | GBM9P GBM15P GBM18R GBM20R ZH802 | GBM9R GBM18P GBM20P ZH654 | 35 | | |
| SDNELQEMSNQGSKYV | 8% P / 13% R | GBM9R GBM18R ZH654 | GBM18P GBM20R ZH802 | 16 | | |
| SDNELQEMSNQGSKYVNK | 11% P / 8% R | GBM9P GBM18R ZH654 | GBM18P GBM20R | 12 | | |
| TVSDNELQEMSNQGSK | 11% P / 8% R | GBM9P GBM18R ZH654 | GBM18P GBM20R ZH802 | 13 | | |
| TVSDNELQEMSNQGSKY | 13% P / 13% R | GBM9P GBM18P GBM20P ZH654 | GBM9R GBM18R GBM20R ZH802 | 17 | | |
| TVSDNELQEMSNQGSKYVNK | 8% P / 8% R | GBM18P GBM20R ZH802 | GBM18R ZH654 | 10 | | |
| VPSGVTEVVVKLFDSDPIT | 8% P / 25% R | GBM4R GBM11R GBM14R GBM16R ZH753 | GBM11P GBM13P GBM16P GBM23R | 17 | | |
| VSDNELQEMSNQGSK | 11% P / 13% R | GBM9P GBM18P GBM20R ZH802 | GBM9R GBM18R ZH654 | 12 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| VSDNELQEMSNQGSKYVN | 8% P / 8% R | GBM18P GBM20R ZH802 | GBM18R ZH654 | 9 | | |
| VSDNELQEMSNQGSKYVNK | 8% P / 8% R | GBM18P GBM20R ZH802 | GBM18R ZH654 | 9 | | |
| VSDNELQEMSNQGSKYVNKE | 8% P / 8% R | GBM9R GBM20R ZH802 | GBM18P ZH654 | 4 | | |
| VTEVVVKLFDSDPIT | 8% P / 17% R | GBM4R GBM14P GBM16R GBM23R | GBM11P GBM14R GBM23P | 5 | | |
| **Epidermal growth factor receptor (EGFR; P00533)** | | | 42% P / 33% R | 9% | | 12% |
| LQRYSSDPTGALTEDS | 5% P / 13% R | GBM10R GBM16P GBM18P | GBM13R GBM16R | 2 | | |
| LRILKETEFKKIKV | 8% P / 8% R | GBM6P GBM16P ZH631 | GBM14R GBM16R | 0 | | |
| **Ephrin type-B receptor 2 (EPHB2; P29323)** | | | 16% P / 21% R | 3% | | 4% |
| TPGMKIYIDPFTYE | 13% P / 17% R | GBM4R GBM7P GBM10R GBM16P ZH791 | GBM6P GBM7R GBM11P GBM16R | 8 | | |
| TPGMKIYIDPFTYEDP | 5% P / 17% R | GBM4R GBM7R GBM11P | GBM7P GBM10R GBM16R | 5 | | |
| **Glypican-1 (GPC1; P35052)** | | | 26% P / 25% R | 4% | | 2% |
| IMQLKIMTNRLRSA | 16% P / 8% R | GBM4R GBM7P GBM14R GBM21P | GBM6P GBM14P GBM16P ZH616 | 1 | | |
| **Hepatocyte cell adhesion molecule (HEPACAM; Q14CZ8)** | | | 47% P / 54% R | 0% | | 1% |
| EPGPPGYSVSPAVPGRSPG | 8% P / 17% R | GBM2R GBM7P GBM12R ZH753 | GBM6P GBM12P GBM20R | 0 | | |
| **Legumain (LGMN; Q99538)** | | | 71% P / 75% R | 29% | | 46% |
| DVTPQNFLAVLRGDAEA | 16% P / 13% R | GBM3P GBM22P ZH678 ZH784 ZH810 | GBM5R GBM22R ZH750 ZH791 | 8 | | |
| EDVTPQNFLAVLRGDAEAVKG | 11% P / 8% R | GBM3P GBM22P ZH678 | GBM5R GBM22R ZH750 | 7 | | |
| GEDVTPQNFLAVLRGDAEAVK | 13% P / 13% R | GBM3R GBM5R GBM22R ZH750 | GBM5P GBM22P ZH678 ZH791 | 6 | | |
| GEDVTPQNFLAVLRGDAEAVKG | 16% P / 8% R | GBM3P GBM22P ZH678 ZH791 | GBM5R GBM22R ZH750 ZH810 | 7 | | |
| HLPDNINVYATTAANPRE | 5% P / 13% R | GBM14P GBM16P GBM21R | GBM14R GBM18R | 24 | | |
| HLPDNINVYATTAANPRES | 5% P / 8% R | GBM16P GBM21R | GBM18R ZH654 | 11 | | |
| INVYATTAANPRES | 11% P / 13% R | GBM4R GBM10R GBM16R ZH757 | GBM9P GBM16P ZH616 | 4 | | |
| KDLNETIHYMYKHKMYRKMVFYI | 8% P / 17% R | GBM2R GBM10R GBM16P ZH631 | GBM4R GBM14R ZH616 | 0 | | |
| NHLPDNINVYATTAANPRE | 8% P / 8% R | GBM14P GBM18R ZH654 | GBM16P GBM21R | 5 | | |
| TGEDVTPQNFLAVLRGDAEAVKG | 5% P / 8% R | GBM5R ZH678 | GBM22R ZH791 | 4 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| VTPQNFLAVLRGDAEA | 16% P / 13% R | GBM3P | GBM5R | | 8 | |
| | | GBM22P | GBM22R | | | |
| | | ZH678 | ZH750 | | | |
| | | ZH784 | ZH791 | | | |
| | | ZH810 | | | | |

**Neuronal cell adhesion molecule (NRCAM; Q92823)** — 79% P / 75% R  5%  6%

Supplementary Table 4

| | | | | | | |
|---|---|---|---|---|---|---|
| ALGAIHHTISVRVKAA | 13% P / 13% R | GBM5P | GBM13P | | 2 | |
| | | GBM13R | GBM17R | | | |
| | | GBM23R | ZH645 | | | |
| | | ZH654 | ZH681 | | | |
| AQLKLSPYVNYSFR | 8% P / 8% R | GBM4R | GBM10R | | 0 | |
| | | GBM11P | GBM16P | | | |
| | | ZH616 | | | | |
| EDGSFIGQYSGKKEKEPA | 5% P / 8% R | GBM4R | GBM6P | | 0 | |
| | | GBM10R | ZH616 | | | |
| GAIHHTISVRVKAAP | 8% P / 8% R | GBM13P | GBM13R | | 2 | |
| | | GBM17R | ZH645 | | | |
| | | ZH681 | | | | |
| GAIHHTISVRVKAAPY | 13% P / 17% R | GBM5P | GBM5R | | 1 | |
| | | GBM13P | GBM13R | | | |
| | | GBM17R | GBM23R | | | |
| | | ZH645 | ZH654 | | | |
| | | ZH681 | | | | |
| NGVPIEIAPDDPSR | 8% P / 8% R | GBM18P | GBM18R | | 1 | |
| | | GBM20R | ZH654 | | | |
| | | ZH802 | | | | |
| NGVPIEIAPDDPSRK | 13% P / 8% R | GBM18P | GBM18R | | 4 | |
| | | GBM20P | GBM20R | | | |
| | | ZH645 | ZH654 | | | |
| | | ZH802 | | | | |
| SPLPTIEWFKGAKGSA | 11% P / 13% R | GBM9P | GBM10R | | 4 | |
| | | GBM11P | GBM14R | | | |
| | | GBM16P | GBM21P | | | |
| | | GBM21R | | | | |
| VPIEIAPDDPSR | 8% P / 8% R | GBM18P | GBM18R | | 3 | |
| | | GBM20R | ZH654 | | | |
| | | ZH802 | | | | |

**Palladin (PALLD; Q8WX93)** — 24% P / 25% R  6%  14%

| | | | | | | |
|---|---|---|---|---|---|---|
| SPSPPPPPPPVFSPTAAFPVPDV | 8% P / 8% R | GBM13P | GBM13R | | 9 | |
| | | GBM22P | GBM23R | | | |
| | | ZH654 | | | | |

**Protocadherin gamma-C5 (PCDHGC5; Q9Y5F6)** — 34% P / 25% R  2%  4%

| | | | | | | |
|---|---|---|---|---|---|---|
| KPSENHYSLLTSQPLDR | 11% P / 8% R | GBM2P | GBM6P | | 0 | |
| | | GBM7P | GBM12P | | | |
| | | GBM12R | ZH753 | | | |
| KPSENHYSLLTSQPLDRE | 11% P / 8% R | GBM2P | GBM6P | | 0 | |
| | | GBM7P | GBM12P | | | |
| | | GBM12R | ZH753 | | | |

**Prostaglandin F2 receptor negative regulator (PTGFRN; Q9P2B2)** — 53% P / 33% R  15%  16%

| | | | | | | |
|---|---|---|---|---|---|---|
| GSDAYRLSVSRALSA | 8% P / 8% R | GBM12P | GBM13P | | 8 | |
| | | GBM13R | GBM22R | | | |
| | | ZH654 | | | | |

**Receptor-type tyrosine-protein phosphatase zeta (PTPRZ1; P23471)** — 79% P / 58% R  2%  4%

Supplementary Table 4

| | | | | | | |
|---|---|---|---|---|---|---|
| DSHIHAYVNALLIPG | 8% P / 13% R | GBM4R | GBM10R | | 0 | |
| | | GBM11P | GBM11R | | | |
| | | GBM16P | ZH791 | | | |
| DSHIHAYVNALLIPGP | 16% P / 21% R | GBM4R | GBM6P | | 0 | |
| | | GBM9R | GBM10R | | | |
| | | GBM11P | GBM11R | | | |
| | | GBM16P | GBM16R | | | |
| | | ZH616 | ZH757 | | | |
| | | ZH791 | | | | |
| IGTKYNEAKTNRSPTR | 11% P / 8% R | GBM8P | GBM9P | | 1 | |
| | | GBM15P | GBM15R | | | |
| | | GBM20P | GBM20R | | | |
| LDSHIHAYVNALLIPG | 21% P / 21% R | GBM4R | GBM6P | | 0 | |
| | | GBM7P | GBM9P | | | |
| | | GBM9R | GBM10R | | | |
| | | GBM11P | GBM11R | | | |
| | | GBM16P | GBM16R | | | |
| | | ZH616 | ZH757 | | | |
| | | ZH791 | | | | |

| | | | | | |
|---|---|---|---|---|---|
| LDSHIHAYVNALLIPGP | 21% P / 21% R | GBM4R GBM6P GBM7P GBM9P GBM9R GBM10R GBM11P GBM11R GBM16P GBM16R ZH616 ZH757 ZH791 | 0 | | |
| LDSHIHAYVNALLIPGPA | 24% P / 21% R | GBM4R GBM6P GBM7P GBM9P GBM9R GBM10P GBM10R GBM11P GBM11R GBM16P GBM16R ZH616 ZH757 ZH791 | 0 | | |
| THYNRIGTKYNEAKT | 13% P / 8% R | GBM8P GBM21P GBM21R GBM22P GBM22R ZH654 ZH761 | 0 | | |
| THYNRIGTKYNEAKTN | 13% P / 8% R | GBM8P GBM21P GBM21R GBM22P GBM22R ZH654 ZH761 | 0 | | |
| Plasminogen activator inhibitor 1 (SERPINE1; P05121) | | | 61% P / 63% R | 14% | 9% |
| APEEIIMDRPFLFVVRHNP | 8% P / 25% R | GBM3R GBM4R GBM11P GBM11R GBM14R GBM15P GBM15R GBM16P GBM16R | 3 | | |
| EIIMDRPFLFVVR | 8% P / 13% R | GBM13P GBM16P GBM16R GBM18R ZH617 ZH753 | 9 | | |
| EIIMDRPFLFVVRHNPT | 5% P / 8% R | GBM3R GBM4R GBM11P GBM16P | 1 | | |
| TQQQIQAAMGFKIDDK | 11% P / 25% R | GBM6P GBM12P GBM12R GBM13R GBM14P GBM14R GBM15R GBM22P GBM22R ZH753 | 7 | | |
| TQQQIQAAMGFKIDDKG | 8% P / 21% R | GBM12P GBM12R GBM14R GBM15R GBM20P GBM22P GBM22R ZH753 | 7 | | |
| Sperm-associated antigen 1 (SPAG1; Q07617) | | | 16% P / 25% R | 1% | 1% |
| EIENSEDEEGKSGRKHED | 8% P / 13% R | GBM4R GBM6P GBM10R GBM16P GBM16R ZH616 | 3 | | |
| ERRKIEIQEVNEGKEEPG | 5% P / 8% R | GBM13R GBM18P GBM23R ZH645 | 3 | | |
| Transforming growth factor beta-2 (TBFB2; P61812) | | | 37% P / 38% R | 4% | 3% |
| Supplementary Table 4 | | | | | |
| STRDLLQEKASRRAA | 8% P / 13% R | GBM6P GBM10R GBM14P GBM14R GBM21R ZH616 | 0 | | |
| Tenascin (TNC; P24821) | | | 76% P / 67% R | 15% | 18% |
| Supplementary Table 4 | | | | | |
| DITGLREATEYEIEL | 11% P / 8% R | GBM12P GBM18P GBM18R GBM19P GBM22P GBM22R | 1 | | |
| EPLEITLLAPERTRD | 11% P / 8% R | GBM6P GBM7P GBM12P GBM14P GBM14R GBM15R | 4 | | |
| GGWIVFLRRKNGREN | 8% P / 13% R | GBM3P GBM3R GBM5P GBM22P GBM22R GBM23R | 3 | | |
| GVIQGYRTPVLS | 8% P / 8% R | GBM10R GBM14P GBM14R GBM16P | 0 | | |
| GVIRGYRTPVL | 18% P / 25% R | GBM4R GBM6P GBM7P GBM9P GBM9R GBM10R GBM11P GBM14R GBM16P GBM16R GBM21R ZH616 ZH791 | 4 | | |

| | | | | |
|---|---|---|---|---|
| GVIRGYRTPVLS | 21% P / 25% R | GBM4R GBM7P<br>GBM9P GBM9R<br>GM10R GBM11P<br>GBM14R GBM16P<br>GBM16R GBM21P<br>GBM21R ZH616<br>ZH757 ZH791 | 3 | |
| GVIRGYRTPVLSA | 16% P / 17% R | GBM4R GBM6P<br>GBM7P GBM9P<br>GBM9R GBM10R<br>GBM14R GBM16P<br>ZH616 ZH757 | 0 | |
| GVIRGYRTPVLSAE | 24% P / 21% R | GBM4R GBM6P<br>GBM7P GBM9P<br>GBM9R GBM10R<br>GBM11P GBM14P<br>GBM14R GBM16P<br>GBM16R GBM21P<br>ZH616 ZH791 | 1 | |
| GVIRGYRTPVLSAEA | 11% P / 8% R | GBM4R GBM6P<br>GBM7P GBM10R<br>GBM16P ZH616 | 1 | |
| GVIRGYRTPVLSAEASTAKEPE | 11% P / 17% R | GBM4R GBM6P<br>GBM7P GBM10R<br>GBM14R GBM16P<br>GBM16R ZH616 | 1 | |
| ITGLREATEYEIEL | 11% P / 8% R | GBM12P GBM18P<br>GBM18R GBM19P<br>GBM22P GBM22R | 0 | |
| IYGVIQGYRTPVLSAE | 5% P / 8% R | GBM4R GBM10R<br>GBM16P ZH616 | 0 | |
| IYGVIRGYRTPVL | 8% P / 8% R | GBM4R GBM6P<br>GBM10R GBM16P<br>ZH616 | 2 | |
| IYGVIRGYRTPVLS | 13% P / 21% R | GBM4R GBM6P<br>GBM7P GBM9P<br>GBM9R GBM10R<br>GBM11P GBM14R<br>GBM16P GBM16R | 3 | |
| IYGVIRGYRTPVLSA | 11% P / 8% R | GBM4R GBM6P<br>GBM7P GBM14R<br>GBM16P ZH616 | 0 | |
| IYGVIRGYRTPVLSAE | 13% P / 8% R | GBM4R GBM6P<br>GBM7P GBM10R<br>GBM11P GBM16P<br>ZH616 | 1 | |
| IYGVIRGYRTPVLSAEA | 5% P / 8% R | GBM4R GBM10R<br>GBM16P ZH616 | 1 | |
| SIYGVIQGYRTPVLSAE | 5% P / 8% R | GBM4R GBM10R<br>GBM16P ZH616 | 0 | |
| TVDSYVISYTGEKVPE | 13% P / 8% R | GBM4R GBM6P<br>GBM7P GBM10R<br>GBM11P GBM16P<br>ZH616 | 1 | |
| VDSYVISYTGEKVPE | 11% P / 13% R | GBM4R GBM9P<br>GBM9R GBM10R<br>GBM11P GBM16P<br>ZH616 | 4 | |
| VDSYVISYTGEKVPEIT | 18% P / 17% R | GBM4R GBM6P<br>GBM7P GBM9P<br>GBM9R GBM10R<br>GBM11P GBM16P<br>GBM16R ZH616<br>ZH791 | 3 | |
| Testis-specific gene 10 protein (TSGA10; Q9BZW7) | | | 8% P / 8% R | 1% 1% |
| KLELITAEAEGNRLKEK | 8% P / 8% R | GBM5P GBM6P<br>GBM7P GBM9R<br>GBM10R | 1 | |

**Supplementary Table 6. Primary and recurrence-exclusive antigens presented on HLA class I and II molecules.** HLA class I restrictions not passing manual assessment as quality control are indicated in italic, whereas HLA class II-presented proteins neither identified with peptides exceeding a length of twelve AA nor with different sequences across patients were not considered for this listing. FTSJ3, TDRD12, and AMER1 were detected with only one HLA class II peptide sequence across all patients. Frequencies of positive tumors are given for n=38 primary (P) and n=24 recurrent (R) glioblastomas.

| Antigen | Frequency of positive tumors | Peptide sequence | HLA restriction | UniProt accession |
|---|---|---|---|---|
| **Primary glioblastoma-associated HLA class I-presented antigens** | | | | |
| PDZ domain-containing protein 2 (PDZD2) | <u>29% P</u> | | | O15018 |
| | GBM5P | LLDDETLNQY | A*01:01 | |
| | GBM13P | GLFHKQVTV | A*02:01 | |
| | GBM15P | NYSRNFSSF | A*24:02 | |
| | GBM22P | NYSRNFSSF | A*24:02 | |
| | ZH613 | HTQPSPVSR | A*31:01 | |
| | ZH631 | ASQEYHIVK | A*11:01 | |
| | ZH645 | SEAPAANAV | B*49:01 | |
| | ZH654 | HTQPSPVSR | A*31:01 | |
| | ZH678 | ASQEYHIVK | A*11:01 | |
| | | SSLQTAIRK | A*11:01 | |
| | ZH761 | AEQEMSRSF | B*44:02 | |
| | | GLFHKQVTV | A*02:01 | |
| | | HLTENLPKA | A*02:01 | |
| | ZH802 | HTQPSPVSR | A*31:01 | |
| Roundabout homolog 1 (ROBO1) | <u>26% P</u> | | | Q9Y6N7 |
| | GBM3P | NSDSNLTTY | A*01:01 | |
| 1 peptide multi-maps to ROBO2 (Q9HCK4) | GBM4P | NPRDPSSSSSM | B*07:02 | |
| | GBM7P | IPFLVPGIRY | B*56:01 | |
| | | KPRDQVVAL | B*07:02 | |
| | | NPRDPSSSSSM | B*07:02 | |
| | GBM16P | NSDSNLTTY | A*01:01 | |
| | GBM18P | NPRDPSSSSSM | B*07:02 | |
| | ZH613 | SSSSIEVHW | B*58:01 | |
| | ZH645 | AEGRPTPTI | B*49:01 | |
| | | NSDSNLTTY | A*01:01 | |
| | ZH681 | NPRDPSSSSSM | B*07:02 | |
| | ZH802 | RQREQANVGR | A*31:01 | |
| | ZH829 | IPFLVPGIRY | B*55:01 | |
| Sodium- and chloride-dependent glycine transporter 1 (SLC6A9) | <u>24% P</u> | | | P48067 |
| | GBM3P | VAYPEALTL | C*02:02 | |
| | GBM5P | VAYPEALTL | C*03:03 | |
| 1 peptide multi-maps to SLC6A14/A7 (Q9UN76 / Q99884) | GBM6P | EFVLTSVGY | *B*35:01* | |
| | | VAYPEALTL | C*12:03 | |
| | | VAYPEALTLL | C*12:03 | |
| | GBM7P | GAFDGIMYY | C*02:02 | |
| | GBM12P | EFVLTSVGY | *B*35:01* | |
| | GBM13P | VAYPEALTL | *B*13:02* | |
| | GBM19P | AFVAYPEALTL | A*24:02 | |
| | GBM20P | AFVAYPEALTL | C*16:01 | |
| | ZH654 | KSSGKVVYF | B*57:01 | |
| Protein FAM65C (FAM65C) | <u>24% P</u> | | | Q96MK2 |
| | GBM6P | GKYPGQLEI | B*15:03 | |
| | GBM8P | DIADFFTTR | A*68:01 | |
| | GBM9P | DIADFFTTR | A*33:05 | |
| | GBM10P | RPLPPPSSL | B*07:02 | |
| | GBM21P | PLQEVLELL | A*02:01 | |
| | ZH617 | SLFGGSQGL | A*02:01 | |
| | ZH720 | PLQEVLELL | A*02:01 | |
| | ZH757 | RPLPPPSSL | B*07:02 | |
| | ZH791 | RPLPPPSSL | B*07:02 | |
| Centrosomal protein of 192 kDa (CEP192) | <u>24% P</u> | | | Q8TEP8 |
| | GBM3P | DELLVTEVY | B*18:02 | |
| | | SAAPFAQRY | C*02:02 | |
| | GBM5P | VLDFGDLTY | A*01:01; B*15:01 | |
| | GBM6P | DVLDFGDLTY | B*35:01 | |
| | GBM9P | VLDFGDLTY | A*01:01 | |
| | GBM11P | TQVELLTRL | *A*02:05* | |
| | GBM15P | SAAPFAQRY | C*02:02 | |
| | GBM23P | HYINMPVQF | A*23:01 | |
| | ZH654 | VRNPRITSL | C*06:02 | |
| | ZH802 | VRNPRITSL | B*14:02 | |
| Actin-related protein 8 (ACTR8) | <u>21% P</u> | | | Q9H981 |
| | GBM4P | VIWSHAIQK | A*03:01 | |
| | GBM5P | IQSNFIIVI | B*13:02 | |

| | | | | |
|---|---|---|---|---|
| | GBM7P | APMALFYPATF | B*07:02 | |
| | | LPASIPHVIA | B*07:02; B*56:01 | |
| | GBM16P | VIWSHAIQK | A*03:01 | |
| | ZH613 | LPASIPHVI | B*51:01 | |
| | ZH631 | LPASIPHVI | B*51:01 | |
| | ZH654 | KSASKPIGF | B*57:01 | |
| | | LPASIPHVI | B*51:01 | |
| | ZH678 | KQQGQPLYK | A*11:01 | |
| | | LPASIPHVI | B*51:01 | |
| Oligophrenin-1 (OPHN1) | 21% P | | | O60890 |
| | GBM3P | DEINIAESF | B*18:01 | |
| | GBM6P | FQFDFIGDTL | C*04:01 | |
| | | LGISWVKYY | C*12:03 | |
| | GBM15P | NYSSAVQKF | A*24:02 | |
| | GBM22P | NYSSAVQKF | A*24:02 | |
| | ZH613 | FQFDFIGDTL | *A*02:05* | |
| | ZH678 | RTVGSNIQVQK | A*11:01 | |
| | ZH720 | DEINIAESF | B*18:01 | |
| | ZH750 | NYSSAVQKF | A*24:02 | |
| Mitotic checkpoint serine/threonine-protein kinase BUB1 beta (BUB1B) | 21% P | | | O60566 |
| | GBM3P | EEYEARENF | B*18:01 | |
| | GBM5P | SQKIPGMTL | B*13:02; B*15:01 | |
| | GBM6P | GIADLAHLL | A*02:05 | |
| | | RAFEYEIRF | B*15:03; C*12:03 | |
| | GBM20P | YLHNQGIGV | A*02:01 | |
| | GBM21P | RAFEYEIRF | C*12:03 | |
| | ZH613 | GIADLAHLL | A*02:05 | |
| | ZH617 | YLHNQGIGV | A*02:01 | |
| | ZH645 | AELTVIKV | B*49:01 | |
| Lymphocyte antigen 6 complex locus protein G6f (LY6G6F) | 21% P | | | Q5SQ64 |
| | GBM2P | RVYDVLVLK | A*03:01 | |
| | GBM18P | RVYDVLVLK | A*03:01 | |
| | GBM20P | AVLGQHHNY | A*29:02 | |
| | GBM23P | RVYDVLVLK | A*03:01 | |
| | ZH616 | AVLGQHHNY | A*29:02 | |
| | ZH631 | RVYDVLVLK | A*11:01 | |
| | ZH678 | RVYDVLVLK | A*11:01 | |
| | ZH757 | SPAAGSFTTL | B*07:02 | |
| Nuclease-sensitive element-binding protein 1 (YBX1)<br><br>7 peptides multi-map to YBX2/3 (Q9Y2T7 / P16989) | 21% P | | | P67809 |
| | GBM3P | RRFPPYYMR (YBX1) | B*27:05 | |
| | | VRNGYGFINR (YBX1/2/3) | B*27:05 | |
| | GBM6P | SKYAADRNHY (YBX1) | B*15:03 | |
| | GBM9P | NVRNGYGFINR (YBX1/2/3) | A*33:05 | |
| | ZH613 | GTVKWFNVR (YBX1/2/3) | A*31:01 | |
| | ZH645 | GEKGAEAANV (YBX1/3) | B*49:01 | |
| | | GETVEFDVV (YBX1/2/3) | B*49:01 | |
| | ZH654 | ATKVLGTVKW (YBX1/3) | B*57:01 | |
| | | GTVKWFNVR (YBX1/2/3) | A*31:01 | |
| | | RNHYRRYPR (YBX1) | A*31:01 | |
| | | RYRRNFNYRRRR (YBX1) | A*31:01 | |
| | ZH757 | RRYRRNFNYRRR (YBX1) | B*27:05 | |
| | ZH802 | GTVKWFNVR (YBX1/2/3) | A*31:01 | |
| | | NVRNGYGFINR (YBX1/2/3) | A*33:01 | |
| | | RNHYRRYPR (YBX1) | A*31:01 | |
| | | RYRRNFNYRRRR (YBX1) | A*31:01 | |
| Tumor necrosis factor receptor superfamily member 21 (TNFRSF21) | 18% P | | | O75509 |
| | GBM3P | SEREVAAF | B*18:01 | |
| | GBM6P | RVIEEIPQA | A*02:05 | |
| | GBM22P | VYSHLPDLL | A*24:02 | |
| | ZH613 | RVIEEIPQA | A*02:05 | |
| | ZH617 | GLMEDTTQL | A*02:01 | |
| | ZH681 | RVIEEIPQA | A*02:01 | |
| | ZH761 | TSPSSSTAL | C*01:02 | |
| Left-right determination factor 1/2 (LEFTY1/2) | 18% P | | | O00292 |
| | GBM3P | SLIDSRLVSV | A*02:01 | O75610 |
| | GBM7P | SLIDSRLVSV | A*02:01 | |
| | GBM21P | SLIDSRLVSV | A*02:01 | |
| | ZH617 | SLIDSRLVSV | A*02:01 | |
| | ZH645 | SLIDSRLVSV | A*02:01 | |
| | ZH681 | SLIDSRLVSV | A*02:01 | |
| | ZH757 | SLIDSRLVSV | A*02:01 | |
| PHD finger protein 1 (PHF1) | 18% P | | | O43189 |
| | GBM3P | WVDVAHLVLY | A*01:01 | |
| | GBM5P | WVDVAHLVLY | A*01:01 | |
| | GBM6P | VQFEDDSQFL | C*04:01 | |
| | GBM9P | WVDVAHLVLY | A*01:01 | |
| | GBM16 | WVDVAHLVLY | A*01:01 | |
| | ZH613 | VQFEDDSQFL | *A*02:05* | |
| | ZH654 | WVDVAHLVLY | A*01:01 | |

| | | | | |
|---|---|---|---|---|
| tRNA (guanine-N(7)-)-methyltransferase subunit WDR4 (WDR4) | <u>18% P</u> | | | P57081 |
| | GBM6P | TFDNVTSYL | C*04:01 | |
| | GBM7P | ATFDNVTSYLK | A*11:01 | |
| | GBM17P | SSGDGTLRLW | B*57:01 | |
| | GBM18P | ATFDNVTSYLK | A*11:01 | |
| | ZH631 | ATFDNVTSYLK | A*11:01 | |
| | ZH678 | ATFDNVTSYLK | A*11:01 | |
| | ZH681 | SSGDGTLRLW | B*57:01 | |
| 5'-AMP-activated protein kinase subunit gamma-2 (PRKAG2) | <u>18% P</u> | | | Q9UGJ0 |
| | GBM3P | SLFDAVYSL | A*02:01 | |
| | ZH613 | DIYSKFDVI | B*51:01 | |
| 2 peptides multi-map to PRKAG1 (P54619) | ZH616 | SESGVYMRF | B*44:02 | |
| | ZH645 | AEVHRLVVV | B*49:01 | |
| | ZH654 | DIYSKFDVI | B*51:01 | |
| | ZH750 | SLFDAVYSL | A*02:01 | |
| | ZH757 | SLFDAVYSL | A*02:01 | |
| Bcl10-interacting CARD protein (C9orf89) | <u>18% P</u> | | | Q96LW7 |
| | GBM8P | DTPFLTGHGR | A*68:01 | |
| | GBM23P | IILQLNRYY | A*03:01 | |
| | ZH645 | QEFYRALYI | B*49:01 | |
| | ZH654 | RYYPQILTNK | A*31:01 | |
| | ZH678 | RLVQDTPFL | A*02:01 | |
| | ZH757 | RLVQDTPFL | A*02:01 | |
| | ZH802 | RYYPQILTNK | A*31:01 | |
| TBC1 domain family member 10B (TBC1D10B) | <u>18% P</u> | | | Q4KMP7 |
| | GBM5P | AQAPVAAVL | B*13:02 | |
| | GBM7P | GTAPLVAPPR | A*11:01 | |
| | ZH631 | RTLPWASVL | A*32:01; C*03:03 | |
| | ZH645 | QEDFLVHEV | B*49:01 | |
| | ZH654 | RTLPWASVL | C*15:06 | |
| | ZH678 | GTAPLVAPPR | A*11:01 | |
| | ZH750 | RTLPWASVL | B*15:17; C*07:01 | |
| Asparagine synthetase domain-containing protein 1 (ASNSD1) | <u>18% P</u> | | | Q9NWL6 |
| | GBM3P | EEKTMPTTF | B*18:01 | |
| | GBM7P | KTMPTTFNR | A*11:01 | |
| | GBM18P | KTMPTTFNR | A*03:01; A*11:01 | |
| | GBM23P | LPIWEKANLTL | B*35:02 | |
| | ZH645 | GEIFSGIKV | B*49:01 | |
| | ZH654 | KTMPTTFNR | A*31:01 | |
| | ZH678 | KTMPTTFNR | A*11:01 | |
| Platelet glycoprotein V (GP5) | <u>18% P</u> | | | P40197 |
| | GBM2P | AMIKIGQLF | B*15:01 | |
| | GBM14P | LPTNLTHIL | B*35:03 | |
| | GBM15P | SEAPVHPAL | B*44:02; B*44:03 | |
| | GBM22P | SEAPVHPAL | B*40:01 | |
| | ZH678 | ALLDKMVLL | A*02:01 | |
| | | LPTNLTHI | B*51:01 | |
| | | SEAPVHPAL | B*44:03 | |
| | | TEVLLGHNSW | B*44:03 | |
| | ZH802 | DRLPNLSSL | B*14:02 | |
| | | LRYLGVTL | B*14:02 | |
| | | RTLPAAAFR | A*31:01 | |
| | ZH829 | SEAPVHPAL | B*44:05 | |

**Recurrence-associated HLA class I-presented antigens**

| | | | | |
|---|---|---|---|---|
| Hormone-sensitive lipase (LIPE) | <u>17% R</u> | | | Q05469 |
| | GBM10R | PLYSSPIVK | A*03:01 | |
| | GBM16R | PLYSSPIVK | A*03:01 | |
| | GBM17R | PLYSSPIVK | A*03:01 | |
| | ZH753 | RLSGVFAGV | A*02:01 | |
| T-cell receptor beta-1/2 chain C region (TRBC1/2) | <u>17% R</u> | | | P01850 |
| | GBM4R | ILYEILLGK | A*03:01 | A0A5B9 |
| | GBM12R | ATILYEILLGK | A*11:01 | |
| | GBM16R | ILYEILLGK | A*03:01 | |
| | GBM18R | ATILYEILLGK | A*11:01 | |
| | | ILYEILLGK | A*03:01 | |

**Primary glioblastoma-associated HLA class II-presented antigens**

| | | | | |
|---|---|---|---|---|
| Receptor-type tyrosine-protein phosphatase gamma (PTPRG) | <u>29% P</u> | | | P23470 |
| | GBM5P | FVCLILLIAVLVYWRGCNKI | | |
| | GBM11P | SSNQLHSYVNSILIPG | | |
| | GBM13P | SSPRVVPNESIPIIPIPD | | |
| | GBM21P | KSVEYLRNNFRPQQ | | |
| | ZH613 | SSPRVVPNESIPIIPIP | | |
| | | SSPRVVPNESIPIIPIPD | | |
| | ZH645 | SPRVVPNESIPIIPIP | | |
| | | SSPRVVPNESIPIIPIP | | |
| | | SSPRVVPNESIPIIPIPD | | |

| | | | |
|---|---|---|---|
| | ZH678 | SSPRVVPNESIPIIPIP | |
| | ZH681 | SPRVVPNESIPIIPIP | |
| | | SPRVVPNESIPIIPIPD | |
| | | SSPRVVPNESIPIIPIP | |
| | | SSPRVVPNESIPIIPIPD | |
| | ZH750 | SPRVVPNESIPIIPIP | |
| | | SSPRVVPNESIPIIPIP | |
| | | SSPRVVPNESIPIIPIPD | |
| | ZH791 | SSNQLHSYVNSILIPG | |
| | ZH802 | SPRVVPNESIPIIPIP | |
| | | SSPRVVPNESIPIIPIP | |
| Zinc finger protein 302 (ZNF302) | <u>18% P</u> | | Q9NR11 |
| | GBM10P | QRIHSMKKKYECNKCLKVFSSFSFL | |
| | GBM11P | QRIHSMKKKYECNKCLKVFSSFSFL | |
| | GBM14P | QRIHSMKKKYECNKCLKVFSSFSFL | |
| | GBM16P | QRIHSMKKKYECNKCLKVFSSFSFL | |
| | ZH631 | QRIHSMKKKYECNKCLKVFSSFSFL | |
| | ZH678 | QRIHSMKKKYECNKCLKVFSSFSFL | |
| | ZH761 | QRIHSMKKKYECNKCLKVFSSFSFL | |
| Pre-rRNA processing protein FTSJ3 (FTSJ3) | <u>18% P</u> | | Q8IY81 |
| | GBM18P | GKKGKVGKSRRDKFY | |
| | GBM23P | GKKGKVGKSRRDKFY | |
| | ZH613 | GKKGKVGKSRRDKFY | |
| | ZH631 | GKKGKVGKSRRDKFY | |
| | ZH645 | GKKGKVGKSRRDKFY | |
| | ZH681 | GKKGKVGKSRRDKFY | |
| | ZH761 | GKKGKVGKSRRDKFY | |
| Patatin-like phospholipase domain-containing protein 7 (PNPLA7) | <u>18% P</u> | | Q6ZV29 |
| | GBM13P | PTPQYRFRKRDKVMFYGRKIMRKVT | |
| | GBM19P | VFYGEEERLKKPPRLQESCDSD | |
| | GBM20P | SFTDLAEIVSRIEPAK | |
| | ZH750 | PTPQYRFRKRDKVMFYGRKIMRKVT | |
| | ZH757 | PTPQYRFRKRDKVMFYGRKIMRKVT | |
| | | SFTDLAEIVSRIEPAK | |
| | ZH761 | SFTDLAEIVSRIEPAK | |
| | ZH802 | PTPQYRFRKRDKVMFYGRKIMRKVT | |
| Maltase-glucoamylase, intestinal (MGAM)<br><br>1 peptide multi-maps to Putative inactive maltase-glucoamylase-like protein LOC93432 (Q2M2H8) | <u>18% P</u> | | O43451 |
| | GBM6P | VVQEYLELIGRPALPS | |
| | GBM12P | KFAGFPALINRMKADG | |
| | GBM16P | DTPEELCRRWMQLGAFYPFSRNHN | |
| | GBM18P | TLMHKAHTEGVTVVRPLLH | |
| | GBM19P | IDSQFLLGPAFLVSPV | |
| | ZH681 | TIGGILDFYVFLGNTPEQ | |
| | ZH810 | RKKLKKFTTLEIVLSVLLLVLFIIS | |

**Recurrence-associated HLA class II-presented antigens**

| | | | |
|---|---|---|---|
| Thy-1 membrane glycoprotein (THY1) | <u>29% R</u> | | Q7Z4Q2 |
| | GBM2R | EIVLKYLSRFPT | |
| | GBM3R | EIVLKYLSRFPT | |
| | GBM9R | EIVLKYLSRFPT | |
| | GBM11R | EIVLKYLSRFPT | |
| | GBM14R | EIVLKYLSRFPT | |
| | GBM16R | VSILGITGSVLAKEDG | |
| | GBM20R | WNLKDIIPCKSQAEII | |
| HEAT repeat-containing protein 3 (HEATR3) | <u>25% R</u> | | P04216 |
| | GBM5R | FSLTRETKKHVLFG | |
| | | LTRETKKHVLFG | |
| | GBM11R | LTRETKKHVLFG | |
| | GBM12R | LTRETKKHVLFG | |
| | GBM14R | YNMKVLYLSAFTSKDE | |
| | GBM16R | LTRETKKHVLFG | |
| | GBM21R | LTRETKKHVLFG | |
| Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 3 (ASAP3) | <u>17% R</u> | | Q8TDY4 |
| | GBM9R | KAAQSLFPFIEKLAASVHA | |
| | GBM10R | PEQFSVAEFL | |
| | GBM18R | KAAQSLFPFIEKLAASVHA | |
| | GBM22R | KAAQSLFPFIEKLAASVHA | |
| | | PEQFSVAEFL | |
| Tudor domain-containing protein 12 (TDRD12) | <u>17% R</u> | | Q587J7 |
| | GBM7R | KNIPVKSKNIRVVVESFM | |
| | GBM10R | KNIPVKSKNIRVVVESFM | |
| | GBM12R | KNIPVKSKNIRVVVESFM | |
| | GBM17R | KNIPVKSKNIRVVVESFM | |
| Serine/threonine-protein kinase LMTK2 (LMTK2) | <u>17% R</u> | | Q8IWU2 |
| | GBM2R | GLVSALSSDSTSQDSLLEDSL | |
| | GBM5R | GLVSALSSDSTSQDSLLEDSL | |
| | GBM9R | GLVSALSSDSTSQDSLLEDSL | |
| | GBM22R | KSEDLPSHQKIFDLMELNGVQADF | |

| | | | |
|---|---|---|---|
| Seipin (BSCL2) | <u>17% R</u> | | Q96G97 |
| | GBM5R | ENSYVPTTGAIIEIH | |
| | GBM13R | ENSYVPTTGAIIEIHS | |
| | GBM14R | SKRIQLYGAYLRIH | |
| | GBM23R | ENSYVPTTGAIIEIH | |
| | | ENSYVPTTGAIIEIHS | |
| APC membrane recruitment protein 1 (AMER1) | <u>17% R</u> | | Q5JTC6 |
| | GBM12R | QQEDSDEEDEEEEEGEWSRDSPLSL | |
| | GBM14R | QQEDSDEEDEEEEEGEWSRDSPLSL | |
| | GBM20R | QQEDSDEEDEEEEEGEWSRDSPLSL | |
| | GBM21R | QQEDSDEEDEEEEEGEWSRDSPLSL | |
| 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase delta-4 (PLCD4) | <u>17% R</u> | | Q9BRC7 |
| | GBM10R | EEAQIWMRGLQLLVDLVTSMDH | |
| | GBM14R | EEAQIWMRGLQLLVDLVTSMDH | |
| | GBM15R | EEAQIWMRGLQLLVDLVTSMD | |
| | ZH753 | EEAQIWMRGLQLLVDLVTSMDH | |
| Cholecystokinin (CCK) | <u>17% R</u> | | P06307 |
| | GBM11R | PAGSGLQRAEEAP | |
| | GBM14R | GALLARYIQQARKA | |
| | GBM17R | PAGSGLQRAEEAP | |
| | GBM21R | GALLARYIQQARK | |
| | | GALLARYIQQARKA | |
| | | GALLARYIQQARKAPS | |
| Treslin (TICRR) | <u>17% R</u> | | Q7Z2Z1 |
| | GBM3R | DSEVPAAYQTPKKS | |
| | GBM12R | KIPSGRTVDKLEDRGRTLRSSKPK | |
| | GBM14R | KSIAEVSQNLRQIEIPK | |
| | GBM18R | KDTVQEVTKVRRNLFNQELLSP | |
| Hematopoietic lineage cell-specific protein (HCLS1) | <u>17% R</u> | | P14317 |
| | GBM4R | KAKFESMAEEKRKRE | |
| | GBM8R | AVALGISAVAV | |
| | GBM10R | KAKFESMAEEKRKR | |
| | | KAKFESMAEEKRKRE | |
| | | KAKFESMAEEKRKREE | |
| | ZH753 | VNDISEKEQRWGAKTIEGSGRTEHI | |
| Proline-rich acidic protein 1 (PRAP1) | <u>17% R</u> | | Q96NZ9 |
| | GBM4R | DQGEERPRLWVMPNHQVLLGPE | |
| | | EERPRLWVMPNHQVLLGPE | |
| | | ERPRLWVMPNHQVL | |
| | | ERPRLWVMPNHQVLL | |
| | | ERPRLWVMPNHQVLLG | |
| | | ERPRLWVMPNHQVLLGPE | |
| | GBM16R | ERPRLWVMPNHQVLL | |
| | GBM18R | AWGARVVEPPEKDDQL | |
| | | WGARVVEPPEKDD | |
| | | WGARVVEPPEKDDQ | |
| | | WGARVVEPPEKDDQL | |
| | | WGARVVEPPEKDDQLV | |
| | GBM23R | WGARVVEPPEKDDQLV | |
| Proteolipid protein 2 (PLP2) | <u>17% R</u> | | Q04941 |
| | GBM3R | PVRQPRHTAAPTDPADGPV | |
| | GBM5R | PVRQPRHTAAPTDPADGPV | |
| | | VTFPVRQPRHTAAPTDPADGPV | |
| | GBM14R | PVRQPRHTAAPTDPADGP | |
| | | PVRQPRHTAAPTDPADGPV | |
| | GBM21R | PVRQPRHTAAPTDPADGPV | |
| Complement factor D (CFD) | <u>17% R</u> | | P00746 |
| | GBM4R | GGVLVAEQWVLSAA | |
| | GBM5R | GGVLVAEQWVLSAA | |
| | | GGVLVAEQWVLSAAH | |
| | | KKPGIYTRVASYAAWI | |
| | | KPGIYTRVASYAAW | |
| | | KPGIYTRVASYAAWI | |
| | | KPGIYTRVASYAAWID | |
| | | RPLPWQRVDRDVAPG | |
| | | VRPLPWQRVDRDVAPG | |
| | GBM18R | VRPLPWQRVDRDVAPG | |
| | GBM20R | GGVLVAEQWVLSAAH | |
| | | VRPLPWQRVDRDVAPG | |
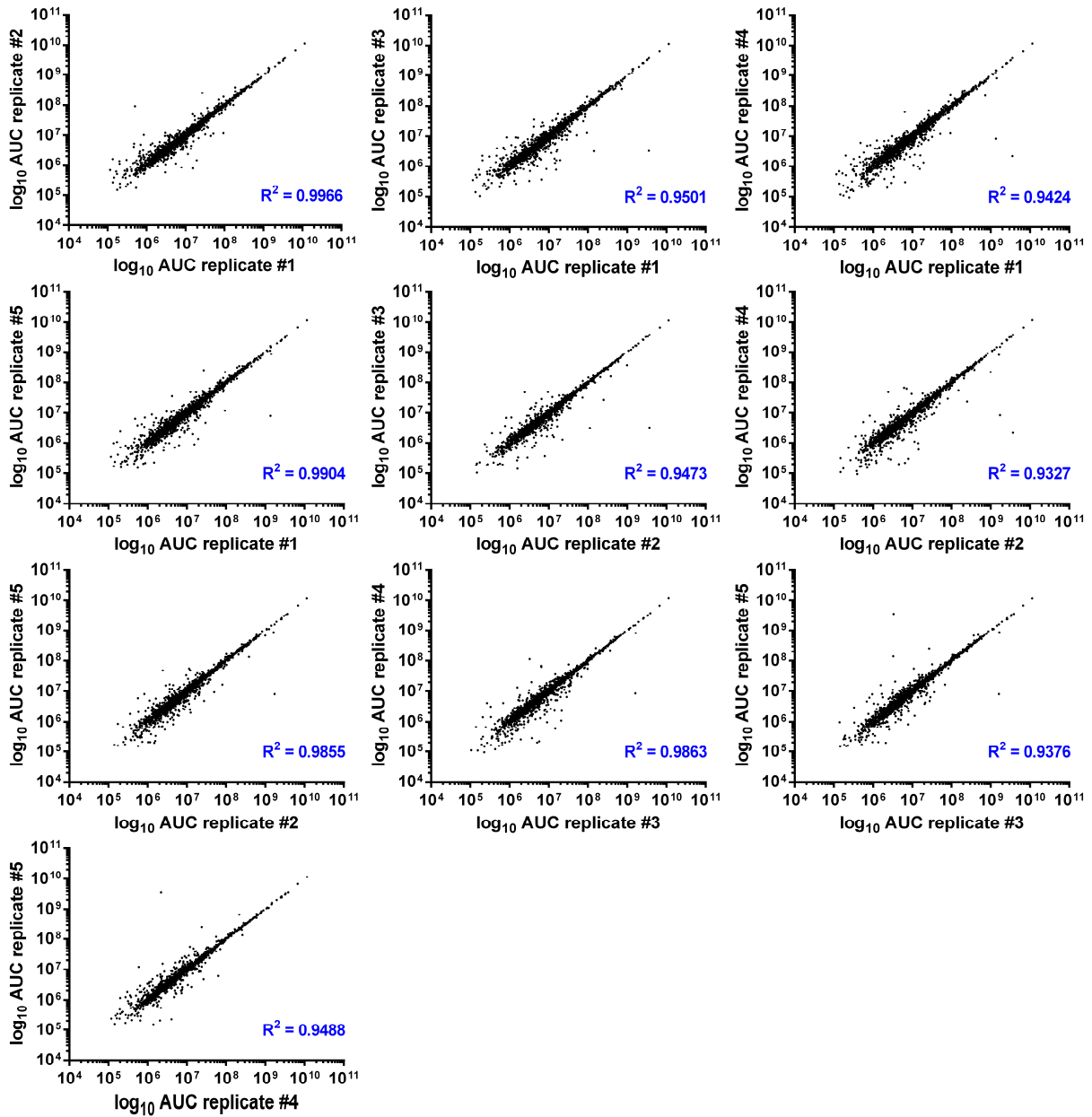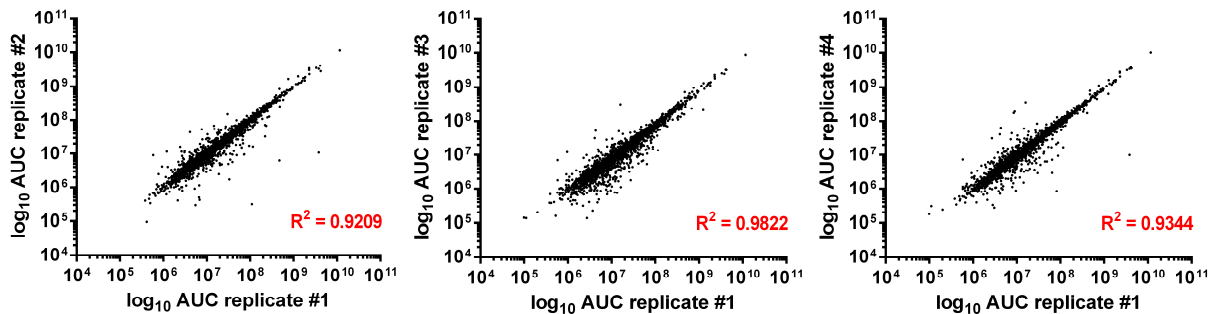
**A**



**B**

C

**D**
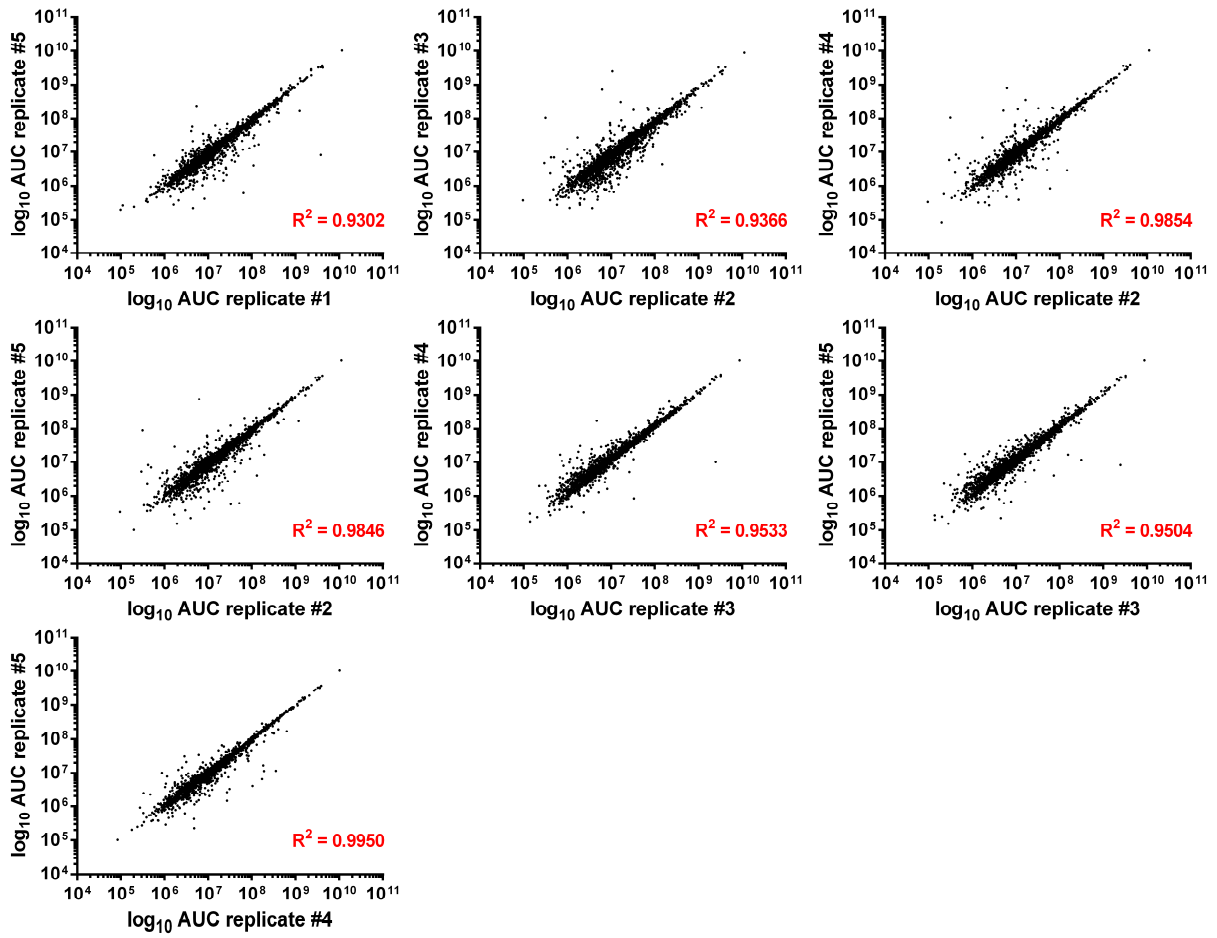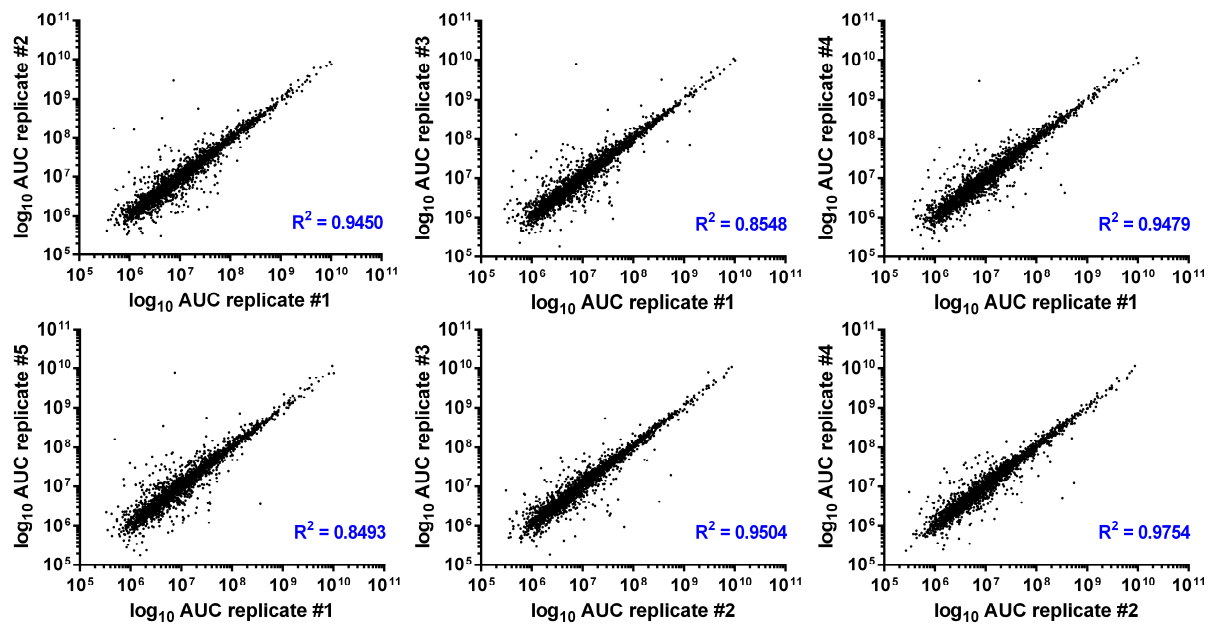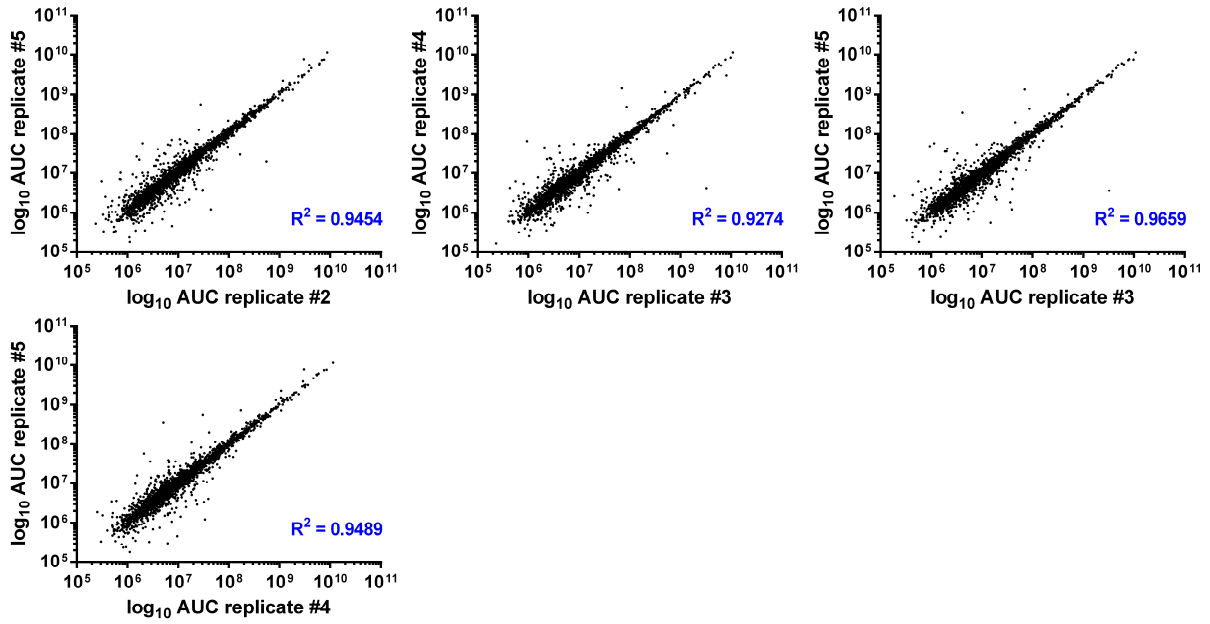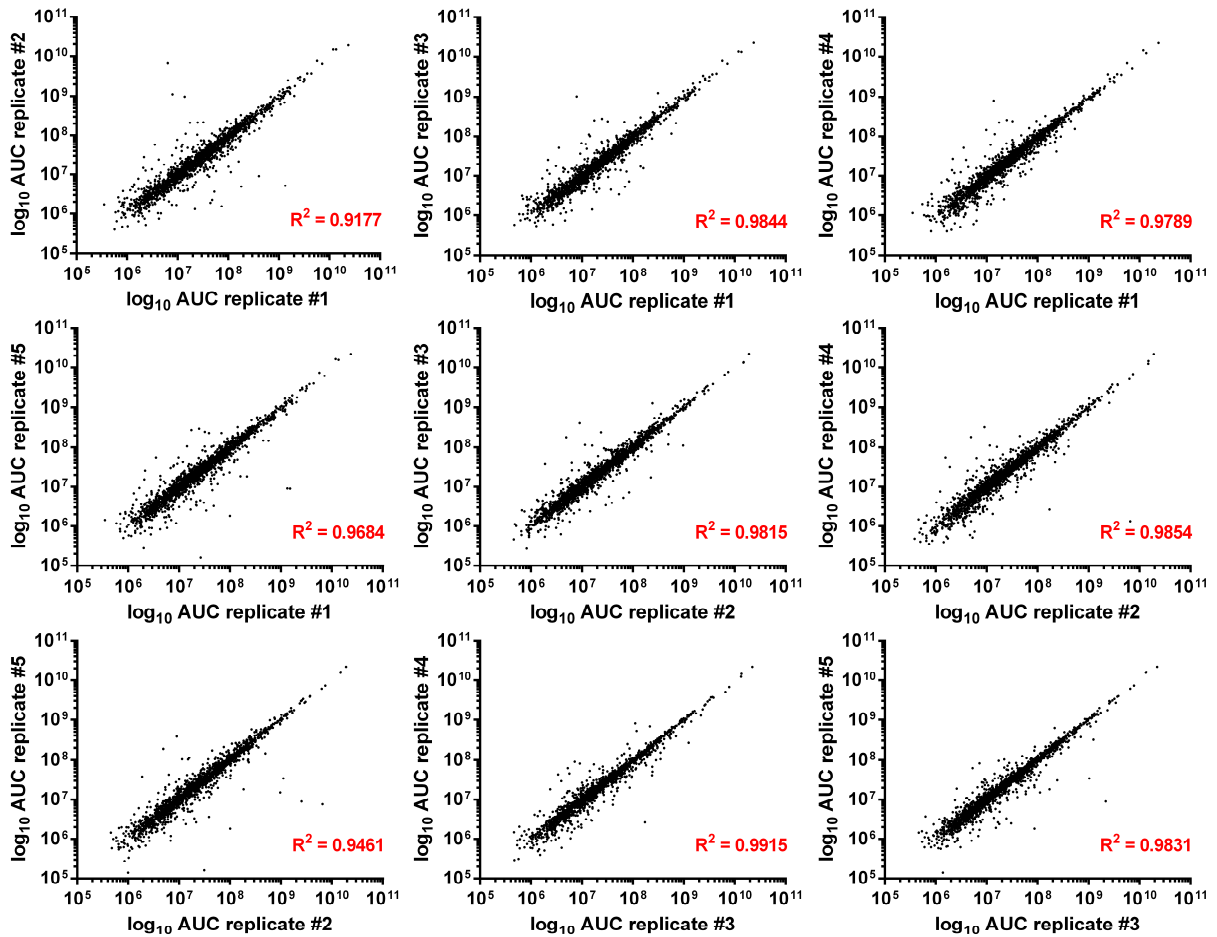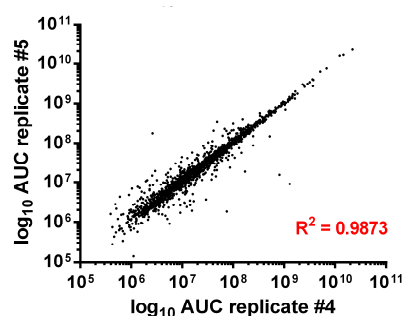
**Supplementary Figure 4. Reproducibility of precursor ion intensities across technical replicates exemplarily shown for GBM16.** Scatter plots depicting raw MS[1] intensities prior to normalization in pairwise comparisons of technical replicates acquired from **(A) HLA class I ligands eluted from primary glioblastoma**, **(B) HLA class I ligands identified at disease recurrence, (C) HLA class II-presented peptides eluted from primary glioblastoma**, and **(D) HLA class II-presented peptides identified at disease recurrence.** Correlation coefficients were calculated by linear regression and amounted to > 0.92 except for HLA class II peptide intensity comparisons between replicate #1 and #5 as well as between replicate #1 and #3 acquired from primary glioblastoma.

**Supplementary Table 7. Peptide sequences and level of modulation for antigens exclusively represented by up- or down-modulated HLA class I- and II-presented peptides on recurrent *versus* primary glioblastoma.** Oxidized (m) and reduced (M) methionine were not treated equally in LFQ-MS and are listed separately. Multi-mapping peptides were censored when the second annotated protein diverged in assignment to groups of modulation according to comparative profiling. HLA restrictions not passing manual assessment as quality control are indicated in italic (HLA class I ligands not matching the motif of any of the patient's HLA allotypes; HLA class II-presented proteins neither identified with peptides exceeding a length of twelve AA nor with different sequences across samples). Abbreviation not introduced in the text above: fold change recurrence/primary (fc R/P).

| UniProt accession | Antigen | Patients with significant modulation | Peptide sequence | HLA restriction | Fc (R/P) | Corrected *p*-value |
|---|---|---|---|---|---|---|
| **Antigens exclusively represented by HLA class I ligands up-modulated at recurrence** | | | | | | |
| P02745 | Complement C1q subcomponent subunit A (C1QA) | 18% | | | | |
| | | GBM4 | QPRPAFSAI | B*07:02 | 173.79 | 0.00009 |
| | | GBM5 | GQVRRSLGF | *B*51:02* | 30.26 | 0.00044 |
| P19878 | Neutrophil cytosol factor 2 (NCF2) | 18% | | | | |
| | | GBM5 | SQVEALFSY | *B*51:01* | 17.08 | 0.00432 |
| | | GBM20 | VLYNIAFmY | A*29:02 | 7.96 | 0.00001 |
| P26572 | Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltrans-ferase (MGAT1) | 18% | | | | |
| | | GBM5 | FRFPAAVVV | C*06:02 | 13.81 | 0.00021 |
| | | GBM6 | DASRPELLY | B*35:01; C*12:03 | 40.85 | 0.00052 |
| Q6P4A8 | Phospholipase B-like 1 (PLBD1) | 18% | | | | |
| | | GBM5 | VTDTASMKY | A*01:01 | 54.44 | 0.00141 |
| | | GBM9 | VTDTASmKY | A*01:01 | 5.01 | 0.00406 |
| Q7Z4Q2 | HEAT repeat-containing protein 3 (HEATR3) | 18% | | | | |
| | | GBM5 | IQIKLLSAL | B*13:02; *B*51:01* | 10.06 | 0.00931 |
| | | GBM14 | VLDNVKMNL | C*08:02 | 6.27 | 0.00002 |
| Q86Y82 | Syntaxin-12 (STX12) | 18% | | | | |
| | | GBM12 | KLQENLQQL | A*02:01 | 7.09 | 0.00014 |
| | | GBM14 | KLQENLQQL | A*02:01 | 4.28 | 0.00117 |
| Q8N7J2 | APC membrane recruitment protein (AMER2) | 18% | | | | |
| | | GBM6 | KKNPGVVAY | *B*15:03* | 19.38 | 0.00010 |
| | | | SKKNPGVVAY | *B*15:03* | 57.84 | 0.00016 |
| | | GBM16 | VPGPGKPAL | B*07:02 | 48.50 | 0.00002 |
| Q8WTW4 | Nitrogen permease regulator 2-like protein (NPRL2) | 18% | | | | |
| | | GBM12 | HPTLGPKITY | B*35:01 | 10.09 | 0.00003 |
| | | GBM20 | EEQSHPARLY | B*44:02 | 4.63 | 0.00839 |
| Q96PV0 | Ras/Rap GTPase-activating protein SynGAP (SYNGAP1) | 18% | | | | |
| | | GBM11 | mLDEDEIHPL | A*02:01 | 16.18 | 0.00001 |
| | | GBM14 | YPDEQTSRTL | B*35:03; C*08:02 | 4.56 | 0.00025 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Antigens exclusively represented by HLA class I ligands down-modulated at recurrence** | | | | | | |
| Q53EL9 | Seizure protein 6 homolog (SEZ6) | 27% | | | | |
| | | GBM5 | QSDPGTSVLGY | A*01:01 | 0.004 | 0.00002 |
| | | GBM9 | QSDPGTSVLGY | A*01:01 | 0.07 | 0.00017 |
| | | GBM16 | QSDPGTSVLGY | A*01:01 | 0.001 | 0.000003 |
| Q9Y2R0 | Cytochrome c oxidase assembly protein 3 homolog, mitochondrial (COA3) | 18% | | | | |
| | | GBM4 | AQWQKVLPR | A*03:01 | 0.02 | 0.00697 |
| | | GBM12 | RQAELAQWQK | A*11:01 | 0.18 | 0.00792 |
| Q9NPD8 | Ubiquitin-conjugating enzyme E2 T (UBE2T) | 18% | | | | |
| | | GBM7 | ASQLVGIEK | A*11:01 | 0.06 | 0.00007 |
| | | GBM14 | EPNPDDPLm | B*35:03 | 0.15 | 0.00005 |
| Q9H095 | IQ domain-containing protein G (IQCG) | 18% | | | | |
| | | GBM5 | TTDQLSILNY | A*01:01 | 0.18 | 0.00604 |
| | | GBM11 | SQNEYIANL | *A*02:01; A*02:05* | 0.10 | 0.00016 |
| Q9BVV7 | Mitochondrial import inner membrane translocase subunit Tim21 (TIMM21) | 18% | | | | |
| | | GBM5 | RSHPEVIGV | B*13:02 | 0.13 | 0.00046 |
| | | GBM12 | RQHVRFTEY | B*15:01 | 0.02 | 0.00020 |
| Q96SC8 | Doublesex- and mab-3-related transcription factor A2 (DMRTA2) | 18% | | | | |
| | | GBM5 | GIIPPRPAY | B*15:01 | 0.02 | 0.00707 |
| | | GBM12 | GIIPPRPAY | B*15:01 | 0.02 | 0.00171 |
| Q8NI35 | InaD-like protein (INADL) | 18% | | | | |
| | | GBM5 | AQGLVQLEI | B*13:02 | 0.05 | 0.00009 |
| | | GBM16 | SPAGKTNAL | B*07:02 | 0.19 | 0.00596 |
| Q86YD5 | Low-density lipoprotein receptor class A domain-containing protein 3 (LDLRAD3) | 18% | | | | |
| | | GBM5 | LLDQRPAWY | A*01:01; B*15:01 | 0.04 | 0.00031 |
| | | | ASEVGSPPSY | A*01:01 | 0.01 | 0.00006 |
| | | GBM16 | LLDQRPAWY | A*01:01 | 0.07 | 0.00020 |
| Q86VE9 | Serine incorporator 5 (SERINC5) | 18% | | | | |
| | | GBM4 | AISPWVQNR | A*03:01 | 0.01 | 0.00315 |
| | | GBM20 | ALSSKPAEV | A*02:01 | 0.06 | 0.000001 |
| Q6P499 | NIPA-like protein 3 (NIPAL3) | 18% | | | | |
| | | GBM5 | AVSEASFSY | B*15:01 | 0.08 | 0.00016 |
| | | GBM7 | APLSLIVPLSA | B*07:02; B*56:01 | 0.08 | 0.00327 |
| Q5VYS8 | Terminal uridylyltrans-ferase 7 (ZCCHC6) | 18% | | | | |
| | | GBM5 | GQVSLILDV | B*13:02 | 0.06 | 0.00383 |
| | | GBM20 | LESFIRQDF | B*44:02 | 0.11 | 0.00020 |
| Q16538 | Probable G-protein coupled receptor 162 (GPR162) | 18% | | | | |
| | | GBM7 | TPREPGSFL | B*07:02 | 0.02 | 0.00023 |
| | | GBM16 | TPREPGSFL | B*07:02 | 0.02 | 0.00034 |
| P62244 | 40S ribosomal protein S15a (RPS15A) | 18% | | | | |
| | | GBM4 | VRmNVLADAL | C*07:02 | 0.07 | 0.00055 |
| | | GBM20 | VLADALKSI | A*02:01 | 0.03 | 0.00002 |
| P28562 | Dual specificity protein phosphatase 1 (DUSP1) | 18% | | | | |
| | | GBM12 | RAAQVFFLK | A*11:01 | 0.03 | 0.00137 |
| | | GBM20 | KLDEAFEFV | A*02:01 | 0.05 | 0.00004 |
| P23743 | Diacylglycerol kinase alpha (DGKA) | 18% | | | | |
| | | GBM9 | DNVPRHLSL | B*08:01 | 0.01 | 0.00017 |
| | | GBM16 | NPRQVFNLL | B*07:02 | 0.14 | 0.00098 |
| P18031 | Tyrosine-protein phosphatase non-receptor type 1 (PTPN1) | 18% | | | | |
| | | GBM9 | DIKSYYTVR | A*33:05 | 0.06 | 0.00008 |
| | | | YRFLFNSNT | *B*14:02* | 0.04 | 0.00075 |
| | | GBM14 | DIKSYYTVR | A*02:01 | 0.07 | 0.00075 |
| P14780 | Matrix metalloproteinase-9 (MMP9) | 18% | | | | |
| | | GBM9 | DAFARAFAL | B*14:02 | 0.04 | 0.00034 |
| | | GBM14 | DAFARAFAL | B*14:02 | 0.02 | 0.00000 |
| P02788 | Lactotransferrin (LTF) | 18% | | | | |
| | | GBM12 | LLFKDSAIGF | B*15:01 | 0.06 | 0.00043 |
| | | GBM20 | TLDGGFIYEA | A*02:01 | 0.02 | 0.00003 |
| O75832 | 26S proteasome non-ATPase regulatory subunit 10 (PSMD10) | 18% | | | | |
| | | GBM16 | LAYSGKLEEL | C*12:03 | 0.01 | 0.00005 |
| | | GBM11 | LAYSGKLEEL | A*02:01 | 0.05 | 0.00040 |
| O15230 | Laminin subunit alpha-5 (LAMA5) | 18% | | | | |
| | | GBM5 | SYSPLLREF | C*06:02 | 0.06 | 0.00019 |
| | 1 peptide multi-maps to LAMA3 (Q16787) | | SSYGGTLRY | A*01:01; B*15:01 | 0.07 | 0.00014 |
| | | GBM16 | SPRPDLWVL | B*07:02 | 0.06 | 0.00004 |
| Q9UQR1 | Zinc finger protein 14 (ZNF148) | 18% | | | | |
| | | GBM5 | QTISPLSTY | B*15:01 | 0.03 | 0.00006 |
| | | GBM6 | ALNVPISVK | A*03:01 | 0.07 | 0.00033 |
| **Antigens exclusively represented by HLA class II-restricted peptides up-modulated at recurrence** | | | | | | |
| O60293 | Zinc finger C3H1 domain-containing protein (ZFC3H1) | 17% | | | | |
| | | GBM11 | ENCVEETFEDLLLK | Class II | 27.90 | 0.00549 |
| | | GBM21 | ENCVEETFEDLLLK | Class II | 6.96 | 0.00046 |

| | | | | | | |
|---|---|---|---|---|---|---|
| P07148 | Fatty acid-binding protein, liver (FABP1) | 17% | | | | |
| | | GBM2 | TITAGSKVIQNEF | Class II | 18.38 | 0.00236 |
| | | | IVQNGKHFKF | Class II | 14.19 | 0.00175 |
| | | | IQKGKDIKGVSE | Class II | 4.76 | 0.00959 |
| | | GBM7 | IQKGKDIKGVSE | Class II | 10.18 | 0.00002 |
| P07492 | Gastrin-releasing peptide (GRP) | 17% | | | | |
| | | GBM11 | RGRAVPLPAG | *Class II* | 8.33 | 0.00013 |
| | | GBM14 | RGRAVPLPAG | *Class II* | 17.06 | 0.0000004 |
| Q5T5U3 | Rho GTPase-activating protein 21 (ARHGAP21) | 17% | | | | |
| | | GBM7 | WSGFTEQDDRRGICERPRQQEIHKS | Class II | 314.25 | 0.00762 |
| | | GBM20 | SIPFIDEPTSPSIDH | Class II | 15.28 | 0.00017 |
| Q7Z2Z2 | Elongation factor Tu GTP-binding domain-containing protein 1 (EFTUD1) | 17% | | | | |
| | | GBM7 | EAYSHLKNILEQINALTGT | Class II | 46.53 | 0.00000003 |
| | | GBM21 | EAYSHLKNILEQINALTGT | Class II | 32.65 | 0.000003 |
| Q8NDZ6 | Transmembrane protein 161B (TMEM161B) | 17% | | | | |
| | | GBM12 | KNIFTPLLFRGLLSFLTWWIAA | Class II | 688.01 | 0.00043 |
| | | GBM16 | KNIFTPLLFRGLLSFLTWWIAA | Class II | 292.53 | 0.00071 |
| Q99767 | Amyloid beta A4 precursor protein-binding family A member 2 (APBA2) | 17% | | | | |
| | | GBM7 | KNIRMMQAQEAVSRVKRmQKA | Class II | 44.65 | 0.00002 |
| | | GBM14 | NPSKNIRMMQAQEAVSR | Class II | 10.02 | 0.00248 |
| Q9ULX3 | RNA-binding protein NOB1 (NOB1) | 17% | | | | |
| | | GBM7 | KNIYTIREVVTEIRDKATR | Class II | 193.58 | 0.00009 |
| | | GBM11 | KNIYTIREVVTEIRDKATR | Class II | 265.85 | 0.00010 |

**Antigens exclusively represented by HLA class II-restricted peptides down-modulated at recurrence**

| | | | | | | |
|---|---|---|---|---|---|---|
| O00469 | Procollagen-lysine,2-oxoglutarate 5-dioxy-genase 2 (PLOD2) | 17% | | | | |
| | | GBM5 | DASTFTINIALNNVG | Class II | 0.10 | 0.00422 |
| | | GBM12 | SPRKGWSFmHPGRLTH | Class II | 0.02 | 0.00116 |
| O95810 | Serum deprivation-response protein (SDPR) | 17% | | | | |
| | | GBM12 | RLENNHAQLLRRNHFKVLIF | Class II | 0.005 | 0.00159 |
| | | GBM14 | RLENNHAQLLRRNHFKVLIF | Class II | 0.003 | 0.000004 |
| P51153 | Ras-related protein Rab-13 (RAB13) | 17% | | | | |
| | | GBM2 | EHGIRFFETSAKSS | Class II | 0.08 | 0.00170 |
| | | GBM16 | ISTIGIDFKIRTVD | Class II | 0.21 | 0.00067 |
| Q5T447 | E3 ubiquitin-protein ligase HECTD3 (HECTD3) | 17% | | | | |
| | | GBM11 | LPARIYIYPDKLGYE | Class II | 0.05 | 0.00152 |
| | | GBM16 | LPARIYIYPDKLGYE | Class II | 0.06 | 0.00098 |
| Q86UP2 | Kinectin (KTN1) | 17% | | | | |
| | | GBM7 | LKAQIQQFHSQIAAQT | Class II | 0.11 | 0.00037 |
| | | | KAQIQQFHSQIAAQT | Class II | 0.17 | 0.00001 |
| | | GBM16 | LKAQIQQFHSQIAAQT | Class II | 0.01 | 0.00009 |
| Q86VH5 | Leucine-rich repeat transmembrane neuronal protein 3 (LRRTM3) | 17% | | | | |
| | | GBM11 | SGNEIEAFSGPSVFQ | Class II | 0.04 | 0.00045 |
| | | GBM13 | SGNEIEAFSGPSVFQ | Class II | 0.06 | 0.00010 |
| Q8N0V3 | Putative ribosome-binding factor A, mitochondrial (RBFA) | 17% | | | | |
| | | GBM2 | KNWLKKFASKTKK | Class II | 0.05 | 0.00372 |
| | | GBM5 | KNWLKKFASKTKK | Class II | 0.07 | 0.00100 |
| Q8NEZ3 | WD repeat-containing protein 19 (WDR19) | 17% | | | | |
| | | GBM11 | QSVIRIYLDHLNNPE | Class II | 0.09 | 0.00098 |
| | | GBM16 | QDLYLASSCPIAALEMRRD | Class II | 0.05 | 0.00024 |
| Q96QR8 | Transcriptional activator protein Pur-beta (PURB) | 17% | | | | |
| | | GBM12 | VKPSYRNAITVPFK | Class II | 0.005 | 0.00230 |
| | | GBM16 | GTSITVDSKRFFFD | Class II | 0.01 | 0.00007 |
| | | | GTSITVDSKRFFFDVG | Class II | 0.07 | 0.00387 |
| | | | ELPEGTSITVDSKRFFFDVG | Class II | 0.12 | 0.00375 |
| Q9BRB3 | Phosphatidylinositol N-acetylglucosaminyltrans-ferase subunit Q (PIGQ) | 17% | | | | |
| | | GBM7 | ANTVASVLLDV | Class II | 0.09 | 0.00003 |
| | | GBM12 | ATTASTGGLAAVF | Class II | 0.04 | 0.00097 |
| Q9H492 | Microtubule-associated proteins 1A/1B light chain 3A (MAP1LC3A) | 17% | | | | |
| | | GBM3 | SELVKIIRRRLQLNPT | Class II | 0.24 | 0.00215 |
| | | GBM5 | SELVKIIRRRLQLNPT | Class II | 0.05 | 0.00493 |
| Q9UPV9 | Trafficking kinesin-binding protein 1 (TRAK1) | 17% | | | | |
| | | GBM12 | KAPVTLTSGILMGAKLSK | Class II | 0.03 | 0.00193 |
| | | GBM14 | KAPVTLTSGILMGAKLSK | Class II | 0.15 | 0.000004 |

**Supplementary Table 8. Functional annotation clustering of source proteins exclusively presented at recurrent or primary disease and/or represented by up- or down-modulated HLA class I- or II-restricted peptides on recurrent *versus* autologous primary glioblastoma.** Clustering for biological processes was performed on the basis of Gene Ontology (GO) terms using DAVID Bioinformatic Resources 6.8 ('Homo sapiens' as background, medium classification stringency, GO BP_ALL). As recommended by Huang *et al.* (Ref. 419 CHAPTER 1), only clusters with an enrichment score ≥ 1.0 were reported.

| Biological process / Gene Ontology IDs | | | | | | Enrichment score |
|---|---|---|---|---|---|---|
| **Primary-exclusive antigens and proteins exclusively represented by HLA class I ligands down-modulated at recurrence** | | | | | | |
| amino acid transport | | | | | | 3.11 |
| GO:0003333 | GO:1905039 | GO:1903825 | GO:0006865 | GO:1902475 | GO:0015804 | GO:0015807 |
| GO:0015849 | GO:0015711 | GO:0046942 | GO:0006820 | | | |
| metal ion transport | | | | | | 2.32 |
| GO:0055085 | GO:0006812 | GO:0098655 | GO:0006811 | GO:0034220 | GO:0030001 | GO:0015672 |
| GO:0098662 | GO:0006813 | GO:0098660 | GO:0034765 | GO:0043269 | GO:0071804 | GO:0071805 |
| GO:0034762 | GO:0006816 | GO:0070838 | GO:0072511 | GO:0010959 | GO:1904062 | |
| intraspecies interaction | | | | | | 1.76 |
| GO:0051705 | GO:0051703 | GO:0035176 | | | | |
| coenzyme biosynthesis | | | | | | 1.59 |
| GO:0051188 | GO:0009108 | GO:0006732 | GO:0051186 | | | |
| protein folding | | | | | | 1.59 |
| GO:0018208 | GO:0000413 | GO:0006457 | | | | |
| synaptic transmission | | | | | | 1.56 |
| GO:0099536 | GO:0099537 | GO:0098916 | GO:0007268 | GO:0050804 | GO:0007267 | |
| tRNA modification | | | | | | 1.49 |
| GO:0030488 | GO:0006400 | GO:0009451 | GO:0006399 | GO:0008033 | GO:0001510 | GO:0034470 |
| adaptive thermogenesis | | | | | | 1.44 |
| GO:0002024 | GO:1990845 | GO:0002021 | | | | |
| response to alkaloid | | | | | | 1.33 |
| GO:0007274 | GO:0007271 | GO:0035094 | GO:0035095 | GO:0043279 | | |
| respiratory chain complex IV assembly | | | | | | 1.31 |
| GO:0008535 | GO:0017004 | GO:0033617 | GO:0097034 | | | |
| insemination | | | | | | 1.29 |
| GO:0007320 | GO:0007620 | GO:0007618 | | | | |
| action potential transmission | | | | | | 1.21 |
| GO:0019228 | GO:0001508 | GO:0035637 | GO:0042391 | GO:0019226 | | |
| quinone biosynthesis | | | | | | 1.20 |
| GO:0006744 | GO:1901663 | GO:0006743 | GO:0042181 | GO:1901661 | GO:0042180 | |
| protein complex disassembly | | | | | | 1.14 |
| GO:0032543 | GO:0070125 | GO:0070126 | GO:0006415 | GO:0006414 | GO:0043624 | GO:0043241 |
| GO:0032984 | GO:0022411 | | | | | |
| mRNA catabolism | | | | | | 1.10 |
| GO:0043928 | GO:0000291 | GO:0000288 | | | | |
| azole transport | | | | | | 1.06 |
| GO:0006867 | GO:1902024 | GO:0089709 | GO:0006868 | GO:0045117 | | |
| postsynaptic transmission | | | | | | 1.01 |
| GO:0060078 | GO:0060079 | GO:0099565 | GO:2000463 | GO:0098815 | | |
| **Recurrence-exclusive antigens and proteins exclusively represented by HLA class I ligands up-modulated at recurrence** | | | | | | |
| negative regulation of fatty acid metabolism | | | | | | 3.98 |
| GO:1904223 | GO:1904224 | GO:2001030 | GO:0052697 | GO:2001029 | GO:0045922 | GO:0009410 |
| GO:0052696 | GO:0071466 | GO:0052695 | GO:0051552 | GO:0006805 | GO:0010677 | GO:0006063 |
| GO:0019585 | GO:0042573 | GO:0019217 | GO:0009812 | GO:0045912 | GO:0009813 | GO:0045833 |
| GO:0034754 | GO:0010565 | GO:0042440 | GO:0010675 | GO:0042180 | GO:0019216 | GO:0006109 |
| GO:0044262 | | | | | | |
| humoral immune response | | | | | | 3.16 |
| GO:0006958 | GO:0002455 | GO:0006956 | GO:0016064 | GO:0072376 | GO:0002250 | GO:0019724 |
| GO:0002449 | GO:0002460 | GO:0038096 | GO:0002433 | GO:0002431 | GO:0038094 | GO:0006959 |
| GO:0002443 | GO:0006909 | GO:0002757 | GO:0038095 | GO:0006898 | GO:0050778 | GO:0002764 |
| GO:0002253 | GO:0050776 | GO:0002429 | GO:0002376 | GO:0006955 | GO:0002684 | GO:0002768 |
| GO:0002682 | GO:0006897 | GO:0038093 | GO:0002252 | GO:0016192 | GO:0048584 | |
| lipid metabolism | | | | | | 2.03 |
| GO:0006631 | GO:0019752 | GO:0043436 | GO:0006082 | GO:0032787 | GO:0044255 | GO:0044281 |
| GO:0006629 | | | | | | |
| isoprenoid metabolism | | | | | | 3.05 |
| GO:0045922 | GO:0042573 | GO:0001523 | GO:0006721 | GO:0016101 | GO:0006720 | GO:0034754 |
| GO:0042445 | GO:0010817 | | | | | |
| drug metabolism | | | | | | 2.69 |
| GO:0017144 | GO:0009698 | GO:0009804 | GO:0019748 | | | |
| citrulline biosynthesis | | | | | | 1.98 |
| GO:0019240 | GO:1901607 | GO:0000052 | | | | |
| drug catabolism | | | | | | 1.73 |
| GO:0017144 | GO:0042738 | GO:0042737 | | | | |
| intermediate filament organization | | | | | | 1.60 |
| GO:0045104 | GO:0045103 | GO:0045109 | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| glutamate biosynthesis | | | | | 1.54 | |
| GO:0009064 | GO:1901605 | GO:0009084 | GO:1901607 | GO:0006541 | GO:0009065 | GO:0006537 |
| GO:0006520 | GO:0043648 | GO:0008652 | GO:1901606 | GO:0043650 | GO:0006536 | |
| amino acid catabolism | | | | | 1.47 | |
| GO:0016054 | GO:1901606 | GO:0009063 | | | | |
| negative regulation of molecular function | | | | | 1.36 | |
| GO:0043086 | GO:0010563 | GO:0045936 | GO:0044092 | GO:0032269 | GO:0051248 | GO:0051338 |
| cellular process | | | | | 1.35 | |
| GO:0044699 | GO:0044763 | GO:0009987 | | | | |
| ion transport | | | | | 1.22 | |
| GO:0006814 | GO:0006813 | GO:0071804 | GO:0071805 | GO:0035725 | GO:0030001 | GO:0055085 |
| GO:0006811 | GO:0042391 | GO:0098662 | GO:0006812 | GO:0098655 | GO:0098660 | GO:0015672 |
| GO:0070588 | GO:0034220 | GO:0043269 | GO:0034762 | GO:0034765 | GO:0006816 | GO:0072511 |
| GO:0070838 | | | | | | |
| fatty acid oxidation | | | | | 1.21 | |
| GO:0016054 | GO:0046395 | GO:0016042 | GO:0044282 | GO:0072329 | GO:0009062 | GO:0044242 |
| GO:0006635 | GO:0019395 | GO:0034440 | GO:0030258 | | | |
| unsaturated fatty acid metabolism | | | | | 1.15 | |
| GO:0019369 | GO:0019373 | GO:0001676 | GO:0006690 | GO:0033559 | | |
| heme metabolism | | | | | 1.10 | |
| GO:0042440 | GO:0006787 | GO:0033015 | GO:0042167 | GO:0046149 | GO:0051187 | GO:0006778 |
| GO:0033013 | GO:0042168 | | | | | |
| glutamate secretion | | | | | 1.03 | |
| GO:0014047 | GO:0006835 | GO:0006820 | GO:0015711 | GO:0046942 | GO:0051966 | |
| **Primary-exclusive antigens and proteins exclusively represented by HLA class II peptides down-modulated at recurrence** | | | | | | |
| organelle organization | | | | | 2.51 | |
| GO:0071840 | GO:0006996 | GO:0016043 | | | | |
| microtubule cytoskeleton organization | | | | | 2.27 | |
| GO:0007017 | GO:0000226 | GO:0007010 | | | | |
| histone H4 acetylation | | | | | 2.15 | |
| GO:0043984 | GO:0043981 | GO:0043982 | GO:0043967 | | | |
| chromatin organization | | | | | 2.07 | |
| GO:0016569 | GO:0016570 | GO:0018205 | GO:0051276 | GO:0006325 | | |
| cell projection assembly | | | | | 2.00 | |
| GO:0070925 | GO:0030031 | GO:0044782 | GO:0042384 | GO:0060271 | GO:0010927 | |
| embryonic morphogenesis | | | | | 1.97 | |
| GO:0043009 | GO:0009792 | GO:0001701 | GO:0009790 | GO:0048568 | GO:0048598 | |
| histone modification | | | | | 1.87 | |
| GO:0016570 | GO:0018205 | GO:0018394 | GO:0018393 | GO:0016573 | GO:0006475 | GO:0006473 |
| GO:0043543 | GO:0043967 | | | | | |
| cell differentiation | | | | | 1.80 | |
| GO:0044767 | GO:0032502 | GO:0048856 | GO:0007275 | GO:0044707 | GO:0048731 | GO:0007399 |
| GO:0048513 | GO:0048869 | GO:0048468 | GO:0032501 | GO:0030154 | | |
| regulation of TOR signaling | | | | | 1.79 | |
| GO:0032006 | GO:0031929 | GO:0032008 | GO:0032007 | | | |
| neural precursor cell proliferation | | | | | 1.76 | |
| GO:0072089 | GO:2000648 | GO:0061351 | GO:1902692 | GO:0007405 | GO:0002052 | GO:0072091 |
| GO:2000177 | GO:2000179 | | | | | |
| DNA-templated transcription | | | | | 1.74 | |
| GO:0010468 | GO:0051171 | GO:0034645 | GO:0010467 | GO:0009059 | GO:0090304 | GO:0044260 |
| GO:0006807 | GO:0060255 | GO:0034641 | GO:0031323 | GO:0016070 | GO:0044249 | GO:2000112 |
| GO:1901576 | GO:0019222 | GO:0008152 | GO:0044237 | GO:0010556 | GO:0071704 | GO:0043170 |
| GO:0031326 | GO:1901360 | GO:0009058 | GO:0046483 | GO:0044271 | GO:0080090 | GO:0006351 |
| GO:0006139 | GO:0009889 | GO:0006725 | GO:0051252 | GO:0019219 | GO:0044238 | GO:0006366 |
| GO:0006355 | GO:0097659 | GO:1903506 | GO:2001141 | GO:1901362 | GO:0018130 | GO:0019438 |
| GO:0006357 | GO:0032774 | GO:0034654 | | | | |
| positive regulation of cellular component biogenesis | | | | | 1.69 | |
| GO:0033043 | GO:0010638 | GO:0044087 | GO:0044089 | GO:0051128 | GO:0051130 | |
| neuronal stem cell division | | | | | 1.68 | |
| GO:0048103 | GO:0017145 | GO:0021846 | GO:0036445 | GO:0055057 | GO:0021873 | GO:0048103 |
| positive regulation of stress-activated MAPK cascade | | | | | 1.63 | |
| GO:0043506 | GO:0032872 | GO:0070302 | GO:0046328 | GO:0032874 | GO:0070304 | GO:0051403 |
| GO:0007254 | GO:0031098 | GO:0043507 | GO:0046330 | GO:0080135 | GO:0043405 | GO:0043406 |
| GO:0043410 | GO:0007257 | | | | | |
| regulation of cytoskeleton organization | | | | | 1.57 | |
| GO:0051493 | GO:0051493 | GO:0051493 | GO:0051493 | | | |
| ribonucleoprotein complex assembly | | | | | 1.54 | |
| GO:0000245 | GO:0022618 | GO:0071826 | GO:0022613 | | | |
| phosphatidylcholine biosynthesis | | | | | 1.50 | |
| GO:0046470 | GO:0006656 | GO:0097164 | | | | |
| positive regulation of NF-kappaB transcription factor activity | | | | | 1.49 | |
| GO:0051091 | GO:0051090 | GO:0051092 | | | | |
| protein localization to microtubule cytoskeleton | | | | | 1.41 | |
| GO:0072698 | GO:0044380 | GO:0035372 | GO:0071539 | | | |
| blastocyst development | | | | | 1.40 | |
| GO:0001832 | GO:0001833 | GO:0001824 | | | | |
| stress fiber assembly | | | | | 1.32 | |
| GO:0051492 | GO:0030038 | GO:0043149 | GO:0032231 | GO:0051493 | GO:0051496 | GO:0061572 |
| GO:0051017 | GO:0032233 | GO:0032970 | GO:0031032 | GO:0032956 | GO:0051495 | GO:0030029 |
| GO:0030036 | GO:0007015 | | | | | |

| hematopoietic stem cell proliferation | | | | | 1.24 | |
|---|---|---|---|---|---|---|
| GO:0071425 | GO:1902033 | GO:1902035 | | | | |
| **negative regulation of proteasomal protein catabolism** | | | | | 1.21 | |
| GO:1903051 | GO:1903363 | GO:1901799 | GO:0031330 | GO:0032435 | GO:0042177 | GO:0032434 |
| GO:0009895 | | | | | | |
| **nonmotile primary cilium assembly** | | | | | 1.20 | |
| GO:0045724 | GO:0035058 | GO:1902855 | GO:1902857 | | | |
| **sensory organ development** | | | | | 1.19 | |
| GO:0007423 | GO:0090596 | GO:0043583 | GO:0048839 | GO:0001654 | GO:0042471 | GO:0042472 |
| GO:0048562 | | | | | | |
| **negative regulation of cyclin-dependent protein kinase activity** | | | | | 1.17 | |
| GO:0045736 | GO:1904030 | GO:0008054 | GO:1904029 | GO:0000079 | GO:0071901 | |
| **neural tube formation** | | | | | 1.17 | |
| GO:0021915 | GO:0014020 | GO:0001841 | GO:0001843 | GO:0060606 | GO:0060562 | GO:0035148 |
| GO:0035239 | GO:0001838 | GO:0072175 | GO:0016331 | | | |
| **regulation of translation** | | | | | 1.16 | |
| GO:0010608 | GO:0006417 | GO:0034248 | GO:0006412 | GO:0043043 | GO:0043604 | GO:0006518 |
| GO:0043603 | GO:1901564 | GO:1901566 | | | | |
| **positive regulation of megakaryocyte differentiation** | | | | | 1.14 | |
| GO:0045654 | GO:0045652 | GO:0030219 | GO:0045639 | | | |
| **mRNA splicing** | | | | | 1.13 | |
| GO:0008380 | GO:0008380 | GO:0008380 | GO:0008380 | GO:0008380 | GO:0008380 | |
| **rRNA modification** | | | | | 1.12 | |
| GO:0000154 | GO:0031167 | GO:0001510 | GO:0070475 | | | |
| **histone deacetylation** | | | | | 1.10 | |
| GO:0016575 | GO:0006476 | GO:0035601 | GO:0070932 | GO:0098732 | | |
| **positive regulation of MAPK cascade** | | | | | 1.09 | |
| GO:0043412 | GO:0044260 | GO:0032874 | GO:0070304 | GO:0006468 | GO:0006464 | GO:0036211 |
| GO:0032268 | GO:0044267 | GO:0046330 | GO:0016310 | GO:0031399 | GO:1902531 | GO:0006793 |
| GO:0051246 | GO:0019220 | GO:0042325 | GO:0051174 | GO:0006796 | GO:0023014 | GO:0000165 |
| GO:0043408 | GO:0001932 | GO:0009967 | GO:0010647 | GO:0023056 | GO:0051338 | GO:0043549 |
| GO:0071900 | GO:0045859 | GO:0019538 | GO:0043405 | GO:0043406 | GO:0043410 | GO:0032270 |
| GO:0051347 | GO:0033674 | GO:1902533 | GO:0051247 | GO:0031401 | GO:0042327 | GO:0045860 |
| GO:0032147 | GO:0001934 | GO:0071902 | GO:0045937 | GO:0010562 | GO:0000187 | |
| **positive regulation of RNA biosynthesis** | | | | | 1.09 | |
| GO:0031323 | GO:0019222 | GO:0051173 | GO:0080090 | GO:0006366 | GO:0031328 | GO:0009891 |
| GO:0010628 | GO:0010557 | GO:0031325 | GO:0045935 | GO:0051254 | GO:0009893 | GO:0006357 |
| GO:1902680 | GO:1903508 | GO:0045893 | GO:0010604 | GO:0048518 | GO:0048522 | GO:0045944 |
| **regulation of mRNA splicing *via* spliceosome** | | | | | 1.08 | |
| GO:0043484 | GO:1903311 | GO:0048024 | GO:0050684 | GO:0000381 | | |
| **smoothened signaling pathway** | | | | | 1.06 | |
| GO:0045880 | GO:0021904 | GO:0007224 | GO:0008589 | GO:0060831 | GO:0021532 | GO:0009953 |
| **positive regulation of GTPase activity** | | | | | 1.06 | |
| GO:0044093 | GO:0043547 | GO:0051345 | GO:0043087 | GO:0043085 | GO:0051336 | GO:0050790 |
| GO:0065009 | | | | | | |
| **protein ubiquitination** | | | | | 1.06 | |
| GO:0070647 | GO:0032446 | GO:0016567 | | | | |
| **leptin-mediated signaling pathway** | | | | | 1.05 | |
| GO:0044321 | GO:0044320 | GO:0033210 | | | | |
| **negative regulation of cell cycle transition due to DNA damage** | | | | | 1.02 | |
| GO:0000075 | GO:0007049 | GO:0000278 | GO:1903047 | GO:1901987 | GO:0010564 | GO:0031570 |
| GO:0000077 | GO:0022402 | GO:0045786 | GO:1901988 | GO:0010389 | GO:0045930 | GO:0044770 |
| GO:0007093 | GO:1901991 | GO:0051726 | GO:0044839 | GO:0000086 | GO:0044772 | GO:0007346 |
| GO:1901990 | GO:0072331 | GO:0010948 | GO:0044773 | GO:0042770 | GO:0044774 | GO:0072395 |
| GO:0030330 | GO:0072401 | GO:0072422 | GO:2000134 | GO:0044783 | GO:0006977 | GO:1902807 |
| GO:1902403 | GO:1902400 | GO:1902402 | GO:0072413 | GO:0072431 | GO:1902806 | GO:0044843 |
| GO:0031571 | GO:0044819 | GO:0007050 | GO:0071158 | GO:0000082 | GO:2000045 | GO:0071156 |
| **regulation of cell cycle process** | | | | | 1.01 | |
| GO:0010564 | GO:0090068 | GO:0045787 | | | | |
| **G2/M DNA damage checkpoint** | | | | | 1.01 | |
| GO:1902749 | GO:0010389 | GO:0031572 | GO:1902750 | GO:0010972 | GO:0007095 | GO:0044818 |
| **mitotic sister chromatid cohesion** | | | | | 1.01 | |
| GO:0007064 | GO:0007063 | GO:0045876 | GO:0034088 | GO:0034086 | GO:0034091 | GO:0034182 |
| GO:0034184 | GO:0034093 | GO:0051984 | GO:0051785 | GO:0045840 | | |

<span style="background-color:#f8c6c6">**Recurrence-exclusive antigens and proteins exclusively represented by HLA class II peptides up-modulated at recurrence**</span>

| positive regulation of hydrolase activity | | | | | 2.25 | |
|---|---|---|---|---|---|---|
| GO:0043085 | GO:0044093 | GO:0051345 | GO:0050790 | GO:0065009 | GO:0051336 | GO:0043547 |
| GO:0043087 | | | | | | |
| **gene expression** | | | | | 1.97 | |
| GO:0034641 | GO:1903506 | GO:0006139 | GO:0006355 | GO:2001141 | GO:0090304 | GO:0044238 |
| GO:0051252 | GO:0046483 | GO:0019219 | GO:0006725 | GO:1901360 | GO:0071704 | GO:0008152 |
| GO:2000112 | GO:0010556 | GO:0006351 | GO:0097659 | GO:0044237 | GO:0051171 | GO:0010468 |
| GO:0006357 | GO:0006807 | GO:0016070 | GO:0043170 | GO:0034654 | GO:0060255 | GO:0032774 |
| GO:0010467 | GO:0006366 | GO:0044271 | GO:0009889 | GO:0031326 | GO:1901362 | GO:0018130 |
| GO:0044260 | GO:0019438 | GO:0080090 | GO:0019222 | GO:0009058 | GO:1901576 | GO:0031323 |
| GO:0009059 | GO:0034645 | GO:0044249 | GO:0009892 | GO:0010605 | GO:0031324 | GO:0048523 |
| GO:0048519 | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| mitotic cell cycle | | | | | 1.73 | |
| GO:0022402 | GO:0044772 | GO:0044770 | GO:1903047 | GO:0000075 | GO:1901990 | GO:0007049 |
| GO:0000278 | GO:1901987 | GO:0010564 | GO:0007067 | GO:0000280 | GO:0048285 | GO:0000070 |
| GO:0000819 | GO:0090068 | GO:0051726 | GO:0010948 | GO:0045787 | GO:0098813 | GO:0007346 |
| GO:0007059 | GO:0045786 | GO:1901991 | GO:1901988 | GO:0007093 | GO:0045930 | GO:0051301 |
| GO:0007062 | | | | | | |
| response to xenobiotic stimulus | | | | | 1.54 | |
| GO:0006805 | GO:0071466 | GO:0009410 | | | | |
| regulation of metaphase/anaphase transition | | | | | 1.43 | |
| GO:0030071 | GO:1902099 | GO:0010965 | GO:0051784 | GO:0051983 | GO:0044784 | GO:0045839 |
| GO:0051306 | GO:0000070 | GO:0000819 | GO:0007091 | GO:0033047 | GO:0051304 | GO:0031577 |
| GO:0007094 | GO:0045841 | GO:0071174 | GO:2000816 | GO:0071173 | GO:1902100 | GO:0033048 |
| GO:0051783 | GO:0033045 | GO:0033046 | GO:0051985 | GO:0007088 | GO:1901991 | GO:1901988 |
| GO:0007093 | GO:0033044 | GO:0010639 | GO:2001251 | | | |
| positive regulation of G1/S phase transition | | | | | 1.42 | |
| GO:1901992 | GO:0090068 | GO:1901989 | GO:1900087 | GO:0007089 | GO:1902808 | GO:0045931 |
| chromatin organization | | | | | 1.36 | |
| GO:0016570 | GO:0016569 | GO:0051276 | GO:0006325 | GO:0018205 | | |
| cerebellum morphogenesis | | | | | 1.32 | |
| GO:0022037 | GO:0030902 | GO:0021549 | GO:0021695 | GO:0021696 | GO:0021575 | GO:0021587 |
| GO:0021533 | GO:0021697 | GO:0021692 | GO:0021702 | GO:0021694 | GO:0021680 | |
| DNA repair | | | | | 1.30 | |
| GO:0006974 | GO:0006259 | GO:0006281 | | | | |
| polyketide metabolism | | | | | 1.21 | |
| GO:0071395 | GO:0009753 | GO:1901661 | GO:0044598 | GO:0044597 | GO:0030638 | GO:0030647 |
| GO:0042448 | GO:0016137 | GO:0019748 | GO:0008207 | GO:0001523 | | |
| triglyceride metabolism | | | | | 1.17 | |
| GO:0006639 | GO:0006638 | GO:0006641 | GO:0046486 | | | |
| negative regulation of DNA-templated transcription | | | | | 1.13 | |
| GO:0006357 | GO:0006366 | GO:0000122 | GO:0045934 | GO:0051253 | GO:1902679 | GO:0051172 |
| GO:1903507 | GO:0009892 | GO:0031327 | GO:0045892 | GO:0010558 | GO:0010605 | GO:0009890 |
| GO:0031324 | GO:0010629 | GO:2000113 | | | | |
| proton-transporting ATP synthase complex assembly | | | | | 1.12 | |
| GO:0043461 | GO:0070272 | GO:0070071 | | | | |
| activation of phospholipase activity | | | | | 1.05 | |
| GO:0007202 | GO:0060193 | GO:1900274 | GO:0060191 | GO:0010518 | GO:0010863 | GO:0010517 |
| negative regulation of muscle cell differentiation | | | | | 1.04 | |
| GO:0051153 | GO:0051154 | GO:0051148 | GO:0051147 | GO:0010832 | GO:0010830 | |
| behavioral defense response | | | | | 1.03 | |
| GO:0042596 | GO:0033555 | GO:0001662 | GO:0002209 | | | |
| positive regulation of pri-miRNA transcription | | | | | 1.01 | |
| GO:1902895 | GO:1902893 | GO:0061614 | | | | |
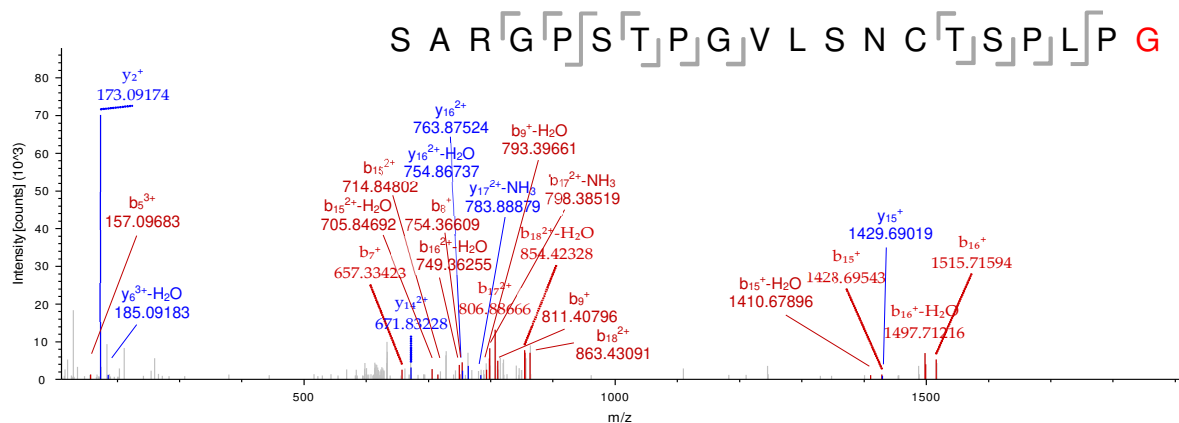


**Supplementary Figure 5. Fragment spectrum of candidate neo-antigenic HLA class II-restricted peptide derived from mutant ATG9B (ENST00000377974 / ENSG00000181652 c.2294A>G; UPI00015E055A p.756E>G) detected in patient ZH750.** The mutated amino acid is marked in red within the sequence, whereas b and y ions are indicated as red and blue peaks in the fragment spectrum, respectively. SARGPSTPGVLSNCTSPLPG was identified with 2.8% FDR (q value = 0.028) and on rank 1 by MHCquant, but annotated on rank 2 by SEQUEST and was therefore excluded from further validation steps.

**Supplementary Table 9. Effect of the p.343P>T mutation in patient ZH681 on the phosphorylation state of PDGFRα.** Using the PhosphoMotif Finder of the Human Protein Reference Database, the presence of phosphorylation motifs in PDGFRα was assessed for the WT sequence, the p.343P>T mutant version of patient ZH681, and the p.343P>S variant listed in the COSMIC database for two glioblastoma patients. Neo-phosphorylated tyrosine, threonine, and serine residues were subsequently investigated for being part of a neo-phosoho binding motif for proteins. Listed phosphorylation motifs and binding sites refer to mutated position 343 plus the first site before and after the mutation.

| Position in PDGFRα | Sequence in PDGFRα Mutated AA in red | Corresponding motif Phosphorylated AA in blue | Features of motif |
|---|---|---|---|
| **Tyrosine phosphorylation motifs** | | | |
| **WT PDGFRα** | | | |
| 288-289 | AE | pY[A/G/S/T/E/D] | Src kinase substrate motif |
| 372-375 | EIRY | [E/D]XXpY | ALK kinase substrate motif |
| **ZH681 PDGFRα p.343P>T: 1 neo-phosphorylation motif** | | | |
| 288-289 | AE | pY[A/G/S/T/E/D] | Src kinase substrate motif |
| 342-343 | YT | pY[A/G/S/T/E/D] | Src kinase substrate motif |
| 372-375 | EIRY | [E/D]XXpY | ALK kinase substrate motif |
| **COSMIC PDGFRα p.343P>S: 1 neo-phosphorylation motif** | | | |
| 288-289 | AE | pY[A/G/S/T/E/D] | Src kinase substrate motif |
| 342-343 | YS | pY[A/G/S/T/E/D] | Src kinase substrate motif |
| 372-375 | EIRY | [E/D]XXpY | ALK kinase substrate motif |
| **Serine / threonine phosphorylation motifs** | | | |
| **WT PDGFRα** | | | |
| 322-325 | SQLE | [pS/pT]XX[E/D] | Casein kinase II substrate motif |
| 346-348 | RIS | RXpS | PKA kinase substrate motif |
| **ZH681 PDGFRα p.343P>T: 8 neo-phosphorylation motifs** | | | |
| 322-325 | SQLE | [pS/pT]XX[E/D] | Casein kinase II substrate motif |
| 339-347 | VRAYTPPRI | [M/V/L/I/F][R/K/H]XX[pS/pT]XXX[M/V/L/I/F] | AMP-activated protein kinase substrate motif |
| 340-343 | RAYT | RXX[pS/pT] | Calmodulin-dependent protein kinase II substrate motif |
| 340-343 | RAYT | [R/K]XX[pS/pT] | PKC kinase substrate motif |
| 340-346 | RAYTPPR | RXX[pS/pT]XXR | CLK1 kinase substrate motif |
| 342-344 | YTP | X[pS/pT]P | GSK-3, ERK1, ERK2, CDK5 substrate motif |
| 343-346 | TPPR | [pS/pT]PX[R/K] | CDK1, 2, 4, 6 kinase substrate motif |
| 343-346 | TPPR | [pS/pT]PX[R/K] | Growth associated histone HI kinase substrate motif |
| 343-346 | TPPR | [pS/pT]PX[R/K] | Cdc2 kinase substrate motif |
| 346-348 | RIS | RXpS | PKA kinase substrate motif |
| **COSMIC PDGFRα p.343P>S: 13 neo-phosphorylation motifs, 1 neo-phosphatase motif** | | | |
| 322-325 | SQLE | [pS/pT]XX[E/D] | Casein kinase II substrate motif |
| 339-347 | VRAYSPPRI | [M/L/V/I/F][R/K/H]XXSXXX[M/L/V/I/F] | HMGCoA reductase kinase substrate motif |
| 339-347 | VRAYSPPRI | [M/L/V/I/F][R/K/H]XX[pS/pT]XXX[M/L/V/I/F] | AMP-activated protein kinase substrate motif |
| 340-343 | RAYS | RXXpS | Calmodulin-dependent protein kinase II substrate motif |
| 340-343 | RAYS | RXXpS | PKA kinase substrate motif |
| 340-343 | RAYS | RXX[pS/pT] | Calmodulin-dependent protein kinase II substrate motif |
| 340-343 | RAYS | [R/K]XX[pS/pT] | PKC kinase substrate motif |
| 340-346 | RAYSPPR | RXX[pS/pT]XXR | CLK1 kinase substrate motif |
| 341-344 | AYSP | XXpSP | GSK-3, ERK1, ERK2, CDK5 substrate motif |
| 342-344 | YSP | X[pS/pT]P | GSK-3, ERK1, ERK2, CDK5 substrate motif |
| 343-344 | SP | pSP | ERK1, ERK2 kinase substrate motif |
| 343-346 | SPPR | [pS/pT]PX[R/K] | CDK1, 2, 4, 6 kinase substrate motif |
| 343-346 | SPPR | [pS/pT]PX[R/K] | Growth associated histone HI kinase substrate motif |
| 343-346 | SPPR | [pS/pT]PX[R/K] | Cdc2 kinase substrate motif |
| 343-347 | SPPRI | pSPX[R/K]X | CDK kinase substrate motif |
| 346-348 | RIS | RXpS | PKA kinase substrate motif |

| **Phospho-tyrosine binding motifs** | | | |
|---|---|---|---|
| **WT PDGFRα: p.342Y not phosphorylated** | | | |
| 136-139 | YLVI | **pY**[M/I/L/V]X[M/I/L/V] | GRB2, 3BP2, Csk, Fes, Syk C-terminal SH2 domain binding motif |
| 555-557 | YEI | **pY**[E/M/V][N/V/I] | 3BP2 SH2 domain binding motif |
| **ZH681 PDGFRα p.343P>T: 2 neo-phospho-tyrosine binding motifs** | | | |
| 136-139 | YLVI | **pY**[M/I/L/V]X[M/I/L/V] | GRB2, 3BP2, Csk, Fes, Syk C-terminal SH2 domain binding motif |
| 342-345 | Y**T**PP | **pY**XXP | Crk SH2 domain binding motif |
| 342-345 | Y**T**PP | **pY**XXP | RasGAP C-terminal SH2 domain binding motif |
| 555-557 | YEI | **pY**[E/M/V][N/V/I] | 3BP2 SH2 domain binding motif |
| **COSMIC PDGFRα p.343P>S: 2 neo-phospho-tyrosine binding motifs** | | | |
| 136-139 | YLVI | **pY**[M/I/L/V]X[M/I/L/V] | GRB2, 3BP2, Csk, Fes, Syk C-terminal SH2 domain binding motif |
| 342-345 | Y**S**PP | **pY**XXP | Crk SH2 domain binding motif |
| 342-345 | Y**S**PP | **pY**XXP | RasGAP C-terminal SH2 domain binding motif |
| 555-557 | YEI | **pY**[E/M/V][N/V/I] | 3BP2 SH2 domain binding motif |
| **Phospho-serine / -threonine binding motifs** | | | |
| **WT PDGFRα** | | | |
| 157-158 | TP | [**pS**/**pT**]P | WW domain binding motif |
| 374-377 | RYRS | RXX**pS** | 14-3-3 domain binding motif |
| **ZH681 PDGFRα p.343P>T: 1 neo-phospho-threonine binding motif** | | | |
| 157-158 | TP | [**pS**/**pT**]P | WW domain binding motif |
| 343-344 | **T**P | [**pS**/**pT**]P | WW domain binding motif |
| 374-377 | RYRS | RXX**pS** | 14-3-3 domain binding motif |
| **COSMIC PDGFRα p.343P>S: 2 neo-phospho-serine binding motifs** | | | |
| 157-158 | TP | [**pS**/**pT**]P | WW domain binding motif |
| 340-343 | RAYS | RXX**pS** | 14-3-3 domain binding motif |
| 343-344 | SP | [**pS**/**pT**]P | WW domain binding motif |
| 374-377 | RYRS | RXX**pS** | 14-3-3 domain binding motif |

## *Supplement of CHAPTER 3*

**Supplementary Table 10. HLA class I allotype and allele frequencies in the medulloblastoma collective comprising n=28 patients.** The top three ranking HLA-A, -B, and -C allotypes within the study cohort are marked in bold.

| HLA allotype | Positive patients | Allele frequency | HLA allotype | Positive patients | Allele frequency |
|---|---|---|---|---|---|
| A*01:01 | 14% | 7% | B*37:01 | 4% | 2% |
| **A*02:01** | **39%** | 21% | B*38:01 | 4% | 2% |
| A*02:02 | 4% | 2% | B*40:02 | 7% | 4% |
| A*02:05 | 4% | 2% | B*41:01 | 4% | 2% |
| A*02:11 | 4% | 2% | B*41:02 | 4% | 2% |
| **A*03:01** | **32%** | 16% | **B*44:02** | **25%** | 13% |
| **A*11:01** | **21%** | 11% | **B*44:03** | **11%** | 5% |
| A*24:02 | 14% | 7% | B*44:05 | 4% | 2% |
| A*25:01 | 7% | 4% | B*49:01 | 4% | 2% |
| A*26:01 | 11% | 5% | B*50:01 | 7% | 4% |
| A*29:02 | 7% | 4% | **B*51:01** | **14%** | 9% |
| A*30:01 | 4% | 2% | B*52:01 | 7% | 4% |
| A*31:01 | 7% | 4% | **B*57:01** | **11%** | 5% |
| A*32:01 | 7% | 4% | B*58:01 | 4% | 2% |
| A*68:01 | 14% | 7% | C*01:02 | 11% | 5% |
| A*68:02 | 4% | 2% | **C*02:02** | **18%** | 9% |
| A*80:01 | 4% | 2% | C*03:03 | 4% | 2% |
| **B*07:02** | **11%** | 5% | **C*04:01** | **21%** | 13% |
| B*08:01 | 7% | 4% | C*05:01 | 14% | 9% |
| **B*13:02** | **11%** | 5% | **C*06:02** | **32%** | 16% |
| B*15:01 | 4% | 2% | **C*07:01** | **21%** | 11% |
| B*15:08 | 4% | 2% | C*07:02 | 11% | 5% |
| B*15:18 | 4% | 2% | C*07:04 | 14% | 7% |
| **B*18:01** | **11%** | 5% | C*12:02 | 7% | 4% |
| B*27:02 | 4% | 2% | C*12:03 | 7% | 4% |
| **B*27:05** | **11%** | 5% | C*14:02 | 7% | 4% |
| **B*35:01** | **11%** | 5% | C*15:02 | 11% | 5% |
| B*35:02 | 4% | 2% | C*16:01 | 7% | 4% |
| B*35:03 | 7% | 4% | C*16:04 | 4% | 2% |
| B*35:08 | 4% | 2% | C*17:01 | 4% | 2% |

**frequency of positive medulloblastoma patients [%]**

0          20          40
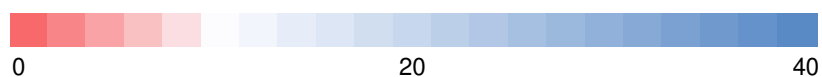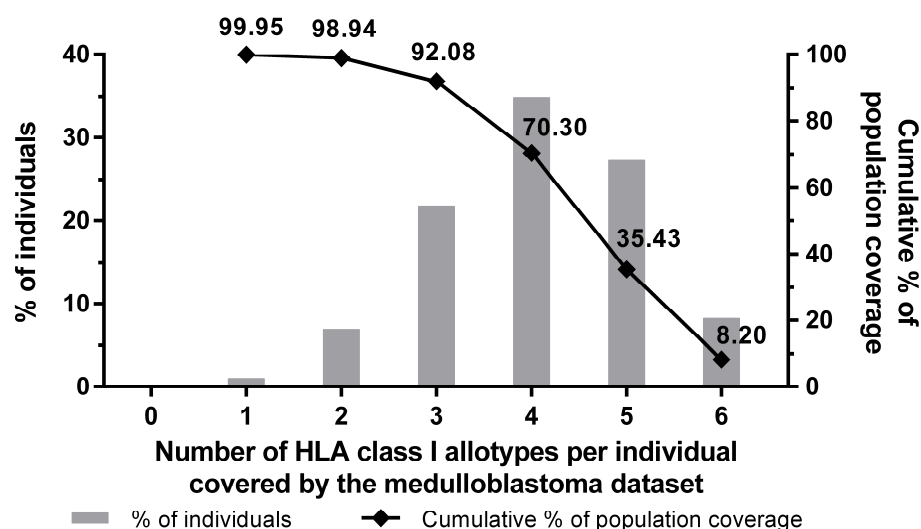
**Supplementary Figure 6. HLA class I allotype population coverage.** Using the population coverage tool provided by the IEDB Analysis Resource, the world population coverage of the 60 distinct HLA-A, -B, and -C allotypes was calculated. The percentage of individuals positive for a specific number of HLA class I allotypes (max. of 6) is indicated by bar charts (associated with the left y-axis). The line diagram (associated with the right y-axis) shows the cumulative percentage of population coverage. The HLA class I allotypes of the medulloblastoma cohort cover 99.95% of the world population (first diamond on line diagram counted from the left) meaning that only 0.05% of all individuals are negative for all HLA-A, -B, and -C allotypes included in the present study (first bar counted from the left). 92.08% of all individuals are positive for at least three HLA class I allotypes (third diamond on line diagram counted from the left).

**Supplementary Table 11. Medulloblastoma-associated HLA class I- and II-presented antigens identified on at least three tumors.** GTEx profiles were assessed from all available datasets excepting EBV-transformed lymphocytes and cultured fibroblasts. Medulloblastoma-exclusive antigens with a brain-specific expression profile > 10 TPM were not reported (HLA class I: n=13; HLA class II: n=6) as candidate targets. Color codes were defined as follows: ■ < 10 TPM in any tissue, ♦ > 10 TPM in testes and < 10 TPM in other tissues (CTA-like expression profile), ■ 10-20 TPM in any tissue, ■ 20-30 TPM in any tissue, ■ > 30 TPM in any tissue. The number of positive tumors other than medulloblastoma was based on n=874 HLA class I and n=626 HLA class II peptidome datasets. HLA restrictions not passing manual assessment as quality control are indicated in italic. These peptides were excluded from downstream analyses such as calculation of peptides matching per patient worldwide. HLA class II-presented proteins neither identified with peptides exceeding a length of twelve AA nor with different sequences across patients were not considered for this listing of candidate target antigens. SYCP3 was detected with only one HLA class II peptide sequence across all patients. To indicate the distribution of antigens across subgroups and age classes, the following abbreviations were used: WNT-activated (W), SHH-activated (S), non-WNT/non-SHH (including those subclassified in Group 3 or Group 4; N), childhood medulloblastoma (≤ 15 years; C), adult medulloblastoma (≥ 25 years; A).

| Antigen | Frequency of positive patients | Peptide sequence | HLA restriction | UniProt accession GTEx profile | Positive non-MB tumors |
|---|---|---|---|---|---|
| **Medulloblastoma-associated HLA class I antigens** | | | | | |
| Serine protease inhibitor Kazal-type 8 (SPINK8) | 29% / NSW / C | | | P0C7L1 ■ | 5 |
| | HH-06 | ILFEGLNITK | A*11:01 | | |
| | SA2 | ILFEGLNITK | A*11:01 | | |
| | SA5 | ILFEGLNITK | A*11:01 | | |
| | Wü-N998/13 | ILFEGLNITK | A*03:01 | | |
| | ZH513 | ILFEGLNITK | A*03:01 | | |
| | ZH713 | ILFEGLNITK | A*03:01 | | |
| | ZH732 | ILFEGLNITK | A*03:01 | | |
| | ZH872 | ILFEGLNITK | A*03:01 | | |

| Protein | Sample | Peptide | HLA | Accession | | Count |
|---|---|---|---|---|---|---|
| Protein Wnt-5a (WNT5A) | 25% / NSW / AC | | | P41221 | 🟨 | 51 |
| | HH-01 | AMSSKFFLV | A*02:01 | | | |
| | Wü-N391/17 | AMSSKFFLV | A*02:01 | | | |
| | ZH703 | AMSSKFFLV | A*02:01 | | | |
| | ZH718 | AMSSKFFLV | A*02:01 | | | |
| | ZH741 | AMSSKFFLV | A*02:01 | | | |
| | ZH868 | AMSSKFFLV | A*02:01 | | | |
| | ZH937 | AMSSKFFLV | A*02:01 | | | |
| Protein shisa-9 (SHISA9) | 18% / N / C | | | B4DS77 | 🟩 | 11 |
| | HH-02 | KVNDDFYTK | A*11:01 | | | |
| | HH-06 | KVNDDFYTK | A*11:01 | | | |
| | SA2 | KVNDDFYTK | A*11:01 | | | |
| | ZH513 | KVNDDFYTK | A*03:01; A*11:01 | | | |
| | ZH732 | KMPPHPLAY | A*80:01 | | | |
| Insulinoma-associated protein 2 (INSM2) | 14% / NS / AC | | | Q96T92 | 🟩 | 1 |
| | HH-06 | STFFSSPGLTR | A*11:01 | | | |
| | SA2 | STFFSSPGLTR | A*11:01 | | | |
| | ZH872 | ESFPGGAAAV | A*68:02 | | | |
| | ZH913 | STFFSSPGLTR | A*68:01 | | | |
| Transcription factor HES-4 (HES4) 1 peptide multi-maps to non-MB-exclusive HES1 (Q14469) and HES5 (Q5TA89) | 14% / NS / C | | | Q9HCC6 | 🟥 | 19 |
| | HH-06 | LEKADILEM | B*41:02 | | | |
| | ZH703 | RINESLAQLK | A*03:01 | | | |
| | ZH872 | RINESLAQLK | A*03:01 | | | |
| | ZH890 | SSKPVMEKR | A*31:01 | | | |
| Solute carrier family 22 member 1 (SLC22A1) peptides multi-map to non-MNG-exclusive SLC22A2 (O15244) | 14% / N / C | | | O15245 | 🟥 | 7 |
| | Wü-N526/17 | RLIQGLVSK | A*03:01 | | | |
| | ZH703 | RLIQGLVSK | A*03:01 | | | |
| | ZH513 | RLLQGLVSK | A*03:01 | | | |
| | ZH732 | RLLQGLVSK | A*03:01 | | | |
| Insulin-like growth factor-binding protein-like 1 (IGFBPL1) | 14% / NS / C | | | Q8WX77 | 🟩 | 24 |
| | HH-02 | FPAPDDRM | B*35:01 | | | |
| | ZH513 | STVTVLDLSK | A*11:01 | | | |
| | ZH859 | FPAPDDRM | B*35:02 | | | |
| | ZH872 | RAVPTPVITW | B*57:01 | | | |
| Mitochondrial coenzyme Q-binding protein COQ10 homolog A (COQ10A) | 14% / N / C | | | Q96MF6 | 🟥 | 16 |
| | HH-06 | ATMFFDEVVK | A*11:01 | | | |
| | SA5 | EVVSNVQEY | A*26:01 | | | |
| | Wü-N526/17 | EVVSNVQEY | A*26:01 | | | |
| | ZH713 | EVVSNVQEY | A*26:01 | | | |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4-like 2 (NDUFA4L2) | 14% / NSW / AC | | | Q9NRX3 | 🟥 | 77 |
| | Wü-N391/17 | DQYKFLAV | B*52:01 | | | |
| | Wü-N998/13 | DQYKFLAV | B*51:01 | | | |
| | | GASLGARFY | A*29:02 | | | |
| | ZH890 | AGASLGARFY | A*01:01 | | | |
| | | AGASLGARFYR | A*31:01 | | | |
| | | ASLGARFYR | A*31:01 | | | |
| | ZH913 | GASLGARFY | A*29:02 | | | |
| Neuronal migration protein doublecortin (DCX) 1 peptide multi-maps to non-MB-exclusive doublecortin-like kinase 1/2 (DCLK1/2; O15075/Q8N568) | 11% / NS / C | | | O43602 | 🟩 | 13 |
| | SA2 | GTSSSQLSTPK | A*11:01 | | | |
| | | SLDDSDSLGDSM | *A*01:01* | | | |
| | ZH703 | LLADLTRSL | A*02:02; *B*13:02* | | | |
| | ZH859 | TAHSFEQVL | B*35:02 | | | |
| Oligodendrocyte transcription factor 3 (OLIG3) 1 peptide multi-maps to MB-exclusive OLIG2 (Q13516) with brain-associated expression | 11% / NS / AC | | | Q7RTU3 | 🟩 | 20 |
| | HH-06 | EVMPYAHGPSVR | A*68:01 | | | |
| | Wü-N526/17 | RLSAESKDLLK | A*03:01 | | | |
| | ZH913 | EVMPYAHGPSVR | A*68:01 | | | |
| BTB/POZ domain-containing protein 17 (BTBD17) | 11% / NSW / AC | | | A6NE02 | 🟩 | 8 |
| | HH-06 | AVFDKFIRY | A*11:01 | | | |
| | Wü-N998/13 | AVFDKFIRY | A*03:01; A*29:02 | | | |
| | ZH913 | AVFDKFIRY | A*29:02 | | | |
| Embryonal Fyn-associated substrate (EFS) | 11% / NSW / AC | | | O43281 | 🟥 | 21 |
| | Wü-N998/13 | STGDLQLLY | A*03:01; A*29:02 | | | |
| | ZH732 | STGDLQLLY | A*80:01; C*02:02 | | | |
| | ZH913 | AESPQELSF | B*44:02; B*44:03 | | | |
| | | STGDLQLLY | A*29:02 | | | |
| Vacuolar ATPase assembly integral membrane protein VMA21 (VMA21) | 11% / SW / C | | | Q3ZAQ7 | 🟨 | 18 |
| | Wü-N998/13 | MSNRDSYFY | A*29:02 | | | |
| | ZH868 | STLKTLLFF | B*57:01 | | | |
| | ZH872 | STLKTLLFF | B*57:01 | | | |

| | | | | | |
|---|---|---|---|---|---|
| SERTA domain-containing protein 4 (SERTAD4) | <u>11% / SW / AC</u> | | | Q9NUC0 ■ | 18 |
| | Wü-N998/13 | FYDYFETGY | A*29:02; C*14:02 | | |
| | ZH872 | GISNPITTSK | A*03:01 | | |
| | ZH913 | FYDYFETGY | A*29:02 | | |

**Medulloblastoma-associated HLA class II antigens**

| | | | | | |
|---|---|---|---|---|---|
| Insulin-like growth factor-binding protein-like 1 (IGFBPL1) | <u>36% / NS / AC</u> | | | Q8WX77 ■ | 7 |
| | HH-03 | AVPTPVITWRKVTKSPE | | | |
| | | VPTPVITWRKVTKSPE | | | |
| | | VPTPVITWRKVTKSPEG | | | |
| | SA2 | ATAWILINPLRKED | | | |
| | | ATAWILINPLRKEDE | | | |
| | | GPSDHEATAWILINPLRKEDE | | | |
| | | GPSDHEATAWILINPLRKEDEG | | | |
| | SA3 | AVPTPVITWRKVTKSPEGTQ | | | |
| | | PTPVITWRKVTKSPEG | | | |
| | | TPVITWRKVTKSPEG | | | |
| | | TPVITWRKVTKSPEGTQ | | | |
| | | VPTPVITWRKVTKSPE | | | |
| | | VPTPVITWRKVTKSPEG | | | |
| | | VPTPVITWRKVTKSPEGT | | | |
| | | VPTPVITWRKVTKSPEGTQ | | | |
| | | VRAVPTPVITWRKVTKSPEGTQA | | | |
| | ZH513 | AVPTPVITWRKVTKSPE | | | |
| | | AVPTPVITWRKVTKSPEG | | | |
| | | VPTPVITWRKVTKSPE | | | |
| | | VPTPVITWRKVTKSPEG | | | |
| | ZH713 | VPTPVITWRKVTKSPE | | | |
| | ZH718 | VPTPVITWRKVTKSPE | | | |
| | ZH732 | AVPTPVITWRKVTKSPE | | | |
| | | AVPTPVITWRKVTKSPEG | | | |
| | | PTPVITWRKVTKSPE | | | |
| | | RAVPTPVITWRKVTKSPEG | | | |
| | | TPVITWRKVTKSPE | | | |
| | | VPTPVITWRKVTK | | | |
| | | VPTPVITWRKVTKSP | | | |
| | | VPTPVITWRKVTKSPE | | | |
| | | VPTPVITWRKVTKSPEG | | | |
| | ZH868 | LLLPLLPPLSP | | | |
| | ZH872 | DLSKYRSFHFPAPDD | | | |
| | | DLSKYRSFHFPAPDDR | | | |
| | | DLSKYRSFHFPAPDDRM | | | |
| | | KYRSFHFPAPDD | | | |
| | | LSKYRSFHFPAPDD | | | |
| | | LSKYRSFHFPAPDDR | | | |
| | | LSKYRSFHFPAPDDRM | | | |
| | | SHSTVTVLDLSKYRSFHFPAPDDRM | | | |
| | | SKYRSFHFPAPDD | | | |
| | | SKYRSFHFPAPDDR | | | |
| | | SKYRSFHFPAPDDRM | | | |
| | | STVTVLDLSKYRSFHFPAPDDRM | | | |
| | | VLDLSKYRSFHFPAPDD | | | |
| | | VLDLSKYRSFHFPAPDDRM | | | |
| | | VTVLDLSKYRSFHFPAPDDRM | | | |
| | ZH913 | ATAWILINPLRKED | | | |
| | | GPSDHEATAWILINPLRKEDE | | | |
| | | GPSDHEATAWILINPLRKEDEG | | | |
| | | LPGDHVNIAVQVRGGPS | | | |
| E3 SUMO-protein ligase CBX4 (CBX4) | <u>21% / NSW / AC</u> | | | O00257 ■ | 10 |
| | HH-02 | GIGGKMKIVKNKNKNGRIVIVMSKY | | | |
| | SA5 | RERQEQLMGYRKRGPK | | | |
| | Wü-N998/13 | GIGGKMKIVKNKNKNGRIVIVMSKY | | | |
| | ZH513 | IVKNKNKNGRIVIVMSKY | | | |
| | ZH718 | GIGGKMKIVKNKNKNGRIVIVMSKY | | | |
| | ZH913 | GIGGKMKIVKNKNKNGRIVIVMSKY | | | |
| N-acetyltransferase ESCO1 (ESCO1) | <u>21% / NSW / AC</u> | | | Q5FWF5 ■ | 17 |
| | HH-02 | KVLEVKSDSKEDENLVINEVINSPK | | | |
| | HH-05 | WVFSMMRRKKIASRMIECLRSNFIY | | | |
| | ZH713 | KVLEVKSDSKEDENLVINEVINSPK | | | |
| | ZH718 | KVLEVKSDSKEDENLVINEVINSPK | | | |
| | ZH859 | KVLEVKSDSKEDENLVINEVINSPK | | | |
| | ZH937 | KVLEVKSDSKEDENLVINEVINSPK | | | |
| Synaptonemal complex protein 3 (SYCP3) | <u>18% / NS / C</u> | | | Q8IZU3 ♦ | 2 |
| | HH-02 | KASLKTSNQKIEHVWKTQQDQRQKL | | | |
| | Wü-N526/17 | KASLKTSNQKIEHVWKTQQDQRQKL | | | |
| | ZH713 | KASLKTSNQKIEHVWKTQQDQRQKL | | | |
| | ZH732 | KASLKTSNQKIEHVWKTQQDQRQKL | | | |
| | ZH859 | KASLKTSNQKIEHVWKTQQDQRQKL | | | |

| Antigen | Frequency | Sample | Peptide sequence | UniProt | Benign |
|---|---|---|---|---|---|
| Thyroid transcription factor 1-associated protein 26 (CCDC59) | 18% / SW / AC | | | Q9P031 ■ | 3 |
| | | HH-01 | RKLKIQQSYKKLL | | |
| | | SA2 | RKLKIQQSYKKLL | | |
| | | Wü-N998/13 | RKLKIQQSYKKLL | | |
| | | | RRKLKIQQSYKKLL | | |
| | | | VREGQGFAFRRKLKIQQSYKKLL | | |
| | | ZH868 | RRKLKIQQSYKKLL | | |
| | | ZH913 | RRKLKIQQSYKKLL | | |
| Protein LLP homolog (LLPH) | 14% / NSW / C | | | Q9BRT6 ■ | 5 |
| | | HH-04 | AKSLRSKWKRKMRAEKRKK | | |
| | | HH-05 | AKSLRSKWKRKMRAEKRKK | | |
| | | SA1 | KNAPKEASRLKSILK | | |
| | | ZH868 | KNAPKEASRLKSILK | | |
| Platelet-activating factor acetylhydrolase (PLA2G7) | 11% / NS / C | | | Q13093 ■ | 30 |
| | | ZH680 | KASLAFLQKHLGLHK | | |
| | | ZH718 | KASLAFLQKHLGLHK | | |
| | | | NKASLAFLQKHLGLHK | | |
| | | ZH919 | KASLAFLQKHLGLHK | | |
| Protocadherin-20 (PCDH20) | 11% / NS / AC | | | Q8N6Y1 ■ | 5 |
| | | ZH703 | KGEKHPREDENLEVQIPLKGKIDLH | | |
| | | ZH913 | KPMDYELQQFYEVAVVAWNSE | | |
| | | ZH919 | IIKVIFRPPEIVPR | | |
| Something about silencing protein 10 (UTP3) | 11% / NW / C | | | Q9NQZ2 ■ | 5 |
| | | HH-02 | RRKKIDRNPRVKHREKFRRAKIRRR | | |
| | | SA1 | SGELSGIRAGVKKSIKLK | | |
| | | Wü-N998/13 | SGELSGIRAGVKKSIKL | | |
| CGG triplet repeat-binding protein 1 (CGGBP1) | 11% / NS / AC | | | Q9UFW8 ■ | 4 |
| | | SA2 | ADHPAVRAFLSRHVKN | | |
| | | ZH680 | DHPAVRAFLSRHVKN | | |
| | | ZH913 | DHPAVRAFLSRHVKN | | |

**Supplementary Table 12. Medulloblastoma-associated HLA class I ligands presented on at least five tumors.** Peptides already reported to derive from medulloblastoma-associated antigens were excluded from this listing. In turn, some of those antigens not designated as medulloblastoma-associated due to a CNS-associated expression profile are listed herein with medulloblastoma-associated peptides. The number of positive tumors other than medulloblastoma was based on n=874 HLA class I peptidome datasets. HLA restrictions not passing manual assessment as quality control are indicated in italic. These combinations of sequence and HLA restriction were excluded from downstream analyses such as calculation of peptides matching per patient worldwide. Peptides with an HLA restriction not covered by the CNS-related subset (brain, cerebellum, and spinal cord) of the benign database were excluded as well, since it is possible that these are false positive tumor-exclusive peptides arising owing to insufficient depth of the benign dataset (marked in grey). To indicate the distribution of antigens across subgroups and age classes, the following abbreviations were used: WNT-activated (W), SHH-activated (S), non-WNT/non-SHH (including those subclassified in Group 3 or Group 4; N), childhood medulloblastoma (≤ 15 years; C), adult medulloblastoma (≥ 25 years; A).

| Peptide sequence | HLA restriction | Frequency of positive patients | | Antigen (UniProt accession) | Protein frequency on medullo-blastomas | Peptide-positive non-MB tumors / Protein frequency on non-MB tumors | Protein frequency on benign samples (n=418) |
|---|---|---|---|---|---|---|---|
| ASYQEALARL | A*24:02 C*02:02 *C*04:01* C*16:01 C*17:01 | 29% HH-04 HH-06 Wü-N526/17 ZH513 | NSW / C HH-05 SA5 Wü-N998/13 ZH859 | Glial fibrillary acidic protein (GFAP; P14136) | 100% | 20 17% | 16% |
| GDRYDGMVGF | B*37:01 B*41:02 B*44:02 B*44:03 C*06:02 *C*07:01* C*07:02 | 25% HH-02 HH-05 SA2 Wü-N998/13 | NW / C HH-04 HH-06 SA4 | Heterogeneous nuclear ribonucleoprotein K (HNRNPK; P61978) | 75% | 2 68% | 64% |
| SAASLLLNR | A*03:01 A*11:01 A*68:01 | 25% HH-03 SA2 ZH513 ZH913 | NSW / AC HH-05 SA5 ZH872 | Sorting nexin-14 (SNX14; Q9Y5W7) | 39% | 3 16% | 16% |
| ALLDGRVQL | A*02:01 | 25% HH-01 SA3 ZH741 ZH937 | NSW / AC SA1 ZH718 ZH868 | Agrin (AGRN; O00468) | 46% | 43 19% | 11% |

| Peptide | HLA | | | Protein | | | |
|---|---|---|---|---|---|---|---|
| GSLNVTLEH | A*03:01<br>A*11:01<br>*A*80:01* | 25%<br>HH-02<br>SA2<br>Wü-N998/13<br>ZH732 | NW / C<br>HH-06<br>SA5<br>ZH513 | DNA (cytosine-5)-methyltransferase 3A (DNMT3A; Q9Y6K1) | 46% | 8<br>11% | 6% |
| LLYPVPLVH | A*03:01 | 25%<br>Wü-N526/17<br>ZH513<br>ZH732<br>ZH872 | NSW / C<br>Wü-N998/13<br>ZH703<br>ZH859 | Kinesin-like protein KIF1A (KIF1A; Q12756) | 57% | 19<br>18% | 15% |
| SVYSTPVFSQK | A*03:01<br>A*11:01 | 25%<br>HH-06<br>Wü-N998/13<br>ZH713<br>ZH872 | NSW / C<br>SA2<br>ZH703<br>ZH732 | Gamma-aminobutyric acid receptor subunit gamma-2 (GABRG2; P18507) | 25% | 1<br>1% | 0.5% |
| AAAGGAFPR | A*11:01<br>A*68:01 | 21%<br>HH-02<br>HH-06<br>SA2 | N / C<br>HH-03<br>SA1<br>ZH513 | Sortilin (SORT1; Q99523) | 29% | 6<br>6% | 5% |
| ATFYSSPGLTR | A*03:01<br>A*11:01<br>A*68:01 | 21%<br>HH-02<br>SA2<br>ZH872 | NS / AC<br>HH-06<br>ZH513<br>ZH913 | Insulinoma-associated protein 1 (INSM1; Q01101) | 21% | 3<br>1% | 0.2% |
| GLAEEVQRL | A*02:01<br>A*02:02<br>A*02:05<br>*B*13:02* | 21%<br>HH-02<br>Wü-N391/17<br>ZH718 | NS / C<br>HH-03<br>ZH703<br>ZH868 | Alpha-internexin (INA; Q16352) | 64% | 6<br>27% | 29% |
| KSYTHPSSLRK | A*03:01 | 21%<br>Wü-N526/17<br>ZH703<br>ZH859 | NS / C<br>ZH513<br>ZH732<br>ZH872 | Zinc finger protein ZIC 1/2/3/5 (ZIC1/2/3/5; Q15915 / O95409 / O60481 / Q96T25) | 61% / 39% / 25% / 25% | 7<br>4% / 5% / 2% / 6% | 1% / 1% / 0% / 1% |
| MIMFPLFGK | A*03:01<br>A*11:01 | 21%<br>HH-02<br>Wü-N998/13<br>ZH713 | N / C<br>SA2<br>ZH513<br>ZH732 | Interphotoreceptor matrix proteoglycan 2 (IMPG2; Q9BZV3) | 25% | 0<br>1% | 1% |
| AEIYVNSSFY | B*18:01<br>B*44:02<br>B*44:03 | 18%<br>Wü-N526/17<br>ZH741<br>ZH913 | NSW / AC<br>ZH718<br>ZH872 | ATP-binding cassette sub-family G member 2 (ABCG2; Q9UNQ0) | 71% | 3<br>8% | 8% |
| FLDEPTTGL | A*02:01<br>C*05:01 | 18%<br>HH-01<br>ZH718<br>ZH937 | S / AC<br>SA3<br>ZH868 | ATP-binding cassette sub-family G member 2 (ABCG2; Q9UNQ0) | 71% | 0<br>8% | 8% |
| AIFDDSYLGY | A*11:01<br>A*25:01<br>A*26:01 | 18%<br>SA5<br>ZH680<br>ZH718 | NS / C<br>Wü-N526/17<br>ZH713 | Integrin alpha-V (ITGAV; P06756) | 89% | 21<br>48% | 41% |
| AILEQILSH | A*03:01<br>A*11:01 | 18%<br>HH-06<br>ZH513<br>ZH872 | NS / C<br>SA2<br>ZH732 | RUN domain-containing protein 3A/3B (RUNDC3A/3B; Q59EK9 / Q96NL0) | 18% / 18% | 1<br>0% / 0% | 0% / 1% |
| ATYGLNVER | A*11:01<br>A*68:01 | 18%<br>HH-02<br>SA2<br>ZH513 | N / C<br>HH-06<br>SA5 | Arf-GAP with GTPase, ANK repeat and PH domain-containing protein 1/3 (AGAP1/3; Q9UPQ3 / Q96P47) | 50% / 54% | 6<br>9% / 11% | 9% / 9% |
| GLYAGDPVSK | A*03:01 | 18%<br>Wü-N526/17<br>ZH703<br>ZH872 | NSW / C<br>Wü-N998/13<br>ZH732 | RNA pseudouridylate synthase domain-containing protein 1 (RPUSD1; Q9UJJ7) | 21% | 14<br>3% | 4% |
| NIYEVVNPK | A*03:01<br>A*11:01<br>A*68:01 | 18%<br>HH-02<br>SA5<br>ZH732 | N / C<br>HH-06<br>ZH513 | Copine-5 (CPNE5; Q9HCH3) | 46% | 1<br>16% | 16% |
| NLDPGAALYLY | A*01:01<br>A*29:02<br>A*80:01 | 18%<br>SA2<br>ZH732<br>ZH913 | NSW / AC<br>Wü-N998/13<br>ZH868 | BarH-like 1 homeobox protein (BARHL1; Q9BZE3) | 18% | 2<br>1% | 0.5% |
| QEADEATLARL | B*40:02<br>B*44:02<br>B*44:03<br>B*44:05 | 18%<br>Wü-N526/17<br>ZH732<br>ZH913 | NSW / AC<br>Wü-N998/13<br>ZH741 | Glial fibrillary acidic protein (GFAP; P14136) | 100% | 8<br>17% | 16% |
| RTYDPEGFK | A*03:01 | 18%<br>Wü-N998/13<br>ZH732<br>ZH872 | NSW / C<br>ZH703<br>ZH859 | Diphosphoinositol polyphosphate phosphohydrolase 3-alpha/beta (NUD10/11; Q8NFP7 / Q96G61) | 36% / 36% | 2<br>4% / 4% | 4% / 4% |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SVDVYQVAK | A*11:01 | <u>18%</u><br>HH-02<br>SA2<br>ZH513 | <u>N / C</u><br>HH-06<br>SA5 | Receptor-type tyrosine-protein phosphatase zeta (PRPRZ1; P23471) | 57% | 7<br>11% | 5% |
| VSLGTPIMK | A*11:01 | <u>18%</u><br>HH-02<br>SA2<br>ZH513 | <u>N / C</u><br>HH-06<br>SA5 | Protein ECT2 (ECT2; Q9H8V3) | 43% | 8<br>19% | 8% |
| YEAPVSYTF | B*18:01<br>B*40:02<br>B*44:02<br>B*44:03 | <u>18%</u><br>SA3<br>ZH718<br>ZH913 | <u>NSW / AC</u><br>ZH703<br>ZH741 | Cingulin-like protein 1 (CGNL1; Q0VF96) | 21% | 6<br>12% | 13% |
| YQDLLNVKL | B*13:02<br>B*38:01<br>*B*52:01*<br>C*02:02<br>C*05:01 | <u>18%</u><br>Wü-N391/17<br>ZH718<br>ZH937 | <u>NSW / AC</u><br>ZH713<br>ZH741 | Glial fibrillary acidic protein (GFAP; P14136) | 100% | 25<br>17% | 16% |
| AVADFLFNV | A*02:01<br>A*68:02 | <u>18%</u><br>Wü-N391/17<br>ZH741<br>ZH872 | <u>SW / C</u><br>ZH718<br>ZH868 | Chemokine-like receptor 1 (CMKKLR1; Q99788) | 25% | 10<br>6% | 1% |
| DVIGTLSGF | A*25:01<br>A*26:01 | <u>18%</u><br>SA5<br>ZH680<br>ZH718 | <u>NS / C</u><br>Wü-N526/17<br>ZH713 | Magnesium transporter NIPA2 (NIPA2; Q8N8Q9) | 39% | 27<br>11% | 14% |
| EAIKIFNSL | A*25:01<br>*B*08:01*<br>B*35:01<br>B*35:02<br>B*35:03 | <u>18%</u><br>HH-02<br>ZH680<br>ZH890 | <u>NS / C</u><br>ZH513<br>ZH859 | Unconventional myosin-X (MYO10; Q9HD67) | 64% | 11<br>25% | 17% |
| ETFPGVTALF | A*25:01<br>A*26:01<br>A*68:02<br>B*57:01 | <u>18%</u><br>SA5<br>ZH680<br>ZH872 | <u>NS / C</u><br>Wü-N526/17<br>ZH718 | Major histocompatibility complex class I-related gene protein (MR1; Q95460) | 25% | 29<br>11% | 5% |
| EVIFSLETY | A*25:01<br>A*26:01 | <u>18%</u><br>SA5<br>ZH680<br>ZH718 | <u>NS / C</u><br>Wü-N526/17<br>ZH713 | Asparagine synthetase [glutamine-hydrolyzing] (ASNS; P08243) | 36% | 17<br>17% | 17% |
| GSVSININR | A*11:01<br>A*68:01 | <u>18%</u><br>HH-03<br>SA1<br>ZH913 | <u>NS / AC</u><br>HH-06<br>SA2 | A-kinase anchor protein 9 AKAP9; Q99996) | 64% | 5<br>35% | 32% |
| QVIEEIEEM | *A*02:05*<br>A*26:01<br>B*35:01 | <u>18%</u><br>HH-02<br>Wü-N526/17<br>ZH859 | <u>NS / C</u><br>SA5<br>ZH713 | Fasciculation and elongation protein zeta-1/2 (FEZ1/2; Q99689 / Q9UHY8) | 29% / 39% | 15<br>6% / 9% | 7% / 9% |
| SGSFVVVQK | A*11:01<br>A*68:01 | <u>18%</u><br>HH-03<br>SA2<br>ZH913 | <u>NS / AC</u><br>HH-06<br>ZH513 | Heterochromatin protein 1-binding protein 3 (HP1BP3; Q5SSJ5) | 36% | 16<br>35% | 24% |
| SIYDDSYLGY | A*25:01<br>A*26:01 | <u>18%</u><br>SA5<br>ZH680<br>ZH718 | <u>NS / C</u><br>Wü-N526/17<br>ZH713 | Integrin alpha-5 (ITGA5; P08648) | 21% | 19<br>10% | 6% |
| STFSGFLVY | A*03:01<br>A*11:01<br>A*29:02<br>B*57:01<br>C*02:02 | <u>18%</u><br>SA5<br>ZH513<br>ZH913 | <u>NSW / AC</u><br>Wü-N998/13<br>ZH872 | Complement C1q tumor necrosis factor-related protein 5 (C1QTBF5; Q9BXJ0) | 21% | 26<br>4% | 1% |
| SVLATVQQV | A*02:01<br>A*02:11 | <u>18%</u><br>HH-01<br>Wü-N391/17<br>ZH868 | <u>SW / C</u><br>SA3<br>ZH741 | Laminin subunit gamma-3 (LAMC3; Q9Y6N6) | 43% | 13<br>4% | 4% |
| TVIIHIPQY | A*25:01<br>A*26:01 | <u>18%</u><br>SA5<br>ZH680<br>ZH718 | <u>NS / C</u><br>Wü-N526/17<br>ZH713 | Integrin alpha-2 (ITGA2; P17301) | 25% | 23<br>7% | 6% |
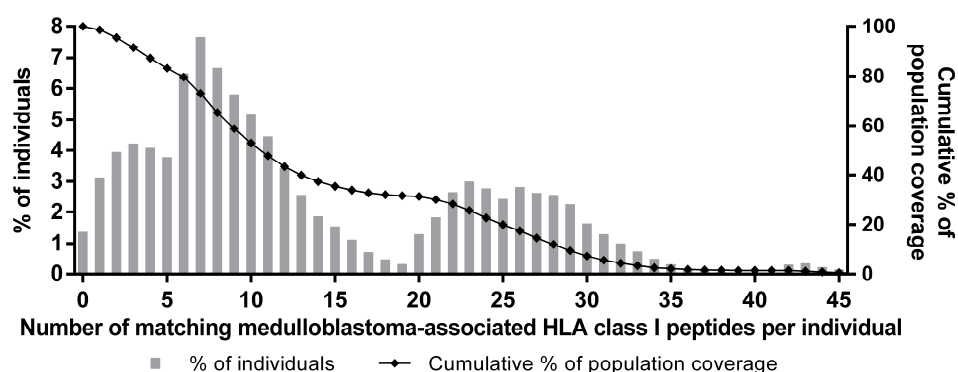
**Supplementary Figure 7. Population coverage of medulloblastoma-associated HLA-A, -B, and -C ligands.** Using the population coverage tool provided by the IEDB Analysis Resource, the world population coverage of the 66 medulloblastoma-associated peptides was calculated. The percentage of individuals with a specific number of matching peptides (max. of 53) is indicated by bar charts (associated with the left y-axis). The line diagram (associated with the right y-axis) shows the cumulative percentage of population coverage. The candidate target peptides cover 98.64% of the world population (first diamond on line diagram counted from the left) meaning that only 1.36% of all individuals are negative for all HLA-A, -B, and -C allotypes for which medulloblastoma-associated peptides were defined. On average, 14 peptides are expected to match per patient worldwide.

**Supplementary Table 13. Medulloblastoma-exclusive HLA class II-presented peptides derived from medulloblastoma-associated HLA presentation hotspots.** Peptides already reported to derive from medulloblastoma-associated antigens were excluded from this listing. The number of positive tumors other than medulloblastoma was based on n=626 HLA class II peptidome datasets. To indicate the distribution of antigens across subgroups and age classes, the following abbreviations were used: WNT-activated (W), SHH-activated (S), non-WNT/non-SHH (including those subclassified in Group 3 or Group 4; N), childhood medulloblastoma (≤ 15 years; C), adult medulloblastoma (≥ 25 years; A).

| Antigen (UniProt accession) | Protein frequency on medullo-blastomas | Peptide sequence | Frequency of positive patients | | Protein frequency on non-MB tumors<br>Peptide-positive non-MB tumors | Protein frequency on benign samples (n=364) |
|---|---|---|---|---|---|---|
| Zinc finger C3H1 domain-containing protein (ZFC3H1; O60293) | 25% | ENCVEETFEDLLLK | <u>NSW</u><br>25%<br>HH-06<br>SA2<br>ZH703 | <u>/ C</u><br>HH-04<br>SA1<br>Wü-N998/13<br>ZH718 | 3%<br>8 | 2% |
| Neural cell adhesion molecule 1 (NCAM1; P13591) | 32% | KDVRFIVLSNNYLQIR<br>KDVRFIVLSNNYLQIRG<br>LSNNYLQIRGIKKTDE<br><br>LSNNYLQIRGIKKTDEG<br>SNNYLQIRGIKKTDE<br><br><br>SNNYLQIRGIKKTDEG | <u>NS</u><br>4%<br>4%<br>14%<br>SA2<br>ZH913<br>4%<br>18%<br>SA1<br>ZH703<br>4% | <u>/ AC</u><br>ZH872<br>ZH872<br>SA1<br>ZH703<br><br>SA2<br>HH-06<br>SA2<br>ZH913<br>ZH913 | 9%<br>3<br>1<br>8<br><br>2<br>4<br><br><br>3 | 6% |
| Coxsackievirus and adenovirus receptor (CXADR; P78310) | 18% | AIPVMIPAQSKDGSIV<br>IPAQSKDGSIV | <u>N</u><br>14%<br>SA4<br>ZH919<br>4% | <u>/ C</u><br>SA2<br>ZH732<br><br>SA1 | 6%<br>0<br><br><br>0 | 3% |
| Follistatin-related protein 4 (FSTL4; Q6MZW2) | 32% | PPVIRVYPESQAQEPGV<br>THVLQVNVPPVIRVYPESQAQEPG<br>VPPVIRVYPESQAQ<br>VPPVIRVYPESQAQEPG | <u>NSW</u><br>4%<br>4%<br>4%<br>14%<br>ZH703<br>ZH872 | <u>/ C</u><br>ZH680<br>ZH718<br>ZH872<br>ZH680<br>ZH741 | 2%<br>0<br>0<br>1<br>3 | 5% |
| Programmed cell death 6-interact-ing protein (PDCD6IP; Q8WUM4) | 54% | AKQQKKFGEEIARLQH<br>ARLQHAAELIKTV<br>FGEEIARLQHAAELIKTV<br>HAAELIKTV | <u>NSW</u><br>4%<br>4%<br>4%<br>11%<br>SA2 | <u>/ AC</u><br>SA3<br>SA1<br>SA1<br>SA1<br>SA3 | 18%<br>0<br>0<br>0<br>0 | 17% |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | KFGEEIARLQHAA | 4% | SA1 | | 0 | |
| | | KFGEEIARLQHAAEL | 7% | SA1 | | 0 | |
| | | | | SA3 | | | |
| | | KFGEEIARLQHAAELIK | 4% | SA1 | | 0 | |
| | | KFGEEIARLQHAAELIKTV | 43% | HH-03 | | 0 | |
| | | | | HH-04 | HH-05 | | |
| | | | | SA1 | SA2 | | |
| | | | | SA3 | SA4 | | |
| | | | | Wü-N391/17 | Wü-N526/17 | | |
| | | | | ZH913 | ZH919 | | |
| | | | | ZH937 | | | |
| | | KQQKKFGEEIARLQH | 4% | SA3 | | 1 | |
| | | QQKKFGEEIARLQH | 4% | SA3 | | 2 | |
| Cadherin-related family member 1 (CDHR1; Q96JP9) | 46% | | | <u>NW</u> / C | | 0.5% | 2% |
| | | ANDEAPRFIQEPYVAL | 4% | ZH680 | | 0 | |
| | | ANDEAPRFIQEPYVALVPEDIPA | 7% | ZH680 | | 0 | |
| | | | | ZH741 | | | |
| | | ANDEAPRFIQEPYVALVPEDIPAG | 4% | ZH680 | | 0 | |
| | | ANDEAPRFIQEPYVALVPEDIPAGS | 4% | ZH680 | | 0 | |
| | | APRFIQEPYVAL | 4% | ZH680 | | 0 | |
| | | APRFIQEPYVALVPE | 14% | ZH680 | | 0 | |
| | | | | ZH713 | ZH732 | | |
| | | | | ZH741 | | | |
| | | DANDEAPRFIQEPYVAL | 4% | ZH680 | | 0 | |
| | | DANDEAPRFIQEPYVALVPE | 4% | ZH680 | | 0 | |
| | | DANDEAPRFIQEPYVALVPED | 4% | ZH680 | | 0 | |
| | | DANDEAPRFIQEPYVALVPEDIPA | 7% | ZH680 | | 0 | |
| | | | | ZH713 | | | |
| | | DANDEAPRFIQEPYVALVPEDIPAG | 4% | ZH680 | | 0 | |
| | | DEAPRFIQEPYVAL | 11% | ZH680 | | 0 | |
| | | | | ZH713 | ZH732 | | |
| | | DEAPRFIQEPYVALVP | 7% | ZH680 | | 0 | |
| | | | | ZH713 | | | |
| | | DEAPRFIQEPYVALVPE | 18% | ZH513 | | 0 | |
| | | | | ZH680 | ZH713 | | |
| | | | | ZH732 | ZH741 | | |
| | | DEAPRFIQEPYVALVPED | 4% | ZH680 | | 0 | |
| | | DEAPRFIQEPYVALVPEDIP | 4% | ZH680 | | 0 | |
| | | DEAPRFIQEPYVALVPEDIPA | 14% | ZH680 | | 0 | |
| | | | | ZH713 | ZH732 | | |
| | | | | ZH741 | | | |
| | | DEAPRFIQEPYVALVPEDIPAG | 4% | ZH680 | | 0 | |
| | | DEAPRFIQEPYVALVPEDIPAGS | 4% | ZH680 | | 0 | |
| | | DEAPRFIQEPYVALVPEDIPAGSII | 4% | ZH680 | | 0 | |
| | | EAPRFIQEPYVAL | 14% | ZH680 | | 0 | |
| | | | | ZH713 | ZH732 | | |
| | | | | ZH741 | | | |
| | | EAPRFIQEPYVALVP | 7% | ZH680 | | 0 | |
| | | | | ZH713 | | | |
| | | EAPRFIQEPYVALVPE | 25% | Wü-N998/13 | | 1 | |
| | | | | ZH513 | ZH680 | | |
| | | | | ZH713 | ZH732 | | |
| | | | | ZH741 | ZH890 | | |
| | | EAPRFIQEPYVALVPED | 4% | ZH680 | | 0 | |
| | | EAPRFIQEPYVALVPEDIPA | 14% | ZH680 | | 0 | |
| | | | | ZH713 | ZH732 | | |
| | | | | ZH741 | | | |
| | | EAPRFIQEPYVALVPEDIPAG | 4% | ZH680 | | 0 | |
| | | GTPVKIEAIDEDAEEP | 4% | HH-02 | | 0 | |
| | | GTPVKIEAIDEDAEEPN | 18% | HH-02 | | 0 | |
| | | | | HH-06 | SA1 | | |
| | | | | SA5 | ZH713 | | |
| | | IQEPYVALVPEDIPA | 4% | Wü-N998/13 | | 0 | |
| | | LGTPVKIEAIDEDAEEPN | 4% | HH-02 | | 0 | |
| | | NDEAPRFIQEPYVALVPEDIPA | 4% | ZH680 | | 0 | |
| | | TPVKIEAIDEDAEEPN | 21% | HH-02 | | 0 | |
| | | | | SA1 | SA4 | | |
| | | | | SA5 | ZH713 | | |
| | | | | ZH732 | | | |
| Insulinoma-associated protein 2 (INSM2; Q96T92) | 25% | | | <u>NSW</u> / AC | | 0% | 1% |
| | | PRGFLVKRTKRTGGL | 11% | SA3 | | 0 | |
| | | | | ZH868 | ZH913 | | |
| | | PRGFLVKRTKRTGGLY | 7% | HH-06 | | 0 | |
| | | | | ZH913 | | | |
| | | PRGFLVKRTKRTGGLYRV | 4% | ZH913 | | 0 | |
| | | PRGFLVKRTKRTGGLYRVR | 7% | Wü-N526/17 | | 0 | |
| | | | | ZH913 | | | |
| | | PRGFLVKRTKRTGGLYRVRL | 21% | SA3 | | 0 | |
| | | | | Wü-N526/17 | Wü-N998/13 | | |
| | | | | ZH513 | ZH868 | | |

| Protein | Protein frequency on medulloblastomas | Peptide sequence | HLA restriction | Frequency of positive patients | Protein frequency on non-MB / Peptide-positive non-MB | Protein frequency on benign samples |
|---|---|---|---|---|---|---|
| | | | | ZH913 | | |
| Insulinoma-associated protein 1 (INSM1; Q01101) | 32% | PRGFLVKRSKKSTPVSY | _NSW_ / _AC_ | 32% Wü-N391/17 ZH513 ZH741 ZH868 / Wü-N998/13 ZH732 ZH859 ZH913 | 0% / 0 | 1% |
| Dihydropyrimidinase-related protein 1 (CRMP1; Q14194) | 68% | | _S_ / _AC_ | | 16% | 14% |
| | | AADDFFQGTRAALVG | | 4% / SA3 | 0 | |
| | | AADDFFQGTRAALVGGT | | 11% SA4 / SA3 ZH868 | 2 | |
| | | AADDFFQGTRAALVGGTT | | 7% ZH868 / SA3 | 1 | |
| | | ADDFFQGTRAALVGG | | 4% / SA3 | 0 | |
| | | ADDFFQGTRAALVGGT | | 11% SA4 / SA3 ZH868 | 5 | |
| | | DDFFQGTRAALVGGT | | 14% ZH868 ZH937 / SA3 ZH872 | 4 | |
| | | DDFFQGTRAALVGGTT | | 4% / SA3 | 0 | |
| | | DFFQGTRAALVGGT | | 11% ZH868 / SA3 ZH872 | 2 | |
| | | DFFQGTRAALVGGTT | | 4% / SA3 | 0 | |
| Nucleolar and coiled-body phosphoprotein 1 (NOLC1; Q14978) | 57% | | _SW_ / _AC_ | | 13% | 2% |
| | | KFTKGKSFRHEKTK | | 7% ZH741 / Wü-N391/17 | 2 | |
| | | KFTKGKSFRHEKTKK | | 18% Wü-N391/17 ZH868 / HH-05 ZH741 ZH913 | 1 | |
| | | KFTKGKSFRHEKTKKK | | 7% ZH913 / ZH741 | 0 | |
| Drebrin (DRBN1; Q16643) | 50% | | _NS_ / _C_ | | 11% | 10% |
| | | FMESAEQAVL | | 4% / ZH513 | 0 | |
| | | MESAEQAVL | | 18% ZH513 ZH718 / SA2 ZH703 ZH732 | 3 | |
| | | MESAEQAVLA | | 14% ZH513 ZH872 / SA2 ZH718 | 2 | |
| | | MFMESAEQAVLA | | 4% / SA2 | 1 | |

**Supplementary Table 14. Established TAAs and CTAs identified as medulloblastoma-exclusive antigens or represented by medulloblastoma-exclusive peptides on ≥ 2 tumors.** HLA restrictions not passing manual assessment as quality control are indicated in italic. The number of positive neoplasias other than medulloblastoma was based on n=874 HLA class I and n=626 HLA class II peptidome datasets. The frequency of positive benign HLA peptidomes was calculated from n=418 (HLA class I) or n=364 (HLA class II) benign human specimens. Medulloblastoma exclusivity of HLA class II-restricted peptides was evaluated for the exact sequence match with none of the peptides arising from a tumor-associated presentation hotspot.

| Peptide sequence | HLA restriction | Frequency of positive patients | Protein frequency on medulloblastomas | Protein frequency on non-MB tumors / Peptide-positive non-MB tumors | Protein frequency on benign samples |
|---|---|---|---|---|---|
| **Medulloblastoma-exclusive HLA class I ligands derived from established TAAs and CTAs** | | | | | |
| Regulator of G-protein signaling 5 (RGS5; O15539) | | | 68% | 31% | 18% |
| KIKSPAKMAEK | A*03:01; A*30:01 | 14% Wü-N998/13 ZH732 ZH872 ZH937 | | 17 | |
| KLLQNNYGL | A*02:01 | 11% HH-01 ZH718 ZH868 | | 18 | |
| Catenin beta-1 (CTNNB1; P35222) | | | 93% | 76% | 72% |
| ATVGLIRNL | _A*02:01_; A*32:01; _B*57:01_; C*02:02; C*12:03 | 14% HH-02 SA3 ZH868 ZH872 | | 25 | |
| IENIQRVAA | B*41:01; B*50:01 | 7% HH-02 Wü-N391/17 | | 9 | |
| VNIMRTYTY | A*29:02 | 7% Wü-N998/13 ZH913 | | 6 | |
| Retinoblastoma-associated protein (RB1; P06400) | | | 46% | 34% | 39% |
| AETQATSAF | B*18:01; B*44:02; B*44:03; B*44:05 | 11% ZH741 ZH872 ZH913 | | 10 | |
| GVLGGYIQK | A*11:01 | 7% SA5 ZH513 | | 10 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| DNA repair protein RAD51 homolog 1 (RAD51; Q06609) | | | | | 14% | 13% | 7% |
| ESFGPQPISR | A*68:01 | 11% | HH-03 ZH913 | HH-06 | | 11 | |
| Arachidonate 12-lipoxygenase, 12S-type (ALOX12; P18054) | | | | | 29% | 6% | 9% |
| KRLDFEWTL | B*27:05 | 7% | ZH513 | ZH680 | | 2 | |
| Axin-2 (AXIN2; Q9Y2T1) | | | | | 14% | 5% | 3% |
| ETVDSGYRSF | A*25:01 | 7% | ZH680 | ZH718 | | 16 | |
| LTSDIYLEY | A*03:01; A*11:01; A*29:02 | 7% | ZH513 | ZH913 | | 15 | |
| G2/mitotic-specific cyclin-B1 (CCNB1; P14635) | | | | | 43% | 15% | 13% |
| EEQAVRPKY | B*44:02; B*44:03 | 7% | Wü-N998/13 | ZH868 | | 13 | |
| Cytochrome P450 1B1 (CYP1B1; Q16678) | | | | | 50% | 28% | 41% |
| DTVVFVNQW | A*25:01 | 7% | ZH680 | ZH718 | | 9 | |
| Glutamate carboxypeptidase 2 (FOLH1; Q04609) | | | | | 14% | 6% | 6% |
| DIVPPFSAF | A*25:01 | 7% | ZH680 | ZH718 | | 4 | |
| UPF0378 protein KIAA0100 (KIAA0100; Q14667) | | | | | 25% | 20% | 17% |
| EVSTAKLTAF | A*25:01 | 7% | ZH680 | ZH718 | | 11 | |
| Leucine zipper putative tumor suppressor 1 (LZTS1; Q9Y250) | | | | | 14% | 3% | 0.5% |
| SPESASHQL | B*35:01; B*35:02; B*35:03 | 7% | ZH859 | ZH890 | | 8 | |
| Outer dense fiber protein 2 (ODF2; Q5BJF6) | | | | | 32% | 3% | 13% |
| NVFGDGPYSTF | A*25:01 | 7% | ZH680 | ZH718 | | 8 | |
| Platelet-derived growth factor receptor beta (PDGFRB; P09619) | | | | | 61% | 23% | 16% |
| YMDLVGFSY | A*29:02 | 7% | Wü-N998/13 | ZH913 | | 4 | |
| Proteasome subunit beta type-9 (PSMB9; P28065) | | | | | 43% | 35% | 29% |
| AGVDHRVIL | *C*07:04; C*16:01* | 7% | ZH872 | ZH913 | | 6 | |
| Inactive rhomboid protein 2 (RHBDF2; Q6PJF5) | | | | | 7% | 5% | 1% |
| ISSTVQRQL | B*57:01 | 7% | SA3 | ZH868 | | 7 | |
| Tyrosine-protein kinase receptor Tie-1 (TIE1; P35590) | | | | | 32% | 10% | 16% |
| SLFENFTYA | A*02:01 | 7% | HH-01 | ZH868 | | 1 | |

**Medulloblastoma-exclusive HLA class II-presented antigens derived from established TAAs and CTAs**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| DNA repair protein RAD51 homolog 1 (RAD51; Q06609) | | | | | 7% | 1% | 0% |
| KLVPMGFTTATEFHQRRSEII | | 7% | HH-01 | ZH680 | | 4 | |
| Glypican-2 (GPC2; Q8N158) | | | | | 7% | 0% | 0% |
| ARGYSLNLIPPALI | | 7% | ZH703 | ZH718 | | 0 | |
| ERVFPLLHPQYSFPPD | | 4% | ZH718 | | | 0 | |
| GARGYSLNLIPPALI | | 4% | ZH703 | | | 0 | |
| GARGYSLNLIPPALIS | | 7% | ZH703 | ZH718 | | 0 | |
| GARGYSLNLIPPALISG | | 7% | ZH703 | ZH718 | | 0 | |
| VLGARGYSLNLIPPALISGEHL | | 4% | ZH703 | | | 0 | |

**Medulloblastoma-exclusive HLA class II-restricted peptides derived from established TAAs and CTAs**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Golgin subfamily A member 6-like protein 2 (GOLGA6L2; Q8N9W4) | | | | | 75% | 24% | 43% |
| EEMWGQEKKMWRQEKMREQEEEMRE | | 14% | HH-06 ZH680 | SA2 ZH872 | | 2 | |
| KMWEQEEKMREQEEKMWRQEERLW | | 7% | Wü-N391/17 | ZH680 | | 2 | |
| QKMRDQEERMWEQDERLREKEERMR | | 7% | ZH718 | ZH741 | | 1 | |
| Breast cancer type 1 susceptibility protein (BRCA1; P38398) | | | | | 7% | 1% | 1% |
| QPEVYKQSLPGSNCKHPEIKKQEY | | 7% | HH-01 | ZH732 | | 2 | |
| Ephrin type-B receptor 2 (EPHB2; P29323) | | | | | 11% | 4% | 4% |
| HRPKFGQIVNTLDK | | 7% | SA5 | ZH713 | | 2 | |
| Legumain (LGMN; Q99538) | | | | | 50% | 31% | 46% |
| HLPDNINVYATTAANPRE | | 7% | ZH868 | ZH872 | | 24 | |
| HLPDNINVYATTAANPRESS | | 7% | ZH680 | ZH868 | | 22 | |
| IPDEQIVVMMYDDIAYSEDNPTPG | | 7% | HH-06 | ZH868 | | 2 | |
| Mucin-1 (MUC1; P15941) | | | | | 7% | 6% | 7% |
| GVSFFFLSFHISNL | | 7% | SA4 | ZH680 | | 0 | |
| Testis-expressed sequence 15 protein (TEX15; Q9BXT5) | | | | | 7% | 2% | 1% |
| KVEMQRSLPGSLLP | | 7% | ZH680 | ZH732 | | 1 | |

## Supplement of CHAPTER 4

**Supplementary Table 15. HLA class I allotype and allele frequencies in the study cohort comprising n=33 meningioma patients.** The HLA-A, -B, and -C allotypes with the top three highest frequencies within the collective are marked in bold.

| HLA allotype | Positive patients | Allele frequency | HLA allotype | Positive patients | Allele frequency |
|---|---|---|---|---|---|
| **A*01:01** | **11%** | 12% | B*39:31 | 2% | 2% |
| **A*02:01** | **20%** | 23% | B*40:01 | 2% | 2% |
| **A*03:01** | **12%** | 12% | B*40:02 | 3% | 3% |
| A*11:01 | 3% | 3% | B*41:01 | 2% | 2% |
| A*23:01 | 3% | 3% | B*44:02 | 2% | 2% |
| **A*24:02** | **11%** | 12% | B*44:03 | 8% | 9% |
| A*26:01 | 2% | 2% | B*49:01 | 2% | 2% |
| A*29:02 | 8% | 8% | B*50:01 | 2% | 2% |
| A*30:01 | 2% | 2% | **B*51:01** | **9%** | 9% |
| A*30:02 | 5% | 5% | B*51:02 | 2% | 2% |
| A*31:01 | 3% | 3% | B*51:08 | 2% | 2% |
| A*32:01 | 5% | 5% | B*55:01 | 3% | 3% |
| A*33:01 | 2% | 2% | B*57:01 | 5% | 5% |
| A*34:01 | 2% | 2% | C*01:02 | 5% | 5% |
| A*66:01 | 2% | 2% | C*02:02 | 8% | 8% |
| A*68:01 | 8% | 8% | C*03:03 | 6% | 6% |
| **B*07:02** | **9%** | 11% | C*03:04 | 3% | 3% |
| B*08:01 | 6% | 6% | **C*04:01** | **14%** | 17% |
| B*13:02 | 5% | 5% | C*05:01 | 2% | 2% |
| B*14:02 | 2% | 2% | **C*06:02** | **11%** | 11% |
| B*15:01 | 5% | 5% | **C*07:01** | **12%** | 14% |
| B*15:35 | 2% | 2% | C*07:02 | 9% | 11% |
| **B*18:01** | **11%** | 11% | C*08:02 | 2% | 2% |
| B*27:05 | 3% | 3% | C*12:03 | 9% | 9% |
| **B*35:01** | **9%** | 11% | C*14:02 | 2% | 2% |
| B*35:03 | 2% | 2% | C*15:02 | 3% | 5% |
| B*37:01 | 2% | 2% | C*16:01 | 6% | 6% |
| B*38:01 | 2% | 2% | C*16:02 | 2% | 2% |
| B*39:01 | 2% | 2% | C*17:01 | 2% | 2% |

**frequency of positive meningioma patients [%]**

0          10          20
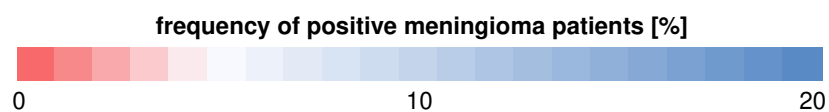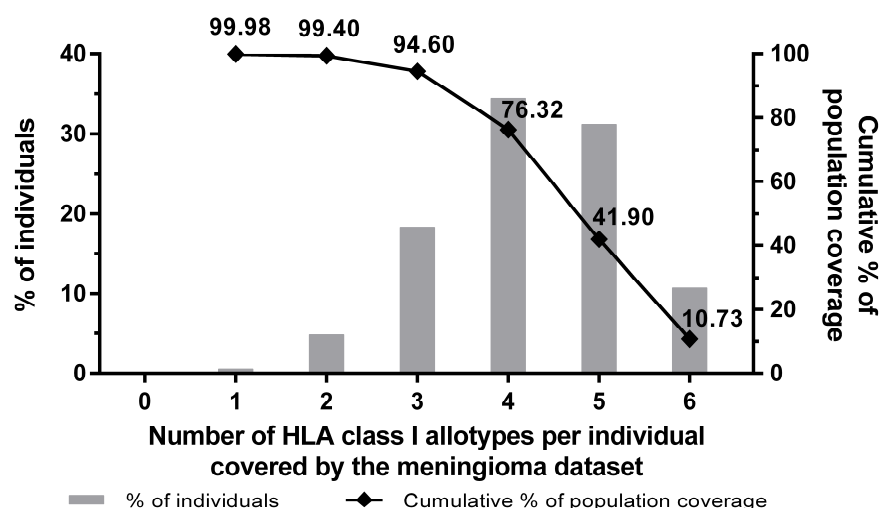
**Supplementary Figure 8. HLA class I allotype population coverage.** Using the population coverage tool provided by the IEDB Analysis Resource, the world population coverage of the 58 distinct HLA-A, -B, and -C allotypes was calculated. The percentage of individuals positive for a specific number of HLA class I allotypes (max. of 6) is indicated by bar charts (associated with the left y-axis). The line diagram (associated with the right y-axis) shows the cumulative percentage of population coverage. The HLA class I allotypes of the meningioma cohort cover 99.98% of the world population (first diamond on line diagram counted from the left) meaning that only 0.02% of all individuals are negative for all HLA-A, -B, and -C allotypes included in the present study (first bar counted from the left). 94.60% of all individuals are positive for at least three HLA class I allotypes (third diamond on line diagram counted from the left).

**Supplementary Table 16. Meningioma-associated HLA class I- and II-presented antigens identified on at least three tumors.** GTEx profiles were assessed from all available datasets excepting EBV-transformed lymphocytes and cultured fibroblasts. Color codes were defined as follows: ■ < 10 TPM in any tissue, ♦ > 10 TPM in testes and < 10 TPM in other tissues (CTA-like expression profile), ■ 10-20 TPM in any tissue, ■ 20-30 TPM in any tissue, ■ > 30 TPM in any tissue. The number of positive non-meningeal tumors was based on n=841 HLA class I and n=593 HLA class II peptidome datasets. HLA restrictions not passing manual assessment as quality control are indicated in italic. These peptides were excluded from downstream analyses such as calculation of peptides matching per patient worldwide. HLA class II-presented proteins neither identified with peptides exceeding a length of twelve AA nor with different sequences across patients were not considered for this listing of candidate target antigens. Eight of the listed HLA class II-presented antigens (DRGX, KIR2DS4, SGK3, PCED1B, STAT5B, ESCO1, ANGEL2, RFWD3) were detected with only one peptide in each case.

| Antigen | Frequency of positive patients | Peptide sequence | HLA restriction | UniProt accession GTEx profile | Positive non-meningeal tumors |
|---|---|---|---|---|---|
| **Meningioma-associated HLA class I antigens** | | | | | |
| Nicotinamide mononucleotide adenylyltransferase 2 (NMNA2) | 30% / WHO I-III | | | Q9BZQ4 ■ | 8 |
| | MNG1 | EEIELRILL | B*18:01 | | |
| | MNG3 | ENANLGTVMR | A*68:01 | | |
| | MNG6 | ENANLGTVMR | A*68:01 | | |
| | | NANLGTVMR | A*68:01 | | |
| | MNG499 | ENANLGTVMR | A*68:01 | | |
| | MNG501 | IVSPVHDSY | B*15:01 | | |
| | MNG636 | EEIELRILL | B*44:03 | | |
| | MNG642 | SVLEHHRDLMK | A*11:01 | | |
| | MNG661 | IVSPVHDSY | A*30:02 | | |
| | MNG734 | SVVSSTKSR | A*66:01 | | |
| | MNG814 | ENANLGTVMR | A*68:01 | | |

| Protein | % / WHO | Sample | Peptide | HLA | UniProt | | # |
|---|---|---|---|---|---|---|---|
| Protein Wnt-5a (WNT5A) | 18% / WHO I+II | | | | P41221 ▪ (orange) | | 43 |
| | | MNG6 | AMSSKFFLV | A*02:01 | | | |
| | | MNG628 | AMSSKFFLV | A*02:01 | | | |
| | | | NPVQMSEVY | B*35:01 | | | |
| | | MNG637 | AMSSKFFLV | A*02:01 | | | |
| | | MNG638 | AMSSKFFLV | A*02:01 | | | |
| | | MNG641 | AMSSKFFLV | A*02:01 | | | |
| | | MNG702 | AMSSKFFLV | A*02:01 | | | |
| T-box transcription factor TBX15/18 (TBX15/18) | 18% / WHO I-III | | | | Q96SF7 ▪ (red) | | 15 |
| 2 peptides multi-map to non-MNG-exclusive TBX20 (Q9UMR3) or to MNG-exclusive TBX22 (Q9Y458) | | MNG7 | GLDPHQQYY (TBX15/18) | A*01:01; A*30:02 | O95935 ▪ (red) | | 9 |
| | | MNG635 | SQMSVHMV (TBX15) | B*13:02 | | | |
| | | MNG636 | FHDIGTEMI (TBX15/22) | B*38:01 | | | |
| | | | THQGSYNTF (TBX18) | B*38:01 | | | |
| | | | DIVPVDNKRYR (TBX15/18/20) | A*33:01 | | | |
| | | | THQGSYNTF (TBX18) | B*38:01 | | | |
| | | MNG646 | GLDPHQQYY(TBX15/18) | A*01:01 | | | |
| | | MNG682 | KTFNFPETVF (TBX15) | B*15:01 | | | |
| | | MNG734 | GLDPHQQYYI (TBX15/18) | A*02:01 | | | |
| | | | YQNQQITRL (TBX15/18) | C*02:02 | | | |
| Protein odd-skipped-related 1 (OSR1) | 15% / WHO I+II | | | | Q8TAX0 ▪ (red) | | 6 |
| | | MNG7 | APVPIHPSL | B*07:02 | | | |
| | | MNG501 | FPWFPHVI | B*51:01 | | | |
| | | MNG628 | SLVDARFQL | A*02:01 | | | |
| | | MNG641 | SLVDARFQL | A*02:01; C*17:01 | | | |
| | | MNG814 | APVPIHPSL | B*07:02 | | | |
| Protein SSX5/9 (SSX5/9) peptides multi-map to MNG-exclusive obsolete UniProt IDs of SSX10 (A6NEJ1) and SSX11 (A6NNU9); not in GTEx | 12% / WHO I+II | | | | O60225 ▪ (green) | | 13 |
| | | MNG1 | SEKIIYVY | B*18:01 | Q7RTT3 ▪ (green) | | 12 |
| | | MNG623 | SEKIIYVY | B*18:01 | | | |
| | | MNG673 | SEKILYVY | B*44:03 | | | |
| | | MNG679 | SEKIIYVY | B*44:03 | | | |
| Frizzled-7 (FZD7) 3 peptides multi-map to non-MNG-exclusive FZD1 (Q9UP38) or FZD2 (Q14332) | 12% / WHO I+II | | | | O75084 ▪ (red) | | 7 |
| | | MNG635 | HQFYPLVKV (FZD1/2/7) | B*13:02 | | | |
| | | MNG661 | TYLVDMRRF (FZD1/7) | A*24:02 | | | |
| | | MNG673 | FSDDGYRTV (FZD7) | C*03:04; C*16:01 | | | |
| | | MNG814 | VPAVKTITI (FZD2/7) | B*07:02 | | | |
| E3 ubiquitin-protein ligase ZNRF2 (ZNRF2) | 12% / WHO I+II | | | | Q8NHG8 ▪ (yellow) | | 8 |
| | | MNG1 | DEMDLHLVM | B*18:01 | | | |
| | | MNG6 | DEMDLHLVM | B*18:01 | | | |
| | | MNG628 | DEMDLHLVM | B*18:01 | | | |
| | | MNG638 | DEMDLHLVM | B*18:01 | | | |
| Tctex1 domain-containing protein 2 (TC1D2) | 12% / WHO I | | | | Q8WW35 ▪ (orange) | | 5 |
| | | MNG7 | KLKEMGFDRY | A*30:02 | | | |
| | | MNG632 | KLKEMGFDRY | A*30:02 | | | |
| | | MNG661 | KLKEMGFDRY | A*30:02 | | | |
| | | MNG702 | LSENIKDKL | *C*06:02* | | | |
| Insulin-like growth factor-binding protein 6 (IGFBP6) | 12% / WHO I+II | | | | P24592 ▪ (red) | | 2 |
| | | MNG1 | DEAPLRAL | B*18:01 | | | |
| | | MNG628 | DEAPLRAL | B*18:01 | | | |
| | | | TPHRLLPPL | B*35:01 | | | |
| | | MNG661 | DEAPLRAL | B*18:01 | | | |
| | | MNG666 | TPHRLLPPL | B*07:02 | | | |
| Uncharacterized protein C8orf34 (C8orf34) | 12% / WHO I+II | | | | Q49A92 ▪ (green) | | 3 |
| | | MNG499 | YPAEPQAKV | B*51:01; B*51:02 | | | |
| | | MNG635 | YPAEPQAKV | B*55:02 | | | |
| | | MNG642 | YPAEPQAKV | B*51:01 | | | |
| | | MNG833 | YPAEPQAKV | B*51:01 | | | |
| Globoside alpha-1,3-N-acetylgalactosaminyltransferase 1 (GBGT1) | 12% / WHO I+II | | | | Q8N5D6 ▪ (yellow) | | 26 |
| | | MNG6 | ESAEEFFMR | A*68:01 | | | |
| | | MNG635 | AVFGGQVAR | A*31:01 | | | |
| | | MNG814 | ESAEEFFMR | A*68:01 | | | |
| | | | RPTQLLTL | B*07:02 | | | |
| | | MNG833 | KYTHFIQSF | A*24:02 | | | |
| Xyloside xylosyltransferase 1 (XXLT1) | 12% / WHO I-III | | | | Q8NBI6 ▪ (green) | | 13 |
| | | MNG3 | ETFSSATKR | A*68:01 | | | |
| | | MNG642 | ETFSSATKR | A*34:01 | | | |
| | | MNG734 | ETFSSATKRL | A*66:01 | | | |
| | | MNG833 | YYSDSIFFL | A*24:02 | | | |
| Solute carrier family 25 member 44 (SLC25A44) | 12% / WHO I-III | | | | Q96H78 ▪ (yellow) | | 10 |
| | | MNG7 | ILQADGLRGFY | A*01:01 | | | |
| | | MNG641 | SLVAQSITV | A*02:01 | | | |
| | | MNG702 | SLVAQSITV | A*02:01 | | | |
| | | MNG734 | SLVAQSITV | A*02:01 | | | |

| Protein | % / WHO | Sample | Peptide | HLA | Accession | | Count |
|---|---|---|---|---|---|---|---|
| Forkhead box protein (FOX) E3, D4-like 1/2/3/5/6 peptides multi-map to FOXB2 (Q5VYV0) and to non-MNG-exclusive FOXD1 (Q16676), FOXD2 (O60548), FOXD3 (Q9UJU5), FOXD4 (Q12950), FOXB1 (Q99853), FOXC1 (Q12948), FOXC2 (Q99958), FOXE1 (O00358), FOXG1 (P55316), FOXI1 (Q12951), FOXI2 (Q6ZQN5), FOXI3 (A8MTJ6), FOXJ1 (Q92949), FOXK1 (P85037), FOXK2 (Q01167), FOXL1 (Q12952), FOXL2 (P58012), FOXS1 (O43638) | 12% / WHO I+II | MNG6 | DMFDNGSFLR | A*68:01 | Q13461 | ■ | 6 |
| | | MNG7 | QNSLRHNL | B*08:01 | Q9NU39 | ■ | 6 |
| | | MNG646 | QNSLRHNL | B*08:01 | Q6VB85 | ■ | 5 |
| | | MNG814 | DMFDNGSFLR | A*68:01 | Q6VB84 | ■ | 4 |
| | | | | | Q5VV16 | ■ | 5 |
| | | | | | Q3SYB3 | ■ | 4 |
| Sterol O-acyltransferase 2 (SOAT2) 2 peptides multi-map to non-MNG-exclusive SOAT1 (P35610) | 9% / WHO I+II | MNG501 | YPVMLILFL | B*51:01 | O75908 | ■ | 7 |
| | | MNG624 | NAFAEMLRF | B*35:01 | | | |
| | | MNG833 | NAFAEMLRF | B*35:01 | | | |
| Cadherin-3 (CDH3) | 9% / WHO I+II | | | | P22223 | ■ | 17 |
| | | MNG641 | LPRGPLASLL | B*07:02 | | | |
| | | MNG666 | LPRGPLASLL | B*07:02 | | | |
| | | MNG814 | LPRGPLASLL | B*07:02 | | | |
| Folate receptor gamma (FOLR3) peptides multi-map to non-MNG-exclusive FOLR2 | 9% / WHO I+II | | | | P41439 | ■ | 9 |
| | | MNG635 | SYFPTPAAL | C*06:02 | | | |
| | | MNG646 | FESYFPTPAA | B*40:02 | | | |
| | | MNG661 | SYFPTPAAL | A*24:02 | | | |
| Transmembrane protein 87A (TMEM87A) | 9% / WHO II+III | | | | Q8NBN3 | ■ | 41 |
| | | MNG642 | DAPYIFIV | B*51:01 | | | |
| | | MNG635 | HPSPLSFFSA | B*55:01 | | | |
| | | MNG734 | AAWLQVLPV | C*12:03 | | | |
| Transmembrane protein 255B (TMEM255B) | 9% / WHO I+II | | | | Q8WV15 | ■ | 16 |
| | | MNG632 | NPAQQILAY | B*35:01 | | | |
| | | MNG819 | YYPGIILGF | A*23:01 | | | |
| | | MNG833 | YYPGIILGF | A*24:02 | | | |
| Ninjurin-2 (NINJ2) | 9% / WHO I+III | | | | Q9NZG7 | ■ | 13 |
| | | MNG7 | EQGPSSHYY | A*30:02 | | | |
| | | MNG632 | EQGPSSHYY | A*30:02 | | | |
| | | MNG734 | NEVEKQWRL | B*40:02 | | | |
| Rho-related GTP-binding protein RhoD (RHOD) | 9% / WHO I-III | | | | O00212 | ■ | 7 |
| | | MNG3 | EVALSSRGR | A*68:01 | | | |
| | | MNG642 | EVALSSRGR | A*34:01 | | | |
| | | MNG734 | EVALSSRGR | A*66:01 | | | |
| Beta-1,4-galactosyltransferase 4 (B4GALT4) | 9% / WHO I | | | | O60513 | ■ | 6 |
| | | MNG673 | FNLTFHLSY | A*29:02 | | | |
| | | MNG679 | FNLTFHLSY | A*29:02 | | | |
| | | MNG682 | FNLTFHLSY | A*29:02 | | | |
| cAMP-responsive element-binding protein-like 2 (CREBL2) | 9% / WHO I-III | | | | O60519 | ■ | 19 |
| | | MNG6 | IPSEIKALL | B*35:03 | | | |
| | | MNG638 | LRYQYLEEL | C*07:01 | | | |
| | | MNG734 | LRYQYLEEL | B*39:31 | | | |
| Mitochondrial tRNA-specific 2-thiouridylase 1 (MTU1) | 9% / WHO I | | | | O75648 | ■ | 10 |
| | | MNG3 | EVFEQKHVKK | A*68:01 | | | |
| | | MNG673 | NFEHFLLQY | A*29:02 | | | |
| | | MNG679 | NFEHFLLQY | A*29:02 | | | |
| NKG2-C type II integral membrane protein (NKG2C) / NKG2-E type II integral membrane protein (NKG2E) | 9% / WHO I+II | | | | P26717 | ■ | 1 |
| | | MNG499 | VTINGLAFK | A*11:01 | Q07444 | ■ | 1 |
| | | MNG501 | LPSSWIGVF | B*51:01 | | | |
| | | MNG642 | VTINGLAFK | A*11:01 | | | |
| Protein AF-9 (AF-9) | 9% / WHO I+II | | | | P42568 | ■ | 3 |
| | | MNG6 | SEALFKSF | B*18:01 | | | |
| | | MNG628 | SEALFKSF | B*18:01 | | | |
| | | MNG661 | SEALFKSF | B*18:01 | | | |
| 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase delta-4 (PLCD4) | 9% / WHO I-III | | | | Q9BRC7 | ■ | 2 |
| | | MNG1 | EELPLEQGF | B*18:01 | | | |
| | | MNG673 | ALSSLVIYL | A*02:01 | | | |
| | | MNG734 | ILFKDVVATV | A*02:01 | | | |
| Paraneoplastic antigen Ma2 (PNMA2) | 9% / WHO I+II | | | | Q9UL42 | ■ | 32 |
| | | MNG1 | AYVLRLETL | A*24:02 | | | |
| | | | SEVQGKGGVW | B*44:03 | | | |
| | | MNG3 | AEIQEVLQETL | B*40:01 | | | |
| | | MNG673 | SEVQGKGGVW | B*44:03 | | | |

| Meningioma-associated HLA class II antigens | | | | |
|---|---|---|---|---|

**Inactive serine protease 35 (PRSS35)** — 30% / WHO I+II — Q8N3Z0 ▪ 3

| | |
|---|---|
| MNG6 | SRFSILDKRFLTNFPFS |
| MNG7 | LPTPSLSELEDY |
| MNG501 | LPTPSLSELEDYL |
| MNG634 | LPTPSLSELEDYL |
| | LPTPSLSELEDYLSYE |
| | SRFSILDKRFLTNFPFS |
| | SRFSILDKRFLTNFPFS |
| MNG638 | KVPRIVSERTFHLTSP |
| | KVPRIVSERTFHLTSPA |
| | LPTPSLSELED |
| | LPTPSLSELEDY |
| | LPTPSLSELEDYL |
| | LPTPSLSELEDYLSY |
| | LPTPSLSELEDYLSYE |
| MNG641 | GTDSRFSILDKRFLTNFPFS |
| | SRFSILDKRFLTNFPFS |
| | SRFSILDKRFLTNFPFST |
| MNG673 | DSRFSILDKRFLTNFPFS |
| | DSRFSILDKRFLTNFPFST |
| | GTDSRFSILDKRFLTNFPF |
| | GTDSRFSILDKRFLTNFPFS |
| | GTDSRFSILDKRFLTNFPFST |
| | SRFSILDKRFLTNFPF |
| | SRFSILDKRFLTNFPFS |
| | SRFSILDKRFLTNFPFST |
| MNG702 | SRFSILDKRFLTNFPFS |
| MNG814 | RFSILDKRFLTNFPFS |
| | SRFSILDKRFLTNFPFS |
| MNG833 | DSRFSILDKRFLTNFPFS |
| | GTDSRFSILDKRFLTNFPFS |
| | RFSILDKRFLTNFPFS |
| | SRFSILDKRFLTNFPF |
| | SRFSILDKRFLTNFPFS |
| | SRFSILDKRFLTNFPFST |

**Lactosylceramide 4-alpha-galactosyltransferase (A4GALT)** — 27% / WHO I-III — Q9NPC4 ▪ 0

| | |
|---|---|
| MNG7 | KPPDLLLRLLRGAP |
| MNG623 | TQSRYVLNGAFLAFERR |
| MNG661 | QSRYVLNGAFLAFER |
| MNG666 | QSRYVLNGAFLAFER |
| | TQSRYVLNGAFLAFER |
| | TQSRYVLNGAFLAFERR |
| MNG673 | GTQSRYVLNGAFLAFERRH |
| | TQSRYVLNGAFLAFER |
| MNG682 | TQSRYVLNGAFLAFER |
| MNG734 | QSRYVLNGAFLAFERR |
| MNG819 | TQSRYVLNGAFLAFER |
| MNG833 | QSRYVLNGAFLAFER |
| | QSRYVLNGAFLAFERR |
| | TQSRYVLNGAFLAFER |
| | TQSRYVLNGAFLAFERR |
| | TQSRYVLNGAFLAFERRH |

**Fibrillin-2 (FBN2)** — 21% / WHO I-III — P35556 ▪ 7

| | |
|---|---|
| MNG1 | DDSVFRIHQRNGLSY |
| | DDSVFRIHQRNGLSYL |
| | GNDDSVFRIHQRNGLSYLH |
| | IQPLNNHIRYVISQG |
| | NDDSVFRIHQRNGLSY |
| MNG3 | DDSVFRIHQRNGLSY |
| MNG612 | LRPAIQPLNNHIRYV |
| MNG641 | LRPAIQPLNNHIRYV |
| MNG700 | KKDSRQKRSI |
| MNG702 | DSVFRIHQRNGLSY |
| | GNDDSVFRIHQRNGLSY |
| | GNDDSVFRIHQRNGLSYLH |
| | IQPLNNHIRYVISQG |
| | NDDSVFRIHQRNGLSY |
| MNG734 | LRPAIQPLNNHIRYV |
| | RPAIQPLNNHIRYV |

**Sushi, nidogen and EGF-like domain-containing protein 1 (SNED1)** — 21% / WHO I-III — Q8TER0 ▪ 1

| | |
|---|---|
| MNG7 | QTVLITDGKLSFTIFN |
| MNG638 | DRFTFRALLPGKRY |
| MNG642 | DRFTFRALLPGKRYT |
| | EPAHLYIITSPRDG |
| | EPAHLYIITSPRDGAD |
| MNG666 | TKSRYVPNGKLASYT |
| MNG679 | TRLFSETKAFPVWE |

| | | | | |
|---|---|---|---|---|
| | MNG702 | TKSRYVPNGKLASYT | | |
| | MNG734 | ASTISVQWALHRIR | | |
| MAGUK p55 subfamily member 6 (MPP6) | <u>18% / WHO I-III</u> | | Q9NZW5 ◾ | 5 |
| | MNG1 | INNQLLPVDAIRILG | | |
| | MNG2 | INNQLLPVDAIRILG | | |
| | | INNQLLPVDAIRILGIH | | |
| | MNG6 | DRHEIQIYEEVAKMPP | | |
| | MNG623 | INNQLLPVDAIRILG | | |
| | MNG641 | DRHEIQIYEEVAKMPP | | |
| | | DRHEIQIYEEVAKMPPF | | |
| | | DRHEIQIYEEVAKMPPFQ | | |
| | | FDRHEIQIYEEVAKMPP | | |
| | | FDRHEIQIYEEVAKMPPFQ | | |
| | MNG734 | DRHEIQIYEEVAKMPP | | |
| | | DRHEIQIYEEVAKMPPF | | |
| Dorsal root ganglia homeobox protein (DRGX) | <u>15% / WHO I+II</u> | | A6NNA5 ◾ | 6 |
| | MNG3 | LAMKINLTEARVQVWF | | |
| | MNG501 | LAMKINLTEARVQVWF | | |
| | MNG666 | LAMKINLTEARVQVWF | | |
| | MNG702 | LAMKINLTEARVQVWF | | |
| | MNG833 | LAMKINLTEARVQVWF | | |
| Melanoma-associated antigen 10 (MAGEA10) 1 peptide multi-maps to non-MNG-exclusive MAGEA9 (P43362) | <u>15% / WHO I+II</u> | | P43363 ◾ | 3 |
| | MNG499 | KLLTQDWVQENYLEYRQVPGSDP | | |
| | MNG624 | KLLTQDWVQENYLEYRQVPGSDP | | |
| | MNG641 | KLLTQDWVQENYLEYRQVPGSDP | | |
| | MNG682 | ESLPRSEIDEKVTDLVQFLLFKYQM | | |
| | MNG700 | KLLTQDWVQENYLEYRQVPGSDP | | |
| Nuclear factor of activated T-cells, cytoplasmic 2 (NFATC2) | <u>15% / WHO I+II</u> | | Q13469 ◾ | 20 |
| | MNG1 | DQTYLDDVNEIIR | | |
| | | LDQTYLDDVNEIIR | | |
| | MNG6 | KDKSQPNMLFVEIPEYR | | |
| | MNG7 | DQTYLDDVNEIIR | | |
| | MNG624 | DQTYLDDVNEIIR | | |
| | | LDQTYLDDVNEIIR | | |
| | MNG702 | DQTYLDDVNEIIR | | |
| | | KPHAFYQVHRITGKT | | |
| | | LDQTYLDDVNEIIR | | |
| Uncharacterized protein KIAA1586 (KIAA1586) | <u>15% / WHO I+II</u> | | Q9HCI6 ◾ | 5 |
| | MNG5 | EVDLNDFREFVNNNIK | | |
| | MNG501 | ELETEIIKIGRVMGPRW | | |
| | MNG637 | ELETEIIKIGRVMGPRW | | |
| | MNG642 | ELETEIIKIGRVMGPRW | | |
| | MNG702 | ELETEIIKIGRVMGPRW | | |
| ZAR1-like protein (ZAR1L) | <u>12% / WHO I+II</u> | | A6NP61 ◾ | 1 |
| | MNG501 | MGPPTFLARPGLLVPANAP | | |
| | MNG638 | SPRLCKPNTKEVGVQVSPRVDKAV | | |
| | MNG702 | MGPPTFLARPGLLVPANAP | | |
| | MNG833 | MGPPTFLARPGLLVPANAP | | |
| G protein-regulated inducer of neurite outgrowth 2 (GPRIN2) | <u>12% / WHO I+II</u> | | O60269 ◾ | 2 |
| | MNG638 | IQKHLEMQFEQLQRAPASEDS | | |
| | MNG679 | IQKHLEMQFEQLQRAPASEDSL | | |
| | MNG682 | IQKHLEMQFEQLQRAPASEDSL | | |
| | MNG702 | IQKHLEMQFEQLQRAPASEDSL | | |
| Bone morphogenetic protein 5 (BMP5) 1 peptide multi-maps to non-MNG-exclusive BMP6 (P18075) | <u>12% / WHO I+II</u> | | P22003 ◾ | 0 |
| | MNG638 | SNVILKKYRNMVVRS | | |
| | MNG642 | AAEFRIYKDRSNN | | |
| | | AAEFRIYKDRSNNR | | |
| | | AEFRIYKDRSNNR | | |
| | | TAAEFRIYKDRSNN | | |
| | | TAAEFRIYKDRSNNR | | |
| | MNG679 | RDADLFLLDTRK | | |
| | | YTNRDADLFLLDTRK | | |
| | MNG702 | AFFKATEVHFR | | |
| | | SSNVILKKYRNMVVR | | |
| Transcription factor SOX-6 (SOX6) | <u>12% / WHO I+II</u> | | P35712 ◾ | 5 |
| | MNG4 | ASMQVSPGAKMPS | | |
| | MNG499 | KTDGGSLAGNEMINGEDEMEMYDDY | | |
| | MNG642 | EMEMYDDYEDDP | | |
| | MNG679 | ITQLISLREQLLAAHDEQKKLAAS | | |
| Zinc finger E-box-binding homeobox 2 (ZEB2) | <u>12% / WHO I+II</u> | | O60315 ◾ | 1 |
| | MNG7 | SVNGRMRNNIKTGSSP | | |
| | MNG623 | EEYFAKRKLEERDGHAVSIEEYLQ | | |
| | MNG624 | EEYFAKRKLEERDGHAVSIEEYLQ | | |
| | MNG635 | EEYFAKRKLEERDGHAVSIEEYLQ | | |
| Mucin-4 (MUC4) | <u>12% / WHO I+II</u> | | Q99102 ◾ | 12 |
| | MNG499 | GRRDLRFQPVSIGR | | |
| | | GRRDLRFQPVSIGRWG | | |
| | MNG501 | DNGQIIFPESDYQIFSYPNPLPT | | |

| | | | | |
|---|---|---|---|---|
| | MNG628 | SNILHASASLPP | | |
| | MNG638 | APIPILPERGVSLFPY | | |
| | | APIPILPERGVSLFPYG | | |
| Kinesin light chain 2 (KLC2) peptides multi-map to non-MNG-exclusive KLC1 (Q07866) | <u>12% / WHO I+III</u> | | Q9H0B6 ■ | 6 |
| | MNG6 | HNLVIQYASQGRYE | | |
| | | LHNLVIQYASQGRYE | | |
| | MNG612 | LHNLVIQYASQGRYE | | |
| | MNG636 | LHNLVIQYASQGRYE | | |
| | MNG734 | HNLVIQYASQGRYE | | |
| Calcium-binding protein 39-like (CAB39L) | <u>12% / WHO I+II</u> | | Q9H9S4 ■ | 2 |
| | MNG638 | GKKDVTQIFNNILRRQ | | |
| | MNG641 | DRHNFAIMTKYISKPE | | |
| | MNG666 | DRHNFAIMTKYISKPE | | |
| | MNG702 | DRHNFAIMTKYISKPE | | |
| Calpain-14 (CAPN14) | <u>9% / WHO I</u> | | A8MX76 ■ | 1 |
| | MNG7 | MLRVENMEDVFQN | | |
| | MNG499 | IMLSDDVCQLMLIRYGGPR | | |
| | MNG673 | LPPEFFQRNTPLSQPDRFLKEKE | | |
| Methylenetetrahydrofolate reductase (MTHFR) | <u>9% / WHO I+II</u> | | P42898 ■ | 9 |
| | MNG499 | IKDVIEPIKDNDAAIR | | |
| | MNG638 | KGENITNAPELQPNAVT | | |
| | MNG814 | IKDVIEPIKDNDAAIR | | |
| Killer cell immunoglobulin-like receptor 2DS4 (KIR2DS4) | <u>9% / WHO I</u> | | P43632 ■ | 1 |
| | MNG7 | MSLMVIIMACVGFFLLQGAW | | |
| | MNG623 | MSLMVIIMACVGFFLLQGAW | | |
| | MNG624 | MSLMVIIMACVGFFLLQGAW | | |
| Probable allantoicase (ALLC) | <u>9% / WHO I+II</u> | | Q8N6M5 ■ | 1 |
| | MNG6 | PSSICLLRPREKPM | | |
| | MNG642 | DFFAPAENLIKSDSPCF | | |
| | MNG673 | IPERGTRTGAAATPEEFEAIAELKS | | |
| Tubulin polyglutamylase TTLL6 (TTLL6) | <u>9% / WHO I+II</u> | | Q8N841 ♦ | 2 |
| | MNG632 | QMKKKVEMQGE | | |
| | MNG702 | QMKKKVEMQGE | | |
| | MNG833 | SEEKGDSSKEDPKETVALAFV | | |
| Serine/threonine-protein kinase Sgk3 (SGK3) | <u>9% / WHO I+II</u> | | Q96BR1 ■ | 4 |
| | MNG682 | TFCGTPEYLAPEVIRKQPYDNT | | |
| | MNG702 | TFCGTPEYLAPEVIRKQPYDNT | | |
| | MNG833 | TFCGTPEYLAPEVIRKQPYDNT | | |
| PC-esterase domain-containing protein 1B (PCED1B) | <u>9% / WHO I-III</u> | | Q96HM7 ■ | 4 |
| | MNG634 | NFMVGPQLPMPFFPTPRYQ | | |
| | MNG641 | NFMVGPQLPMPFFPTPRYQ | | |
| | MNG734 | NFMVGPQLPMPFFPTPRYQ | | |
| Mitochondrial import inner membrane translocase subunit TIM44 (TIMM44) | <u>9% / WHO I</u> | | O43615 ■ | 1 |
| | MNG4 | KRTEFAGDKFKEEKVFE | | |
| | MNG6 | KWYQQWKDFKENNVVFNRFFEMKMK | | |
| | MNG682 | KWYQQWKDFKENNVVFNRFFEMKMK | | |
| Ubiquitin conjugation factor E4 B (UBE4B) | <u>9% / WHO I+II</u> | | O95155 ■ | 5 |
| | MNG641 | KDLIGQILMEVLMMSTQTRDEN | | |
| | MNG666 | VDMFHILTKQVQKPF | | |
| | MNG702 | VDMFHILTKQVQKPF | | |
| Protein SGT1 (ECD) | <u>9% / WHO I</u> | | O95905 ■ | 4 |
| | MNG5 | DSDDLDDEDFECLDSDDDLDF | | |
| | MNG6 | LRDPIDLRACRVFKTFLPETRIMTS | | |
| | MNG632 | EKIQASLHRAHCFL | | |
| T-lymphocyte activation antigen CD86 (CD86) | <u>9% / WHO I</u> | | P42081 ■ | 22 |
| | MNG7 | DKTRLLSSPFSIELEDPQPPP | | |
| | | TDKTRLLSSPFSIELEDPQPPP | | |
| | MNG623 | DKTRLLSSPFSIELEDPQPPP | | |
| | MNG624 | DKTRLLSSPFSIELEDPQPPP | | |
| Signal transducer and activator of transcription 5B (STAT5B) | <u>9% / WHO I+II</u> | | P51692 ■ | 13 |
| | MNG3 | DGVMEVLKKHLKPH | | |
| | MNG702 | DGVMEVLKKHLKPH | | |
| | MNG833 | DGVMEVLKKHLKPH | | |
| Nucleoporin GLE1 (GLE1) | <u>9% / WHO I+II</u> | | Q53GS7 ■ | 3 |
| | MNG628 | ISGIIRASSESSYPTAE | | |
| | MNG634 | KEEGQIRLRALYALQEEML | | |
| | MNG814 | KQAEQERLRKEEGQI | | |
| N-acetyltransferase ESCO1 (ESCO1) | <u>9% / WHO I+II</u> | | Q5FWF5 ■ | 14 |
| | MNG499 | KVLEVKSDSKEDENLVINEVINSPK | | |
| | MNG635 | KVLEVKSDSKEDENLVINEVINSPK | | |
| | MNG641 | KVLEVKSDSKEDENLVINEVINSPK | | |
| Protein angel homolog 2 (ANGEL2) | <u>9% / WHO I-III</u> | | Q5VTE6 ■ | 0 |
| | MNG634 | DHYGAEIRPSLESLGY | | |
| | MNG734 | DHYGAEIRPSLESLGY | | |
| | MNG833 | DHYGAEIRPSLESLGY | | |

| | | | | | |
|---|---|---|---|---|---|
| Meteorin-like protein (METRNL) | <u>9% / WHO I+II</u> | | | Q641Q3 ■ | 3 |
| | MNG666 | GFQYELVRRHRASD | | | |
| | | TGFQYELVRRHRASD | | | |
| | MNG702 | GFQYELVRRHRASD | | | |
| | | TGFQYELVRRHRASDLH | | | |
| | MNG833 | GFQYELVRRHRASD | | | |
| E3 ubiquitin-protein ligase RFWD3 (RFWD3) | <u>9% / WHO I</u> | | | Q6PCD5 ■ | 2 |
| | MNG3 | TNFISGLQRLHGMLEFL | | | |
| | MNG666 | TNFISGLQRLHGMLEFL | | | |
| | MNG702 | TNFISGLQRLHGMLEFL | | | |
| Highly divergent homeobox (HDX) | <u>9% / WHO I+II</u> | | | Q7Z353 ■ | 2 |
| | MNG634 | QRSYKPEHTGPALHNLC | | | |
| | MNG635 | FIENELEIQKQKYFKLQ | | | |
| | MNG666 | SEMTVPQKPSVCHRPCKIEP | | | |
| Signal-regulatory protein delta (SIRPD) | <u>9% / WHO I+II</u> | | | Q9H106 ♦ | 3 |
| | MNG1 | PLPSLLLYLLLELA | | | |
| | | PLPSLLLYLLLELAG | | | |
| | MNG3 | PLPSLLLYLLLELA | | | |
| | MNG673 | PLPSLLLYLLLELA | | | |
| Uncharacterized protein C1orf112 (C1orf112) | <u>9% / WHO I+II</u> | | | Q9NSG2 ♦ | 1 |
| | MNG3 | FVSSLGKLF | | | |
| | MNG6 | FVSSLGKLF | | | |
| | MNG642 | KAVFYSFEQCSGELSLP | | | |

**Supplementary Table 17. Meningioma-associated HLA class I ligands presented on at least five tumors.** Peptides already reported to derive from meningioma-associated antigens were excluded from this listing. The number of positive non-meningeal tumors was based on n=841 HLA class I peptidome datasets. HLA restrictions not passing manual assessment as quality control are indicated in italic. These combinations of sequence and HLA restriction were excluded from downstream analyses such as calculation of peptides matching per patient worldwide.

| Peptide sequence | HLA restriction | Frequency of positive patients | | Antigen (UniProt accession) | Protein frequency on meningiomas | Peptide-positive non-meningeal tumors<br><br>Protein frequency on non-meningeal tumors | Protein frequency on dura (n=9) / benign samples (n=418) |
|---|---|---|---|---|---|---|---|
| TLQSTLLLL | A*02:01<br>C*17:01 | <u>30%</u><br>MNG501<br>MNG628<br>MNG641<br>MNG673<br>MNG702 | <u>WHO I-III</u><br>MNG612<br>MNG638<br>MNG666<br>MNG700<br>MNG734 | Mimecan (OGN; P20774) | 61% | 1<br>2% | 11%<br>5% |
| QTFPNVREM | B*57:01<br>C*03:04<br>C*06:02<br>C*12:03 | <u>30%</u><br>MNG2<br>MNG5<br>MNG634<br>MNG636<br>MNG666 | <u>WHO I-III</u><br>MNG3<br>MNG628<br>MNG635<br>MNG661<br>MNG734 | Forkhead box protein C2 (FOXC2; Q99958) | 85% | 0<br>7% | 67%<br>8% |
| IPISNILMV | B*07:02<br>B*51:01<br>B*51:02<br>B*55:01 | <u>24%</u><br>MNG499<br>MNG612<br>MNG642<br>MNG814 | <u>WHO I-III</u><br>MNG501<br>MNG635<br>MNG702<br>MNG833 | Interferon-induced protein 44-like (IFI44L; Q53G44) | 30% | 37<br>12% | 11%<br>3% |
| LLLPVVSFA | A*02:01 | <u>21%</u><br>MNG612<br>MNG641<br>MNG673<br>MNG734 | <u>WHO I-III</u><br>MNG628<br>MNG666<br>MNG702 | Cathepsin K (CTSK; P43235) | 21% | 29<br>7% | 0%<br>4% |
| SLPELVHAV | A*02:01 | <u>21%</u><br>MNG6<br>MNG641<br>MNG673<br>MNG734 | <u>WHO I-III</u><br>MNG628<br>MNG666<br>MNG702 | Sestrin-3 (SESN3; P58005) | 11% | 23<br>11% | 33%<br>4% |
| YSLEKVFGI | A*02:01<br>C*02:02<br>*C*06:02*<br>*C*07:01*<br>C*12:03<br>C*17:01 | <u>21%</u><br>MNG2<br>MNG612<br>MNG641<br>MNG734 | <u>WHO I-III</u><br>MNG5<br>MNG638<br>MNG666 | Melanoma-associated antigen D2 (MAGED2; Q9UNF1) | 94% | 24<br>52% | 56%<br>48% |
| EVYGTGVASTR | A*34:01<br>A*66:02<br>A*68:01 | <u>21%</u><br>MNG3<br>MNG499<br>MNG642<br>MNG814 | <u>WHO I-III</u><br>MNG6<br>MNG632<br>MNG734 | Serine palmitoyl-transferase 3 (SPTLC3; Q9NUV7) | 76% | 2<br>20% | 33%<br>17% |

| Peptide | HLA | | | Protein | | | | |
|---|---|---|---|---|---|---|---|---|
| KAIDYIRFL | C*03:04<br>C*12:03<br>C*16:01 | 21%<br>MNG1<br>MNG628<br>MNG666<br>MNG734 | WHO I-III<br>MNG4<br>MNG636<br>MNG673 | Sterol regulatory element-binding protein 1 (SREBF1; P36956) | 55% | 12<br>23% | 11%<br>13% | |
| KLNNLTFLY | A*03:01<br>A*29:02<br>A*30:02<br>B*15:01 | 21%<br>MNG1<br>MNG641<br>MNG673<br>MNG814 | WHO I+II<br>MNG632<br>MNG646<br>MNG682 | Mimecan (OGN; P20774) | 61% | 2<br>2% | 11%<br>5% | |
| SSFPTVVIY | C*02:02<br>C*12:03 | 21%<br>MNG628<br>MNG637<br>MNG666<br>MNG734 | WHO I-III<br>MNG636<br>MNG661<br>MNG702 | GPN-loop GTPase 1 (GPN1; Q9HCN4) | 49% | 32<br>25% | 22%<br>15% | |
| YPSGIHLEL | B*07:02<br>B*35:01 | 21%<br>MNG2<br>MNG632<br>MNG642<br>MNG814 | WHO I+II<br>MNG7<br>MNG634<br>MNG666 | Sulfate transporter (SLC26A2; P50443) | 70% | 2<br>4% | 33%<br>3% | |
| LPYNTSLVEM | B*07:02<br>B*35:01 | 18%<br>MNG7<br>MNG632<br>MNG641 | WHO I+II<br>MNG628<br>MNG634<br>MNG814 | Tropomodulin-1 (TMOD1; P28289) | 67% | 2<br>8% | 22%<br>11% | |
| NYAGALMYF | A*24:02 | 18%<br>MNG1<br>MNG635<br>MNG661 | WHO I+II<br>MNG628<br>MNG638<br>MNG833 | Sodium leak channel non-selective protein (NALCN; Q8IZF0) | 24% | 2<br>2% | 0%<br>1% | |
| SELLVVKM | B*18:01<br>B*40:02 | 18%<br>MNG1<br>MNG628<br>MNG646 | WHO I+II<br>MNG6<br>MNG638<br>MNG661 | Obscurin-like protein 1 (OBSL1; O75147) | 45% | 5<br>17% | 33%<br>15% | |
| VYGLYTSFF | A*24:02 | 18%<br>MNG5<br>MNG635<br>MNG661 | WHO I+II<br>MNG628<br>MNG638<br>MNG833 | Sulfate transporter (SLC26A2; P50443) | 70% | 3<br>4% | 33%<br>3% | |
| DGYIEVIGF | B*35:01<br>*B*51:01* | 18%<br>MNG624<br>MNG632<br>MNG682 | WHO I+II<br>MNG628<br>MNG634<br>MNG833 | Diacylglycerol kinase iota / zeta (DGKI / DGKZ; O75912 / Q13574) | 18% / 39% | 34<br>6% / 19% | 0% / 0%<br>3% / 16% | |
| DVIDGPISQR | A*66:01<br>A*68:01 | 18%<br>MNG3<br>MNG499<br>MNG734 | WHO I-III<br>MNG6<br>MNG632<br>MNG814 | Signal-induced proliferation-associated 1-like protein 1 (SIPA1L1; O43166) | 49% | 7<br>20% | 33%<br>18% | |
| EVQDRVMLTGR | A*33:01<br>A*68:01 | 18%<br>MNG3<br>MNG499<br>MNG634 | WHO I<br>MNG6<br>MNG632<br>MNG636 | Protein yippee-like 5 (YPEL5; P62699) | 70% | 28<br>47% | 56%<br>41% | |
| SMVEDITGLRL | A*02:01 | 18%<br>MNG501<br>MNG638<br>MNG666 | WHO I-III<br>MNG612<br>MNG641<br>MNG673 | Desmoplakin (DSP; P15924) | 97% | 26<br>34% | 56%<br>32% | |
| SVFAGVVGV | A*02:01 | 18%<br>MNG6<br>MNG641<br>MNG673 | WHO I-III<br>MNG612<br>MNG666<br>MNG702 | Guanylate cyclase soluble subunit alpha-3 (GUCY1A3; Q02108) | 64% | 42<br>17% | 56%<br>16% | |
| VPYSRALIM | B*35:03<br>B*51:01<br>B*51:02 | 18%<br>MNG6<br>MNG612<br>MNG702 | WHO I-III<br>MNG499<br>MNG642<br>MNG833 | Lysoplasmaloge-nase-like protein TMEM86A (TMEM86A; Q8N2M4) | 24% | 27<br>5% | 11%<br>2% | |
| YENLLKASF | B*18:01<br>B*15:35 | 18%<br>MNG1<br>MNG628<br>MNG642 | WHO I+II<br>MNG6<br>MNG638<br>MNG661 | Desmoplakin (DSP; P15924) | 97% | 12<br>34% | 56%<br>32% | |
| AANGVFHVV | C*12:03 | 15%<br>MNG628<br>MNG661<br>MNG734 | WHO I-III<br>MNG636<br>MNG666 | Stabilin-1 (STAB1; Q9NY15) | 79% | 19<br>34% | 56%<br>31% | |
| AEFPELAAF | B*18:01<br>B*40:02<br>B*44:03 | 15%<br>MNG1<br>MNG636<br>MNG646 | WHO I+II<br>MNG6<br>MNG638 | Uncharacterized protein KIAA1755 (KIAA1755; Q5JYT7) | 45% | 0<br>2% | 0%<br>2% | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| DEDRFMMQF | B*18:01 | <u>15%</u> MNG1 MNG628 MNG661 | <u>WHO I+II</u> MNG6 MNG638 | Formin-like protein 2 (FMNL2; Q96PY5) | 48% | 3 18% | 11% 14% |
| DEKFHIAY | B*18:01 | <u>15%</u> MNG1 MNG623 MNG661 | <u>WHO I+II</u> MNG6 MNG628 | Sestrin-1 (SESN1; Q9Y6P5) | 67% | 6 28% | 56% 34% |
| DMFATPQYR | A*33:01 A*68:01 | <u>15%</u> MNG3 MNG499 MNG814 | <u>WHO I</u> MNG6 MNG636 | Adiponectin receptor protein 2 (ADIPOR2; Q86V24) | 24% | 18 8% | 0% 3% |
| DVFQHSQSR | A*33:01 A*34:01 A*66:01 A*68:01 | <u>15%</u> MNG3 MNG636 MNG734 | <u>WHO I-III</u> MNG632 MNG642 | Constitutive coactivator of PPAR-gamma-like protein 1 (FAM120A; Q9NZB2) | 85% | 3 42% | 33% 36% |
| DYTIGFGKF | A*24:02 | <u>15%</u> MNG1 MNG628 MNG833 | <u>WHO I+II</u> MNG5 MNG661 | Integrin beta-4 (ITGB4; P16144) | 76% | 7 21% | 22% 27% |
| EIVGNLPSAMR | A*68:01 | <u>15%</u> MNG3 MNG499 MNG814 | <u>WHO I+II</u> MNG6 MNG632 | Kelch-like protein 23 (KLHL23; Q8NBE8) | 21% | 3 3% | 0% 2% |
| ETASVLVNYR | A*66:01 A*68:01 | <u>15%</u> MNG3 MNG632 MNG814 | <u>WHO I-III</u> MNG6 MNG734 | Intersectin-2 (ITSN2; Q9NZM3) | 24% | 6 19% | 11% 13% |
| EVASEIQPFLR | A*68:01 | <u>15%</u> MNG3 MNG499 MNG814 | <u>WHO I+II</u> MNG6 MNG632 | Desmoplakin (DSP; P15924) | 97% | 1 34% | 56% 23% |
| FEGNVFMY | B*18:01 | <u>15%</u> MNG1 MNG628 MNG661 | <u>WHO I+II</u> MNG6 MNG638 | Cell cycle control protein 50A (TMEM30A; Q9NV96) | 64% | 7 30% | 44% 18% |
| GLFQKLENI | A*02:01 | <u>15%</u> MNG501 MNG666 MNG700 | <u>WHO I+II</u> MNG641 MNG673 | Desmoplakin (DSP; P15924) | 97% | 1 34% | 56% 32% |
| HSIGAVGPTGR | A*68:01 | <u>15%</u> MNG3 MNG499 MNG814 | <u>WHO I+II</u> MNG6 MNG632 | Serine palmitoyl-transferase 3 (SPTLC3; Q9NUV7) | 76% | 0 20% | 33% 17% |
| IYNGKVTSI | A*24:02 | <u>15%</u> MNG1 MNG635 MNG833 | <u>WHO I+II</u> MNG628 MNG661 | ATP-dependent RNA helicase DHX8 (DHX8; Q14562) | 58% | 6 23% | 11% 18% |
| LAFPGEMLL | C*03:03 C*12:03 | <u>15%</u> MNG628 MNG661 MNG734 | <u>WHO I-III</u> MNG636 MNG666 | Neutral amino acid transporter A (SLC1A4; P43007) | 18% | 23 9% | 0% 3% |
| LPIGIALMF | B*51:01 | <u>15%</u> MNG499 MNG632 MNG833 | <u>WHO I+II</u> MNG501 MNG682 | Multidrug and toxin extrusion protein 1 (SLC47A1; Q96FL8) | 70% | 1 5% | 33% 5% |
| LPIIANMI | B*51:01 | <u>15%</u> MNG499 MNG612 MNG833 | <u>WHO I-III</u> MNG501 MNG642 | Armadillo repeat-containing X-linked protein 2 (ARMCX2; Q7L311) | 45% | 11 12% | 33% 11% |
| MEYFPTTRF | B*18:01 | <u>15%</u> MNG1 MNG623 MNG638 | <u>WHO I+II</u> MNG6 MNG628 | Phospholipase D4 (PLD4; Q96BZ4) | 39% | 1 5% | 33% 4% |
| PYFDKPLFI | A*24:02 | <u>15%</u> MNG628 MNG638 MNG833 | <u>WHO I+II</u> MNG635 MNG661 | Sodium leak channel non-selective protein (HSBP1; Q8IZF0) | 24% | 1 2% | 0% 1% |
| QTMSDQIIGR | A*68:01 | <u>15%</u> MNG3 MNG499 MNG814 | <u>WHO I+II</u> MNG6 MNG632 | Heat shock factor-binding protein 1 (O75506) | 15% | 8 3% | 0% 3% |

| Peptide | HLA | | | Protein | | | |
|---|---|---|---|---|---|---|---|
| STITPTSTR | A*66:01<br>A*68:01 | 15%<br>MNG3<br>MNG499<br>MNG734 | WHO I+III<br>MNG6<br>MNG632 | Prolow-density lipoprotein receptor-related protein 1 (LRP1; Q07954) | 88% | 7<br>32% | 67%<br>38% |
| TPHDFIEHF | B*35:01 | 15%<br>MNG624<br>MNG632<br>MNG833 | WHO I+II<br>MNG628<br>MNG682 | G1/S-specific cyclin-D1 (CCND1; P24385) | 79% | 13<br>36% | 67%<br>39% |
| VALPVYLLI | B*51:01 | 15%<br>MNG499<br>MNG612<br>MNG833 | WHO I-III<br>MNG501<br>MNG702 | Phosphatidylinositol N-acetylgluco-saminyltransferase subunit P (PIGP; P57054) | 30% | 27<br>9% | 11%<br>2% |
| VAVGFPMMI | C*12:03 | 15%<br>MNG628<br>MNG661<br>MNG734 | WHO I-III<br>MNG636<br>MNG666 | Putative sodium-coupled neutral amino acid transporter 10 (SLC38A10; Q9HBR0) | 48% | 19<br>24% | 33%<br>19% |
| VEVQLPELY | B*18:01<br>B*44:03 | 15%<br>MNG1<br>MNG636<br>MNG661 | WHO I+II<br>MNG6<br>MNG638 | Voltage-gated potassium channel subunit beta-1 (KCNAB1; Q14722) | 21% | 9<br>5% | 11%<br>4% |
| YGYSNPKIL | C*03:04<br>C*12:03 | 15%<br>MNG3<br>MNG636<br>MNG734 | WHO I-III<br>MNG628<br>MNG661 | Transcription factor AP-1 (JUN; P05412) | 33% | 27<br>24% | 11%<br>26% |
| YNFEYSVVF | B*38:01<br>C*12:03 | 15%<br>MNG628<br>MNG637<br>MNG734 | WHO I-III<br>MNG636<br>MNG666 | Renin receptor (RENR; O75787) | 70% | 16<br>42% | 33%<br>41% |
| YPNAKVELV | B*51:01<br>B*51:08 | 15%<br>MNG4<br>MNG501<br>MNG702 | WHO I+II<br>MNG499<br>MNG702 | PDZ and LIM domain protein 4 (PDLIM4; P50479) | 28% | 17<br>8% | 0%<br>4% |
| YYASAFSMM | A*24:02 | 15%<br>MNG628<br>MNG638<br>MNG833 | WHO I+II<br>MNG635<br>MNG661 | Dolichyl-diphospho-oligosaccharide-protein glycosyl-transferase 48 kDa subunit (DDOST; P39656) | 58% | 0<br>39% | 44%<br>41% |
| YYKSTSSAF | A*24:02 | 15%<br>MNG1<br>MNG635<br>MNG833 | WHO II<br>MNG628<br>MNG638 | Galactose-3-O-sulfotransferase 4 (GAL3ST4; Q96RP7) | 30% | 9<br>6% | 11%<br>1% |
| DAFDGHAAR | A*33:01<br>A*34:01<br>A*66:01<br>A*68:01 | 15%<br>MNG3<br>MNG636<br>MNG734 | WHO I-III<br>MNG632<br>MNG642 | CDP-diacylglycerol-inositol 3-phospha-tidyltransferase (CDIPT; O14735) | 28% | 6<br>9% | 0%<br>6% |
| DSERFFIRY | A*01:01 | 15%<br>MNG4<br>MNG7<br>MNG646 | WHO I<br>MNG5<br>MNG624 | Tubulin-specific chaperone cofactor E-like protein (TBCEL; Q5QJ74) | 18% | 30<br>7% | 11%<br>9% |
| EAENTLQSFR | A*68:01 | 15%<br>MNG3<br>MNG499<br>MNG814 | WHO I+II<br>MNG6<br>MNG632 | Vimentin (VIM; P08670) | 100% | 1<br>88% | 100%<br>96% |
| EAIFLEVKY | A*26:01<br>A*29:02<br>B*35:01 | 15%<br>MNG624<br>MNG679<br>MNG833 | WHO I+II<br>MNG634<br>MNG682 | Vacuolar protein sorting-associated protein 13D (VPS13D; Q5THJ4) | 61% | 1<br>30% | 33%<br>35% |
| EVFAGSGTSGQR | A*66:01<br>A*68:01 | 15%<br>MNG3<br>MNG632<br>MNG814 | WHO I-III<br>MNG6<br>MNG734 | Procollagen C-endopeptidase enhancer 1 (PCOLCE; Q15113) | 21% | 1<br>1% | 0%<br>1% |
| EVPSFTMGR | A*34:01<br>A*66:01<br>A*68:01 | 15%<br>MNG3<br>MNG642<br>MNG814 | WHO I+II<br>MNG6<br>MNG734 | PRKR-interacting protein 1 (PRKRIP1; Q9H875) | 15% | 5<br>1% | 0%<br>0.5% |
| FFNIPQIQY | A*29:02 | 15%<br>MNG1<br>MNG673<br>MNG682 | WHO I+II<br>MNG623<br>MNG679 | 28S ribosomal protein S25, mitochondrial (MRPS25; P82663) | 15% | 12<br>4% | 0%<br>5% |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| FLMEMGFRM | A*02:01 | 15% MNG6 MNG628 MNG673 | WHO I-III MNG612 MNG638 | Mediator of RNA polymerase II transcription subunit 18 (MED18; Q9BUE0) | 24% | 13 7% | 11% 4% |
| FVFGENMVER | A*68:01 | 15% MNG3 MNG499 MNG814 | WHO I+II MNG6 MNG632 | Ran-binding protein 3-like (RANBP3L; Q86VV4) | 24% | 1 1% | 0 1% |
| GAFGLPITV | C*02:02 C*12:03 | 15% MNG636 MNG666 MNG734 | WHO I+III MNG661 MNG702 | Glutathione S-transferase kappa 1 (GSTK1; Q9Y2Q3) | 24% | 10 27% | 11% |
| GLLPLLREA | A*02:01 | 15% MNG641 MNG673 MNG734 | WHO I-III MNG666 MNG702 | Stabilin-1 (STAB1; Q9NY15) | 79% | 34 34% | 56% |
| HTNDTIGSVR | A*66:01 A*68:01 | 15% MNG3 MNG632 MNG814 | WHO I-III MNG6 MNG734 | Probable ubiquitin carboxyl-terminal hydrolase FAF-X / FAF-Y (USP9X/Y; Q93008 / O00507) | 82% / 64% 53% / 41% | 18 | 67% / 44% 57%/ 41% |
| NLMGKTSER | A*34:01 A*66:01 A*68:01 | 15% MNG3 MNG632 MNG734 | WHO I-III MNG6 NBG642 | Transcription factor 12 (TCF12; Q99081) | 61% 43% | 3 | 33% 29% |
| RVYDIPPKF | A*30:02 A*32:01 B*15:35 C*12:03 | 15% MNG7 MNG634 MNG661 | WHO I+II MNG632 MNG642 | Cytochrome b-245 heavy chain (CYBB; P04839) | 88% 34% | 4 | 56% 32% |
| SLSLENVLYY | A*29:02 B*15:01 | 15% MNG1 MNG673 MNG682 | WHO I+II MNG623 MNG679 | Sortilin-related receptor (SORL1; Q92673) | 45% 23% | 14 | 11% 17% |
| SPNNFLSYY | B*35:01 | 15% MNG628 MNG634 MNG833 | WHO I+II MNG632 MNG682 | G1/S-specific cyclin-D1 (CCND1; P24385) | 79% 36% | 8 | 67% 39% |
| SVDSNLLSDY | A*01:01 | 15% MNG3 MNG624 MNG702 | WHO I MNG7 MNG646 | Oxidative stress-induced growth inhibitor 2 (OSGIN2; Q9Y236) | 42% 12% | 41 | 33% 7% |
| SVIEGVSRSR | A*66:01 A*68:01 | 15% MNG3 MNG632 MNG814 | WHO I-III MNG6 MNG734 | BTB/POZ domain-containing protein 9 (BTBD9; Q96Q07) | 27% 12% | 13 | 0% 6% |
| SYFPTVPGVYI | A*24:02 | 15% MNG1 MNG635 MNG833 | WHO I+II MNG5 MNG638 | Filamin-B (FLNB; O75369) | 94% 46% | 7 | 67% 60% |
| TEEPLKQSF | B*18:01 | 15% MNG1 MNG628 MNG661 | WHO I+II MNG6 MNG638 | GRAM domain-containing protein 3 (GRAMD3; Q96HH9) | 42% 12% | 9 | 11% 13% |
| VELLMHNDY | B*18:01 | 15% MNG1 MNG628 MNG661 | WHO I+II MNG6 MNG638 | Importin subunit alpha-5/6/7 (KPNA1/5/6; P52294 / O15131 / O60684) | 73% / 58 % / 64% 52% / 45% / 46% | 12 | 78% / 67% / 78% 53% / 45% / 46% |
| VGVDFALKV | C*12:03 | 15% MNG628 MNG661 MNG734 | WHO I-III MNG636 MNG666 | Ras-related protein Rab-7L1 (RAB7L1; O14966) | 27% 4% | 12 | 11% / 2% |
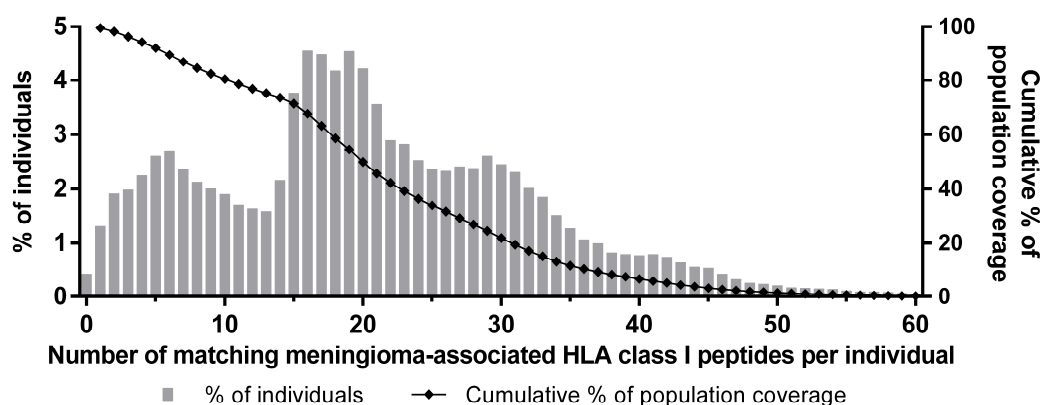
**Supplementary Figure 9. Population coverage of meningioma-associated HLA-A, -B, and -C ligands.** Using the population coverage tool provided by the IEDB Analysis Resource, the world population coverage of the 141 meningioma-associated peptides was calculated. The percentage of individuals with a specific number of matching peptides (max. of 74) is indicated by bar charts (associated with the left y-axis). The line diagram (associated with the right y-axis) shows the cumulative percentage of population coverage. The candidate target peptides cover 99.57% of the world population (first diamond on line diagram counted from the left) meaning that only 0.43% of all individuals are negative for all HLA-A, -B, and -C allotypes for which meningioma-associated peptides were defined. On average, 21 peptides are expected to match per patient worldwide.
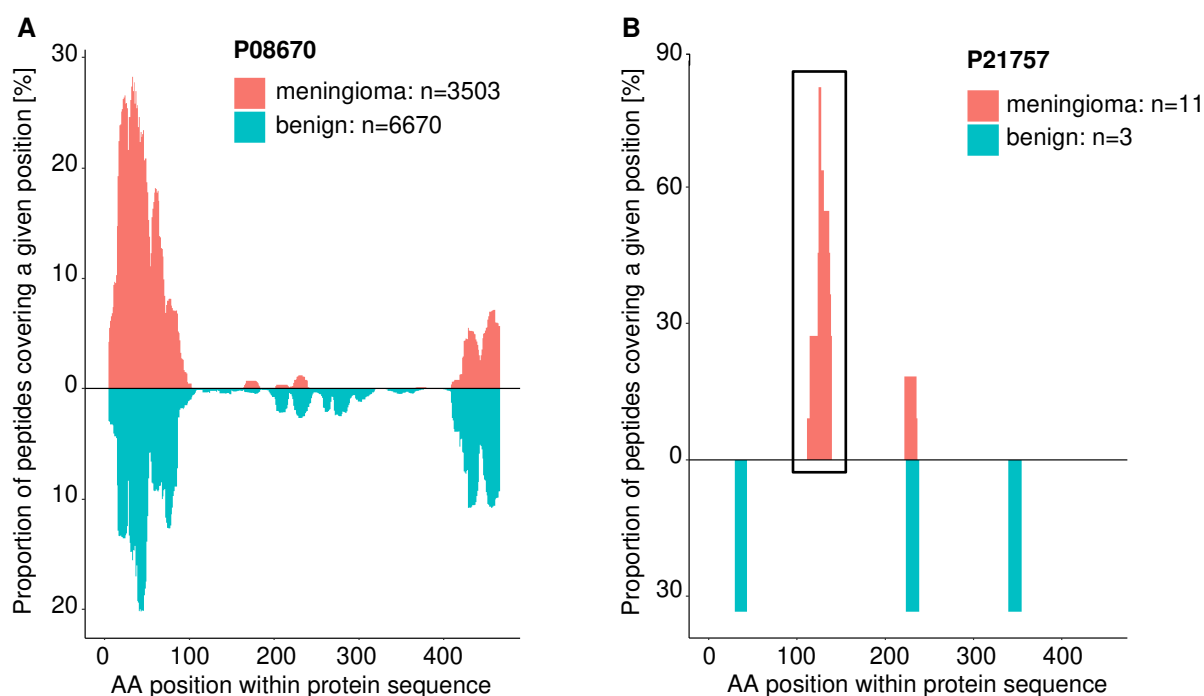


**Supplementary Figure 10. Hotspot analysis of antigens represented by meningioma-exclusive HLA class II-restricted peptides.** Peptides were aligned to source protein sequences, whereby identifications on meningioma (n=33) or autologous tumor-free dura (n=9) / benign tissues (n=364) are shown in red or green, respectively. The proportion of all peptides covering a given position is indicated on the y-axis (separately for malignant and benign hits). **(A) Vimentin (VIM; P08670).** Comparative profiling identified a total of 29 meningioma-exclusive peptides derived from vimentin. However, identical peptide sequences differing only in 1 AA in length were identified on benign tissues, which cannot be reflected by comparative profiling. HLA class II molecules are renowned for promiscuous binding of peptides due to decreased length preferences and degenerate peptide motifs and TCRs of CD4+ T cells rather recognize the core sequence of HLA class II-presented peptides than distinguishing between length variants. This refutes all meningioma-exclusive peptides originating from vimentin to be candidate targets for cancer immunotherapy, as they derived from regions presented on both benign and

malignant tissues. **(B) Macrophage scavenger receptor types I and II (MSR1; P21757).** Despite one part of the protein sequence was covered by both malignant and benign peptide identifications, eight distinct sequences derived from a meningioma-associated presentation hotspot were identified between position 112 and 139 (indicated by box).

**Supplementary Table 18. Meningioma-exclusive HLA class II-presented peptides derived from meningioma-associated HLA presentation hotspots.** Peptides already reported to derive from meningioma-associated antigens were excluded from this listing. The number of positive non-meningeal tumors was based on n=593 HLA class II peptidome datasets.

| Antigen (UniProt accession) | Protein frequency on meningiomas | Peptide sequence | Frequency of positive patients | Protein frequency on non-meningeal tumors / Peptide-positive non-meningeal tumors | Protein frequency on dura (n=9) / benign samples (n=364) |
|---|---|---|---|---|---|
| Unconventional myosin-Ic (MYI1C; O00159) | 49% | | WHO I+II | 11% | 0% / 17% |
| | | ENQLKYLTRLLSVE | 12% MNG623 MNG702 MNG666 | 1 | |
| | | ENQLKYLTRLLSVEG | 6% MNG623 MNG6 | 5 | |
| | | NQLKYLTRLLSVEG | 6% MNG623 MNG6 | 0 | |
| | | TENQLKYLTRLLSVE | 3% MNG623 | 0 | |
| | | TENQLKYLTRLLSVEG | 12% MNG623 MNG666 MNG6 MNG635 | 5 | |
| E3 ubiquitin-protein ligase MARCH6 (MARCH6; O60337) | 15% | | WHO I+II | 2% | 0% / 2% |
| | | DQTPLFYPWQDWALGVLH | 15% MNG642 MNG673 MNG833 MNG637 MNG666 | 0 | |
| | | TPLFYPWQDWALGVLH | 6% MNG833 MNG637 | 0 | |
| Slit homolog 2 protein (SLIT2; O94813) | 52% | | WHO I-III | 7% | 22% / 7% |
| | | DHIAVELYRGRVRAS | 12% MNG634 MNG734 MNG6 MNG641 | 0 | |
| | | DHIAVELYRGRVRASYD | 6% MNG641 MNG6 | 0 | |
| | | DKDHIAVELYRGRVRASYD | 6% MNG641 MNG6 | 0 | |
| | | HIAVELYRGRVRAS | 3% MNG636 | 0 | |
| | | HIAVELYRGRVRASYD | 3% MNG6 | 1 | |
| | | KGDKDHIAVELYRGRVRASY | 3% MNG636 | 0 | |
| EGF-containing fibulin-like extracellular matrix protein 2 (EFEMP2; O95967) | 45% | | WHO I-III | 8% | 33% / 6% |
| | | DVFQIQATSVYPG | 9% MNG638 MNG734 MNG6 | 0 | |
| | | ERSVPADVFQIQATSVYPG | 3% MNG636 | 0 | |
| | | ITSERSVPADVFQIQATSVYPGAYN | 3% MNG814 | 1 | |
| | | SVPADVFQIQATSVYPG | 6% MNG638 MNG636 | 1 | |
| | | VPADVFQIQATSVYPG | 24% MNG6 MNG636 MNG641 MNG734 MNG3 MNG624 MNG638 MNG682 | 12 | |
| Coagulation factor XIII A chain (F13A1; P00488) | 79% | | WHO I+III | 23% | 44% / 32% |
| | | LSANITFYTGVPKAEF | 9% MNG634 MNG6 MNG636 | 1 | |
| | | LSANITFYTGVPKAEFK | 6% MNG734 MNG636 | 0 | |
| | | SANITFYTGVPKAEF | 6% MNG734 MNG612 | 0 | |
| Antithrombin-III (SERPINC1; P01008) | 85% | | WHO I-III | 50% | 89% / 48% |
| | | SDQIHFFFAKLN | 15% MNG612 MNG641 MNG6 MNG636 MNG734 | 1 | |
| Insulin-like growth factor II (IGF2; P01344) | 70% | | WHO I+II | 16% | 44% / 18% |
| | | PAHGGAPPEM | 3% | MNG637 0 | |
| | | PAHGGAPPEMA | 3% | MNG637 1 | |
| | | PAHGGAPPEMAS | 6% MNG702 | MNG637 0 | |

| | | | | | | WHO | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PAHGGAPPEMASN | 33% | MNG3 | 8 | | | | |
| | | | | MNG624 MNG637 | | | | | |
| | | | | MNG638 MNG641 | | | | | |
| | | | | MNG666 MNG673 | | | | | |
| | | | | MNG679 MNG702 | | | | | |
| | | | | MNG833 | | | | | |
| Fibronectin (FN1; P02751) | 94% | | | | | WHO I-III | 54% | 100% / 65% | |
| | | LTPGVEYVYTIQVLRDGQERDAPI | 3% | MNG632 | 0 | | | | |
| | | TPGVEYVYTIQVLRDG | 6% | MNG638 | 6 | | | | |
| | | | | MNG679 | | | | | |
| | | TPGVEYVYTIQVLRDGQE | 9% | MNG1 | 1 | | | | |
| | | | | MNG638 MNG679 | | | | | |
| | | TPGVEYVYTIQVLRDGQER | 3% | MNG638 | 1 | | | | |
| | | TPGVEYVYTIQVLRDGQERDAPI | 3% | MNG632 | 0 | | | | |
| | | YVYTIQVLRDGQERDAPI | 3% | MNG642 | 2 | | | | |
| | | VYTIQVLRDGQERDAPI | 3% | MNG642 | 3 | | | | |
| | | VGQQMIFEEHGFRRTTPPT | 6% | MNG635 | 1 | | | | |
| | | | | MNG638 | | | | | |
| | | GQQMIFEEHGFRRTTPP | 12% | MNG612 | 2 | | | | |
| | | | | MNG635 MNG638 | | | | | |
| | | | | MNG641 | | | | | |
| | | GQQMIFEEHGFRRTTPPT | 6% | MNG635 | 1 | | | | |
| | | | | MNG638 | | | | | |
| | | QQMIFEEHGFRRTTPP | 12% | MNG6 | 9 | | | | |
| | | | | MNG612 MNG635 | | | | | |
| | | | | MNG734 | | | | | |
| | | IFEEHGFRRTTPP | 3% | MNG734 | 0 | | | | |
| Collagen alpha-2(V) chain (COL5A2; P05997) | 36% | | | | | WHO I-III | 11% | 11% / 3% | |
| | | GNVGKTVFEYRTQNVAR | 6% | MNG636 | 3 | | | | |
| | | | | MNG638 | | | | | |
| | | VGKTVFEYRTQNVAR | 12% | MNG612 | 10 | | | | |
| | | | | MNG636 MNG638 | | | | | |
| | | | | MNG734 | | | | | |
| | | VARLPIIDLAPVDVGGTD | 9% | MNG499 | 13 | | | | |
| | | | | MNG642 MNG700 | | | | | |
| | | ARLPIIDLAPVDVGGTD | 9% | MNG1 | 21 | | | | |
| | | | | MNG499 MNG642 | | | | | |
| | | RLPIIDLAPVDVGGTD | 6% | MNG499 | 28 | | | | |
| | | | | MNG642 | | | | | |
| | | LPIIDLAPVDVGGT | 3% | MNG499 | 25 | | | | |
| | | LPIIDLAPVDVGGTD | 9% | MNG1 | 35 | | | | |
| | | | | MNG499 MNG642 | | | | | |
| Secretogranin-2 (SCG2; P13521) | 30% | | | | | WHO I-III | 9% | 0% / 5% | |
| | | LSDDVSKVIAYLKRLVNAAGSG | 3% | MNG4 | 1 | | | | |
| | | SDDVSKVIAYLKRLVNAAG | 3% | MNG4 | 2 | | | | |
| | | SDDVSKVIAYLKRLVNAAGSG | 3% | MNG4 | 1 | | | | |
| | | DDVSKVIAYLKRLVNAA | 3% | MNG4 | 1 | | | | |
| | | DDVSKVIAYLKRLVNAAG | 9% | MNG4 | 3 | | | | |
| | | | | MNG6 MNG702 | | | | | |
| | | DDVSKVIAYLKRLVNAAGSG | 6% | MNG4 | 1 | | | | |
| | | | | MNG702 | | | | | |
| | | DVSKVIAYLKRLVNAA | 6% | MNG4 | 4 | | | | |
| | | | | MNG6 | | | | | |
| | | DVSKVIAYLKRLVNAAG | 9% | MNG4 | 8 | | | | |
| | | | | MNG6 MNG702 | | | | | |
| | | VSKVIAYLKRL | 3% | MNG6 | 0 | | | | |
| | | VSKVIAYLKRLVNA | 6% | MNG4 | 4 | | | | |
| | | | | MNG6 | | | | | |
| | | VSKVIAYLKRLVNAA | 15% | MNG4 | 16 | | | | |
| | | | | MNG6 MNG635 | | | | | |
| | | | | MNG641 MNG702 | | | | | |
| | | VSKVIAYLKRLVNAAG | 15% | MNG4 | 16 | | | | |
| | | | | MNG6 MNG635 | | | | | |
| | | | | MNG641 MNG702 | | | | | |
| | | SKVIAYLKRLVNAA | 3% | MNG6 | 2 | | | | |
| | | SGYPKTPGRAGTEALPDG | 3% | MNG682 | 0 | | | | |
| | | YPKTPGRAGTEALPDG | 9% | MNG6 | 6 | | | | |
| | | | | MNG682 MNG734 | | | | | |
| | | TPGRAGTEALPDG | 9% | MNG6 | 12 | | | | |
| | | | | MNG682 MNG734 | | | | | |
| Prolyl 4-hydroxylase subunit alpha-1 (P4HA1; P13674) | 24% | | | | | WHO I+II | 7% | 0% / 3% | |
| | | DKVSVLDYLSYA | 6% | MNG501 | 5 | | | | |
| | | | | MNG638 | | | | | |
| | | DKVSVLDYLSYAVYQ | 9% | MNG3 | 7 | | | | |
| | | | | MNG7 MNG638 | | | | | |
| | | DKVSVLDYLSYAVYQQ | 9% | MNG501 | 9 | | | | |
| | | | | MNG635 MNG638 | | | | | |
| | | DKVSVLDYLSYAVYQQG | 3% | MNG3 | 11 | | | | |

| Protein | % | Peptide | % | MNG samples | n | WHO | Grade | % | Ratio |
|---|---|---|---|---|---|---|---|---|---|
| Inter-alpha-trypsin inhibitor heavy chain H2 (ITIH2; P19823) | 73% | | | | | WHO | I+II | 35% | 33% / 32% |
| | | FEIPINGLSE | 6% | MNG702 MNG638 | 3 | | | | |
| | | FEIPINGLSEF | 18% | MNG501 MNG702 MNG833 MNG6 MNG638 MNG814 | 1 | | | | |
| | | FEIPINGLSEFVD | 6% | MNG501 MNG702 | 0 | | | | |
| Macrophage scavenger receptor types I and II (MSR1; P21757) | 15% | | | | | WHO | I-III | 3% | 0% / 1% |
| | | EVFMEHMSNMEKRIQH | 3% | MNG642 | 3 | | | | |
| | | MEHMSNMEKRIQH | 3% | MNG635 | 2 | | | | |
| | | MEHMSNMEKRIQHILD | 3% | MNG6 | 0 | | | | |
| | | RIQHILDMEANLMDTE | 6% | MNG638 MNG734 | 1 | | | | |
| | | IQHILDMEANLM | 3% | MNG734 | 0 | | | | |
| | | IQHILDMEANLMD | 3% | MNG734 | 0 | | | | |
| | | IQHILDMEANLMDT | 3% | MNG638 | 0 | | | | |
| | | IQHILDMEANLMDTE | 3% | MNG638 | 1 | | | | |
| Cellular retinoic acid-binding protein 1 (CRABP1; P29762) | 27% | | | | | WHO | I+II | 2% | 0% / 1% |
| | | INFKVGEGFEEETVD | 15% | MNG623 MNG661 MNG7 MNG642 MNG679 | 2 | | | | |
| | | INFKVGEGFEEETVDG | 3% | MNG642 | 0 | | | | |
| | | KVGEGFEEETVD | 3% | MNG7 | 0 | | | | |
| Replication factor C subunit 4 (RFC4; P35249) | 18% | | | | | WHO | I+II | 1% | 11% / 1% |
| | | KDRGVAASAGSSGENKKAKPVP | 18% | MNG499 MNG634 MNG682 MNG7 MNG628 MNG638 | 3 | | | | |
| Disintegrin and metalloproteinase domain-containing protein 17 (ADAM17; P78536) | 24% | | | | | WHO | I-III | 6% | 0% / 3% |
| | | HVETLLTFSALKRH | 3% | MNG734 | 1 | | | | |
| | | HVETLLTFSALKRHF | 3% | MNG641 | 0 | | | | |
| | | HVETLLTFSALKRHFK | 15% | MNG612 MNG641 MNG6 MNG634 MNG734 | 3 | | | | |
| | | TSTHVETLLTFSALKRHFK | 3% | MNG641 | 0 | | | | |
| | | VETLLTFSALKRH | 6% | MNG641 MNG734 | 0 | | | | |
| | | VETLLTFSALKRHF | 9% | MNG641 MNG6 MNG734 | 1 | | | | |
| | | VETLLTFSALKRHFK | 12% | MNG612 MNG634 MNG641 MNG734 | 3 | | | | |
| Probable cation-transporting ATPase 13A5 (ATP13A5; Q4VNC0) | 18% | | | | | WHO | I-III | 0.3% | 0% / 1% |
| | | VDSCKFGTSVSNIIKP | 18% | MNG612 MNG641 MNG734 MNG6 MNG634 MNG642 | 1 | | | | |
| Tetratricopeptide repeat protein 27 (TTC27; Q6P3X3) | 18% | | | | | WHO | I-III | 2% | 0% / 2% |
| | | EIAIILGICTNFQKN | 15% | MNG636 MNG682 MNG624 MNG638 MNG734 | 1 | | | | |
| Chondroitin sulfate proteoglycan 4 (CSPG4; Q6UVK1) | 24% | | | | | WHO | I-III | 8% | 0% / 6% |
| | | DFIYVDIFEGHLRA | 9% | MNG634 MNG6 MNG734 | 2 | | | | |
| | | DFIYVDIFEGHLRAVV | 3% | MNG634 | 0 | | | | |
| | | DFIYVDIFEGHLRAVVE | 6% | MNG612 MNG734 | 1 | | | | |
| | | FIYVDIFEGHLRA | 15% | MNG6 MNG612 MNG636 MNG641 MNG734 | 5 | | | | |
| | | FIYVDIFEGHLRAV | 3% | MNG734 | 1 | | | | |
| | | FIYVDIFEGHLRAVV | 6% | MNG612 MNG734 | 2 | | | | |
| | | FIYVDIFEGHLRAVVE | 3% | MNG734 | 0 | | | | |
| | | IYVDIFEGHLRA | 18% | MNG6 MNG612 MNG634 MNG636 MNG641 MNG734 | 4 | | | | |
| | | IYVDIFEGHLRAVV | 6% | MNG634 MNG734 | 2 | | | | |
| | | IYVDIFEGHLRAVVE | 6% | MNG634 MNG734 | 0 | | | | |
| Protocadherin Fat 4 (FAT4; Q6V0I7) | 70% | | | | | WHO | I+II | 4% | 11% / 20% |
| | | GGNSQFTINPSTGQIIT | 3% | MNG702 | 0 | | | | |
| | | GNSQFTINPSTGQII | 3% | MNG702 | 0 | | | | |
| | | GNSQFTINPSTGQIIT | 9% | MNG666 MNG3 MNG702 | 0 | | | | |

| Protein | | Peptide | | MNG | | | |
|---|---|---|---|---|---|---|---|
| | | IGGNSQFTINPSTGQIIT | 6% | MNG635 MNG673 | 0 | | |
| Interleukin-34 (IL34; Q6ZMJ4) | 18% | | WHO I+II | | 0% | 0% / 1% | |
| | | DKLQYRSRLQYMKHY | 3% | MNG666 | 0 | | |
| | | LRDKLQYRSRLQYMKHY | 15% MNG3 MNG673 | MNG1 MNG666 MNG702 | 0 0 | | |
| | | RDKLQYRSRLQYMKHY | 6% MNG833 | MNG666 | | | |
| Thrombospondin type-1 domain-containing protein 4 (THSD4; Q6ZMP0) | 82% | | WHO I+III | | 9% | 56% / 12% | |
| | | EMYKSNNYLALRSRSGRSIIN | 3% | MNG682 | 0 | | |
| | | KSNNYLALRSRSG | 3% | MNG682 | 1 | | |
| | | KSNNYLALRSRSGR | 3% | MNG682 | 4 | | |
| | | KSNNYLALRSRSGRS | 9% MNG636 | MNG624 MNG682 | 5 | | |
| | | KSNNYLALRSRSGRSI | 15% MNG624 MNG702 | MNG6 MNG682 MNG734 | 6 | | |
| | | KSNNYLALRSRSGRSIIN | 3% | MNG682 | 0 | | |
| | | KSNNYLALRSRSGRSIING | 3% | MNG682 | 1 | | |
| | | SNNYLALRSRSG | 3% | MNG682 | 2 | | |
| | | SNNYLALRSRSGR | 3% | MNG682 | 2 | | |
| | | SNNYLALRSRSGRS | 3% | MNG682 | 1 | | |
| | | SNNYLALRSRSGRSI | 9% MNG682 | MNG624 MNG734 | 4 | | |
| | | SNNYLALRSRSGRSIIN | 6% MNG702 | MNG3 | 1 | | |
| | | YKSNNYLALRSRSGRS | 3% | MNG682 | 1 | | |
| | | YKSNNYLALRSRSGRSI | 3% | MNG682 | 0 | | |
| | | YKSNNYLALRSRSGRSIIN | 3% | MNG682 | 0 | | |
| Transmembrane protease serine 9 (TMPRSS9; Q7Z410) | 21% | SDYHRTLTPTLEALLH | WHO I+II 21% MNG635 MNG637 MNG666 | MNG499 MNG636 MNG646 MNG682 | 1% 1 | 0% / 1% | |
| Epidermal growth factor-like protein 6 (EGFL6; Q8IUX8) | 27% | | WHO I+II | | 1% | 0% / 1% | |
| | | GKGKTGEIAVDGVLLVSG | 3% | MNG7 | 1 | | |
| | | GKTGEIAVDGVLLVSG | 9% MNG7 | MNG5 MNG624 | 1 | | |
| | | GKTGEIAVDGVLLVSGL | 6% MNG637 | MNG7 | 3 | | |
| | | KSIIFEAERGKGKTG | 6% MNG638 | MNG6 | 0 | | |
| | | KTGEIAVDGVLLVS | 3% | MNG7 | 0 | | |
| | | KTGEIAVDGVLLVSG | 12% MNG7 MNG646 | MNG5 MNG624 | 2 | | |
| | | KTGEIAVDGVLLVSGL | 12% MNG7 MNG637 | MNG5 MNG624 | 4 | | |
| | | TKSIIFEAERGKGKTG | 3% | MNG6 | 0 | | |
| E3 ubiquitin-protein ligase UBR1 (UBR1; Q8IWV7) | 21% | TKDQDLIKQYNTLIEEMLQV | WHO I+II 18% MNG6 MNG641 MNG682 | MNG3 MNG499 MNG702 | 1% 2 | 0% / 1% | |
| Phosphatidyl-inositol 3,4,5-trisphosphate-dependent Rac exchanger 1 protein (PREX1; Q8TCU6) | 24% | | WHO I+II | | 3% | 0% / 3% | |
| | | LRNDFKLVENILAKR | 15% MNG634 MNG637 | MNG6 MNG636 MNG641 | 5 | | |
| | | LRNDFKLVENILAKRL | 3% | MNG637 | 2 | | |
| Protein KIAA1199 (KIAA1199; Q8WUJ3) | 70% | | WHO I-III | | 10% | 11% / 5% | |
| | | DPLKPREPAIIRHFIAYKNQD | 3% | MNG638 | 0 | | |
| | | DPLKPREPAIIRHFIAYKNQDH | 3% | MNG638 | 0 | | |
| | | DPLKPREPAIIRHFIAYKNQDHG | 9% MNG641 | MNG638 MNG734 | 0 | | |
| | | KPREPAIIRHFIAYKNQD | 12% MNG636 MNG734 | MNG6 MNG641 | 0 | | |
| | | KPREPAIIRHFIAYKNQDH | 6% MNG734 | MNG6 | 0 | | |
| | | KPREPAIIRHFIAYKNQDHG | 3% | MNG734 | 0 | | |
| | | EPAIIRHFIAYK | 3% | MNG734 | 0 | | |
| | | EPAIIRHFIAYKN | 6% MNG734 | MNG641 | 0 | | |
| | | EPAIIRHFIAYKNQ | 6% MNG734 | MNG641 | 0 | | |

| Protein | % | Sequence | % | MNG samples | n | WHO | % | %/% |
|---|---|---|---|---|---|---|---|---|
|  |  | EPAIIRHFIAYKNQD | 3% | MNG734 | 0 |  |  |  |
|  |  | IIRHFIAYKNQDHG | 3% | MNG642 | 0 |  |  |  |
|  |  | IRHFIAYKNQDHG | 3% | MNG642 | 0 |  |  |  |
|  |  | IPDNSIVLMASKGRYV | 36% | MNG1 MNG3 MNG6 MNG7 MNG634 MNG636 MNG638 MNG641 MNG642 MNG666 MNG702 MNG734 | 0 |  |  |  |
|  |  | IPDNSIVLMASKGRYVS | 45% | MNG1 MNG3 MNG6 MNG7 MNG612 MNG634 MNG635 MNG636 MNG638 MNG641 MNG642 MNG666 MNG702 MNG833 MNG833 | 1 |  |  |  |
|  |  | IPDNSIVLMASKGRYVSR | 27% | MNG1 MNG6 MNG634 MNG636 MNG638 MNG641 MNG666 MNG702 MNG734 | 0 |  |  |  |
|  |  | PDNSIVLMASKGRYVS | 3% | MNG702 | 0 |  |  |  |
| Cortactin-binding protein 2 (CTNNBP2; Q8WZ74) | 18% |  |  |  |  | <u>WHO</u> I | 1% | 0% / 1% |
|  |  | QKKLEMEKLQLQALEQEHKK | 18 % | MNG499 MNG636 MNG679 MNG682 MNG700 MNG702 | 1 |  |  |  |
| SLIT and NTRK-like protein 6 (SLIRK6; Q9H5Y7) | 18% |  |  |  |  | <u>WHO</u> I-III | 2% | 0% / 2% |
|  |  | NDSRMSTKTTSILKLP | 18% | MNG6 MNG612 MNG634 MNG641 MNG642 MNG682 | 8 |  |  |  |
| Stabilin-1 (STAB1; Q9NY15) | 85% |  |  |  |  | <u>WHO</u> I+II | 32% | 44% / 32% |
|  |  | DELARIRAHRQLVFR | 9% | MNG1 MNG666 MNG702 | 3 |  |  |  |
|  |  | DELARIRAHRQLVFRYH | 15% | MNG3 MNG666 MNG673 MNG702 MNG833 | 5 |  |  |  |
|  |  | ELARIRAHRQLVFRYH | 15% | MNG1 MNG3 MNG666 MNG702 MNG833 | 2 |  |  |  |
|  |  | GLVPQIEAATAYTIFVPT | 3% | MNG638 | 1 |  |  |  |
|  |  | IEAATAYTIFVPT | 3% | MNG638 | 0 |  |  |  |
|  |  | LARIRAHRQLVFR | 15% | MNG1 MNG3 MNG666 MNG702 MNG833 | 4 |  |  |  |
|  |  | LARIRAHRQLVFRYH | 15% | MNG1 MNG3 MNG666 MNG702 MNG833 | 1 |  |  |  |
|  |  | LVPQIEAATAYTIF | 3% | MNG638 | 0 |  |  |  |
|  |  | LVPQIEAATAYTIFVP | 3% | MNG638 | 3 |  |  |  |
|  |  | LVPQIEAATAYTIFVPT | 12% | MNG623 MNG624 MNG638 MNG700 | 6 |  |  |  |
|  |  | VPQIEAATAYTIF | 3% | MNG638 | 0 |  |  |  |
|  |  | VPQIEAATAYTIFV | 3% | MNG638 | 2 |  |  |  |
|  |  | VPQIEAATAYTIFVP | 6% | MNG638 | 4 |  |  |  |
|  |  | VPQIEAATAYTIFVPT | 9% | MNG623 MNG636 MNG638 | 4 |  |  |  |
| Carboxypeptidase A4 (CPA4; Q9UI42) | 45% |  |  |  |  | <u>WHO</u> I+II | 1% | 22% / 0% |
|  |  | IVSDYQRDPAITS | 3% | MNG702 | 0 |  |  |  |
|  |  | SREWISQATAIWTARK | 3% | MNG638 | 0 |  |  |  |
|  |  | TARKIVSDYQRDPA | 9% | MNG661 MNG624 MNG642 | 1 |  |  |  |
|  |  | TARKIVSDYQRDPAI | 3% | MNG624 | 0 |  |  |  |
|  |  | TARKIVSDYQRDPAIT | 3% | MNG624 | 0 |  |  |  |
|  |  | WTARKIVSDYQRDPA | 3% | MNG642 | 0 |  |  |  |
| Carboxypeptidase Q (CPQ; Q9Y646) | 48% |  |  |  |  | <u>WHO</u> I+II | 10% | 22% / 7% |
|  |  | AGVPGASLLDDL | 15% | MNG499 MNG632 MNG642 MNG666 MNG673 | 0 |  |  |  |
| Olfactomedin-like protein 2B (OLFM2B; Q68BL8) | 55% |  |  |  |  | <u>WHO</u> I+II | 11% | 11% / 1% |
|  |  | FSQEVIVLSKLNAAD | 12% | MNG623 MNG635 MNG641 MNG661 | 2 |  |  |  |
|  |  | FSQEVIVLSKLNAADL | 9% | MNG623 | 6 |  |  |  |

| Protein | | Peptide | % | MNG codes | # | WHO I+II |
|---|---|---|---|---|---|---|
| | | | | MNG635 MNG641 | | |
| | | GAFYYNRAFTRNII | 9% | MNG3 | 4 | |
| | | | | MNG501 MNG642 | | |
| | | GAFYYNRAFTRNIIK | 6% | MNG642 | 0 | |
| | | | | MNG700 | | |
| | | GFSQEVIVLSKLNAAD | 3% | MNG623 | 1 | |
| | | GFSQEVIVLSKLNAADL | 6% | MNG623 | 3 | |
| | | | | MNG641 | | |
| | | NGAFYYNRAFTRNII | 6% | MNG638 | 0 | |
| | | | | MNG642 | | |
| | | NGAFYYNRAFTRNIIK | 6% | MNG638 | 0 | |
| | | | | MNG642 | | |
| | | SQEVIVLSKLNAAD | 9% | MNG635 | 3 | |
| | | | | MNG641 MNG661 | | |
| | | SQEVIVLSKLNAADL | 15% | MNG623 | 6 | |
| | | | | MNG635 MNG641 | | |
| | | | | MNG661 MNG702 | | |
| | | VIVLSKLNAAD | 3% | MNG628 | 1 | |
| | | VIVLSKLNAADL | 3% | MNG702 | 2 | |
| | | VYNGAFYYNRAFTRNIIK | 3% | MNG642 | 0 | |
| | | YNGAFYYNRAFTRNII | 12% | MNG3 | 1 | |
| | | | | MNG501 MNG638 | | |
| | | | | MNG642 | | |
| | | YNGAFYYNRAFTRNIIK | 9% | MNG501 | 2 | |
| | | | | MNG638 MNG642 | | |
| | | YYNRAFTRNII | 6% | MNG638 | 0 | |
| | | | | MNG642 | | |
| Inter-alpha-trypsin inhibitor heavy chain H5 (ITIH5; Q86UX2) | 55% | | | | | WHO I+II 8%  22% / 12% |
| | | GHKKQRTYLRTITILINKPE | 6% | MNG623 | 0 | |
| | | | | MNG679 | | |
| | | KQRTYLRTITILINKPE | 15% | MNG1 | 1 | |
| | | | | MNG2 MNG5 | | |
| | | | | MNG623 MNG679 | | |
| Immunoglobulin superfamily member 21 (IGSF21; Q96ID5) | 21% | | | | | WHO I+II 1%  0% / 1% |
| | | APKGPKIVMTPSRARVGDT | 15% | MNG1 | 1 | |
| | | | | MNG3 MNG666 | | |
| | | | | MNG702 MNG833 | | |
| CUB and sushi domain-containing protein 1 (CSMD1; Q96PZ7) | 18% | | | | | WHO I+II 1%  0% / 1% |
| | | KSPVCKSKGVREVNETVTKTPVP | 15% | MNG7 | 1 | |
| | | | | MNG499 MNG638 | | |
| | | | | MNG641 MNG666 | | |
| Hermansky-Pudlak syndrome 3 protein (HPS3; Q969F9) | 21% | | | | | WHO I+II 2%  11% / 1% |
| | | ERGLIFYINHSLYE | 3% | MNG7 | 0 | |
| | | ERGLIFYINHSLYEN | 9% | MNG3 | 4 | |
| | | | | MNG634 MNG638 | | |
| | | KYERGLIFYINHSLY | 12% | MNG3 | 3 | |
| | | | | MNG501 MNG702 | | |
| | | | | MNG814 | | |
| Fibromodulin (FMOD; Q06828) | 94% | | | | | WHO I+II 26%  56% / 18% |
| | | MPGPLPRSLRELHLDHNQI | 9% | MNG499 | 10 | |
| | | | | MNG642 MNG700 | | |
| | | MPGPLPRSLRELHLDHNQISR | 9% | MNG499 | 21 | |
| | | | | MNG646 MNG700 | | |
| | | MPGPLPRSLRELHLDHNQISRVPN | 3% | MNG499 | 18 | |
| | | NLTRMPGPLPRSLRE | 6% | MNG635 | 0 | |
| | | | | MNG646 | | |
| | | NLTRMPGPLPRSLRELH | 3% | MNG635 | 0 | |
| | | NNLTRMPGPLPRSLRE | 6% | MNG635 | 0 | |
| | | | | MNG646 | | |
| | | NNLTRMPGPLPRSLRELH | 6% | MN635 | 0 | |
| | | | | MNG646 | | |
| EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1; Q12805) | 61% | | | | | WHO I+II 11%  33% / 16% |
| | | GRNNFVIRRNPADPQR | 9% | MNG682 | 0 | |
| | | | | MNG702 | | |
| | | GRNNFVIRRNPADPQRIP | 3% | MNG833 | 0 | |
| | | GRNNFVIRRNPADPQRIPS | 9% | MNG702 | 1 | |
| | | | | MNG666 | | |
| | | GRNNFVIRRNPADPQRIPSN | 3% | MNG682 | 0 | |
| | | GRNNFVIRRNPADPQRIPSNP | 3% | MNG702 | 0 | |
| | | GRNNFVIRRNPADPQRIPSNPS | 3% | MNG702 | 0 | |
| | | NFVIRRNPADPQR | 9% | MNG628 | 1 | |
| | | | | MNG666 MNG702 | | |
| | | NFVIRRNPADPQRIPS | 12% | MNG628 | 3 | |
| | | | | MNG682 MNG702 | | |
| | | | | MNG833 | | |
| | | NNFVIRRNPAD | 6% | MNG628 | 0 | |
| | | | | MNG702 | | |

| Protein | Cov. | Sequence | Freq. | Samples | Count | WHO | Ratio |
|---|---|---|---|---|---|---|---|
| | | NNFVIRRNPADPQR | 6% | MNG682 MNG702 | 0 | | |
| | | NNFVIRRNPADPQRIPS | 12% | MNG628 MNG682 MNG833 MNG702 | 2 | | |
| | | NNFVIRRNPADPQRIPSNPS | 3% | MNG702 | 0 | | |
| | | PQRIPSNPS | 6% | MNG628 MNG702 | 0 | | |
| | | TGRNNFVIRRNPADPQR | 3% | MNG666 | 0 | | |
| | | TGRNNFVIRRNPADPQRIPS | 3% | MNG666 | 0 | | |
| | | TGRNNFVIRRNPADPQRIPSN | 3% | MNG702 | 0 | | |
| | | TGRNNFVIRRNPADPQRIPSNPS | 3% | MNG702 | 0 | | |
| | | TGRNNFVIRRNPADPQRIPSNPSH | 3% | MNG702 | 0 | | |
| | | VIRRNPADPQR | 3% | MNG628 | 0 | | |
| | | VIRRNPADPQRIPS | 6% | MNG628 MNG702 | 1 | | |
| Collagen alpha-3(IX) chain (COL9A3; Q14050) | 24% | WHO | I-III | | 2% | | 0% / 2% |
| | | EQIAQLAAHLRKPL | 6% | MNG636 MNG734 | 1 | | |
| | | EQIAQLAAHLRKPLAPG | 3% | MNG734 | 0 | | |
| | | GGMISEQIAQLAAHLRKPLAPG | 6% | MNG641 MNG734 | 0 | | |
| | | IAQLAAHLRKPLAPG | 6% | MNG501 MNG702 | 1 | | |
| | | ISEQIAQLAAHLRKP | 9% | MNG612 MNG636 MNG734 | 2 | | |
| | | ISEQIAQLAAHLRKPL | 18% | MNG6 MNG612 MNG636 MNG638 MNG641 MNG734 | 6 | | |
| | | ISEQIAQLAAHLRKPLA | 6% | MNG641 MNG734 | 3 | | |
| | | ISEQIAQLAAHLRKPLAP | 15% | MNG6 MNG612 MNG636 MNG641 MNG734 | 6 | | |
| | | ISEQIAQLAAHLRKPLAPG | 21% | MNG6 MNG501 MNG612 MNG636 MNG638 MNG641 MNG734 | 6 | | |
| | | QIAQLAAHLRKP | 3% | MNG734 | 1 | | |
| | | QIAQLAAHLRKPL | 3% | MNG734 | 1 | | |
| | | SEQIAQLAAHLRKP | 6% | MNG636 MNG734 | 2 | | |
| | | SEQIAQLAAHLRKPL | 15% | MNG6 MNG612 MNG636 MNG641 MNG734 | 4 | | |
| | | SEQIAQLAAHLRKPLA | 3% | MNG734 | 1 | | |
| | | SEQIAQLAAHLRKPLAP | 3% | MNG734 | 1 | | |
| Nidogen-2 (NID2; Q14112) | 73% | WHO | I-III | | 14% | | 11% / 13% |
| | | DTSPAVLGLAARYVRAGFPR | 3% | MNG1 | 0 | | |
| | | EDTSPAVLGLAARYVRAGFPR | 12% | MNG1 MNG666 MNG673 MNG833 | 0 | | |
| | | LPSGELNTFQAVLASDG | 15% | MNG3 MNG6 MNG638 MNG641 MNG734 | 2 | | |
| | | LPSGELNTFQAVLASDGSDSYAL | 3% | MNG6 | 0 | | |
| | | SPAVLGLAARYVRAG | 6% | MNG3 MNG638 | 1 | | |
| | | SPAVLGLAARYVRAGFP | 9% | MNG1 MNG3 MNG833 | 0 | | |
| | | SPAVLGLAARYVRAGFPR | 9% | MNG1 MNG702 MNG833 | 0 | | |
| | | TSPAVLGLAARYVRAG | 3% | MNG638 | 1 | | |
| Importin subunit beta-1 (KPNB1; Q14974) | 18% | WHO | I+II | | 11% | | 0% / 8% |
| | | KTTLVIMERLQQ | 3% | MNG6 | 1 | | |
| | | KTTLVIMERLQQVL | 3% | MNG623 | 3 | | |
| | | KTTLVIMERLQQVLQ | 15% | MNG6 MNG623 MNG641 MNG666 MNG702 | 16 | | |
| Splicing factor 1 (SF1; Q15637) | 15% | WHO | I+II | | 6% | | 0% / 2% |
| | | GVAGMPPFGMPPAPPPPPPQN | 12% | MNG6 MNG628 MNG638 MNG702 | 13 | | |
| | | MGMGVAGMPPFGMPPAPPPPPPQN | 6% | MNG666 MNG702 | 20 | | |
| Proteoglycan 4 (PRG4; Q92954) | 42% | WHO | I+II | | 3% | | 0% / 5% |
| | | GGLTGQIVAALSTAKYKNWPE | 3% | MNG6 | 0 | | |
| | | GLTGQIVAALSTAKYKN | 6% | MNG3 MNG638 | 0 | | |

| | | | |
|---|---|---|---|
| GLTGQIVAALSTAKYKNWPE | 6% | MNG6 MNG666 | 1 |
| GQIVAALSTAKYKN | 3% | MNG3 | 0 |
| TGQIVAALSTAKYK | 3% | MNG642 | 0 |
| TGQIVAALSTAKYKN | 9% | MNG3 MNG638 MNG642 | 0 |

**Supplementary Table 19. Established TAAs and CTAs identified as meningioma-exclusive antigens or represented by meningioma-exclusive peptides on ≥ 2 tumors.** HLA restrictions not passing manual assessment as quality control are indicated in italic. The number of positive non-meningeal tumors was based on n=841 HLA class I and n=593 HLA class II peptidome datasets. The frequency of positive benign HLA peptidomes was calculated from n=9 tumor-free duras and n=418 (HLA class I) or n=364 (HLA class II) benign human specimens. Meningioma exclusivity of HLA class II-restricted peptides was evaluated for the exact sequence match with none of the peptides arising from a tumor-associated presentation hotspot.

| Peptide sequence | HLA restriction | Frequency of positive patients | | Protein frequency on meningiomas | Protein frequency on non-meningeal tumors Peptide-positive non-meningeal tumors | Protein frequency on dura / benign samples |
|---|---|---|---|---|---|---|
| **Meningioma-exclusive HLA class I-presented antigens derived from established TAAs and CTAs** | | | | | | |
| Protein SSX5/SSX9 (SSX5/9; O60225 / Q7RTT3) | | | | | | |
| Supplementary Table 16 | | | | | | |
| Probable ATP-dependent RNA helicase DDX43/54 (DDX43/53; Q9NXZ2 / Q86TM3) | | | | 6% / 6% | 0.5% / 0.5% | 0% / 0% 0.5% / 0.5% |
| YLMPGFIHL | A*02:01; C*17:01 | 6% | MNG628 MNG641 | | 0 | |
| **Meningioma-exclusive HLA class I ligands derived from established TAAs and CTAs** | | | | | | |
| Neurofibromin (NF1; P21359) | | | | 64% | 25% | 56% / 22% |
| LPYLFHVV | B*51:01 | 12% | MNG499 MNG612 MNG642 MNG833 | | 13 | |
| NYPDEFTKL | A*24:02 | 12% | MNG1 MNG628 MNG635 MNG661 | | 8 | |
| AETVLADRF | B*44:03 | 6% | MNG1 MNG636 | | 2 | |
| DEFDQRILY | B*18:01 | 6% | MNG1 MNG661 | | 5 | |
| AVIAFRSSY | A*30:02 | 6% | MNG7 MNG632 | | 3 | |
| DYAELIVKF | A*24:02 | 6% | MNG628 MNG833 | | 5 | |
| Catenin beta-1 (CTNNB1; P35222) | | | | 100% | 75% | 100% / 72% |
| ATVGLIRNL | A*32:01; *B*13:02 C*02:02* | 9% | MNG634 MNG635 MNG702 | | 22 | |
| KTNVKFLAI | C*15:02 | 6% | MNG499 MNG501 | | 7 | |
| TYTYEKLLW | A*23:01; A*24:02 | 6% | MNG638 MNG679 | | 9 | |
| Platelet-derived growth factor receptor beta (PDGFRB; P09619) | | | | 70% | 21% | 33% / 16% |
| YMDLVGFSY | A*29:02 | 9% | MNG623 MNG673 MNG682 | | 1 | |
| MPYHIRSI | B*51:01 | 9% | MNG499 MNG833 | | 7 | |
| Regulator of G-protein signaling 5 (RGS5; O15539) | | | | 61% | 29% | 11% / 18% |
| KAKQIYEEF | *B*15:35*; B*57:01 | 9% | MNG3 MNG642 MNG673 | | 11 | |
| KIKSPAKMAEK | A*03:01 | 6% | MNG636 MNG814 | | 15 | |
| Mothers against decapentaplegic homolog 3 (SMAD3; P84022) | | | | 33% | 11% | 11% / 12% |
| HASQPSMTV | B*51:01; C*15:02 | 9% | MNG499 MNG702 MNG833 | | 11 | |
| Werner syndrome ATP-dependent helicase (WRN; Q14191) | | | | 24% | 4% | 11% / 4% |
| ILQDLQPFL | A*02:01 | 9% | MNG612 MNG638 MNG673 | | 5 | |
| Cytochrome P450 1B1 (CYP1B1; Q16678) | | | | 73% | 27% | 89% / 41% |
| NRNFSNFIL | B*39:01; B*39:31 | 6% | MNG666 MNG734 | | 0 | |
| RVMIFSVGK | A*03:01 | 6% | MNG636 MNG666 | | 3 | |
| SMMRNFFTR | A*31:01 | 6% | MNG623 MNG635 | | 2 | |
| Zinc phosphodiesterase ELAC protein 2 (ELAC2; Q9BQ52) | | | | 61% | 30% | 22% / 22% |
| EISSPAVER | A*68:01 | 6% | MNG3 MNG632 | | 1 | |
| Retinoblastoma-associated protein (RB1; P06400) | | | | 52% | 33% | 33% / 39% |
| DEVKNVYF | B*18:01 | 6% | MNG6 MNG661 | | 13 | |
| TEINSALVL | B*40:01; B*40:02 | 6% | MNG3 MNG646 | | 5 | |
| Cytochrome c oxidase subunit 1 (MT-CO1; P00395) | | | | 39% | 21% | 11% / 18% |
| LPVLAAGITM | B*07:02; B*35:01 | 6% | MNG628 MNG666 | | 3 | |

| Peptide / Protein | HLA | % | MNG samples | | | |
|---|---|---|---|---|---|---|
| DNA polymerase epsilon catalytic subunit A (POLE; Q07864) | | | | 33% | 19% | 11% / 25% |
| FYVDTVRAF | A*24:02 | 6% | MNG661 MNG833 | | 18 | |
| EPSSLTYVTR | A*68:01 | 6% | MNG3 MNG814 | | 2 | |
| Short transient receptor potential channel 1 (TRPC1; P48995) | | | | 33% | 5% | 0% / 4% |
| NEIRDLLGF | B*18:01 | 6% | MNG1 MNG628 | | 1 | |
| DNA mismatch repair protein Msh2 (MSH2; P43246) | | | | 30% | 34% | 11% / 32% |
| SAAEVGFVR | A*68:01 | 6% | MNG3 MNG814 | | 3 | |
| Epidermal growth factor receptor (EGFR; P00533) | | | | 24% | 13% | 0% / 6% |
| YQDPHSTAV | C*02:02; B*39:31 | 6% | MNG702 MNG734 | | 28 | |
| Adenomatous polyposis coli protein (APCP; P25054) | | | | 24% | 18% | 0% / 19% |
| GELDTPINY | B*18:01; B*44:03 | 6% | MNG1 MNG636 | | 1 | |
| LPSSSSSRGSL | B*07:02 | 6% | MNG7 MNG814 | | 30 | |
| Mismatch repair endonuclease PMS2 (PMS2; P54278) | | | | 18% | 7% | 11% / 7% |
| DALHNLFYI | B*51:01 | 6% | MNG501 MNG833 | | 4 | |
| Mothers against decapentaplegic homolog 4 (SMAD4; Q13485) | | | | 18% | 11% | 0% / 5% |
| KGFPHVIYA | C*12:03 | 6% | MNG636 MNG661 | | 6 | |
| HRQGGESETF | B*38:01 B*39:31 | 6% | MNG636 MNG734 | | 6 | |
| POTE ankyrin domain family member A (POTEA; Q6S8J7) | | | | 30% | 3% | 11% / 5% |
| EVPRADLIVM | B*35:01 | 6% | MNG628 MNG833 | | 2 | |

**Meningioma-exclusive HLA class II-presented antigens derived from established TAAs and CTAs**

| Peptide / Protein | HLA | % | MNG samples | | | |
|---|---|---|---|---|---|---|
| Melanoma-associated antigen 10 (MAGEA10; P43363) | | | | | | |
| Supplementary Table 16 | | | | | | |
| A/G-specific adenine DNA glycosylase (MUTYH; Q9UIF7) | | | | 6% | 1% | 0% / 0% |
| WRRRAEDEMDLDRRAYAVWVSEVM | | 6% | MNG1 MNG679 | | 2 | |
| Calcium-binding tyrosine phosphorylation-regulated protein (CABYR; O75952) | | | | 6% | 0.5% | 0% / 0% |
| DVLMVDVATSMPVVIKEVPS | | 3% | MNG666 | | 0 | |
| PVTEGVVYIEQLPEQIV | | 3% | MNG636 | | 2 | |

**Meningioma-exclusive HLA class II-restricted peptides derived from established TAAs and CTAs**

| Peptide / Protein | HLA | % | MNG samples | | | |
|---|---|---|---|---|---|---|
| Platelet-derived growth factor receptor-like protein (PDGFRL; Q15198) | | | | 48% | 5% | 33% / 2% |
| APKTQSIMMQVLDKGRFQKPA | | 15% | MNG499 MNG623 MNG673 MNG679 MNG700 | | 5 | |
| TQSIMMQVLDKGRFQKPA | | 15% | MNG499 MNG623 MNG637 MNG679 MNG700 | | 5 | |
| TQSIMMQVLDKGRFQKP | | 15% | MNG499 MNG623 MNG637 MNG642 MNG679 | | 5 | |
| IQDTWRLIHRGLGHTT | | 9% | MNG666 MNG702 MNG833 | | 3 | |
| QDTWRLIHRGLGHT | | 6% | MNG666 MNG833 | | 2 | |
| Alpha-1,6-mannosylglycoprotein 6-beta-N-acetylglucosaminyl-transferase A (MGAT5; Q09328) | | | | 30% | 15% | 0% / 7% |
| DNILQRIGKLESKVDN | | 12% | MNG6 MNG623 MNG642 MNG666 | | 15 | |
| LDLSKRYIKALAEENR | | 9% | MNG642 MNG673 MNG679 | | 13 | |
| Cadherin-1 (CDH1; P12830) | | | | 64% | 22% | 56% / 32% |
| GTDGVITVKRPLRFHNPQ | | 9% | MNG1 MNG673 MNG702 | | 3 | |
| TGRQRTAYFSLDTRF | | 6% | MNG7 MNG623 | | 2 | |
| Serine/threonine-protein kinase ATR (ATR9; Q13535) | | | | 15% | 4% | 11% / 5% |
| GLPLSIEGHVHYLIQEATDE | | 9% | MNG501 MNG700 MNG702 | | 0 | |
| Neurofibromin (NF1; P21359) | | | | 12% | 3% | 0% / 4% |
| GNPIFYYVARRFK | | 9% | MNG6 MNG641 MNG734 | | 0 | |
| Legumain (LGMN; Q99538) | | | | 49% | 31% | 22% / 46% |
| VPKDYTGEDVTPQNFLAVLR | | 6% | MNG1 MNG833 | | 21 | |
| Matrix-remodeling-associated protein 5 (MXRA5; Q9NR99) | | | | 24% | 16% | 11% / 5% |
| DPWPRILWRLPSK | | 6% | MNG666 MNG702 | | 10 | |
| GDPWPRILWRLPSK | | 6% | MNG666 MNG702 | | 12 | |
| DPWPRILWRLPSKR | | 6% | MNG666 MNG702 | | 12 | |
| C-1-tetrahydrofolate synthase, cytoplasmic (MTHFD1; P11586) | | | | 6% | 15% | 0% / 18% |
| GVSGALTVLMKDAIKPNLMQ | | 6% | MNG624 MNG682 | | 2 | |

| Macrophage scavenger receptor types I and II (MSR1; P21757) | | | | 15% | 3% | 0% / 1% |
|---|---|---|---|---|---|---|
| RIQHILDMEANLMDTE | 6% | MNG638 | MNG734 | 1 | | |
| Zinc phosphodiesterase ELAC protein 2 (ELAC2; Q9BQ52) | | | | 12% | 1% | 0% / 1% |
| LSGMILTLKETGLPKCVLSGPP | 6% | MNG7 | MNG499 | 0 | | |
| IIFLGTGSAIPMKIRNVSATLVNI | 6% | MNG638 | MNG702 | 0 | | |
| Poly(A) polymerase alpha (PAPOLA; P51003) | | | | 9% | 2% | 0% / 1% |
| LSVDLTYDIQSFTD | 6% | MNG1 | MNG3 | 6 | | |
| Ephrin type-B receptor 2 (EPHB2; P29323) | | | | 9% | 3% | 0% / 4% |
| TPGMKIYIDPFTYED | 6% | MNG3 | MNG638 | 1 | | |
| Tyrosine-protein kinase receptor Tie-1 (TIE1; P35590) | | | | 9% | 2% | 0% / 15% |
| QTDVIWKSNGSYFYT | 6% | MNG499 | MNG814 | 0 | | |
| A/G-specific adenine DNA glycosylase (MUTYH; Q9UIF7) | | | | 6% | 1% | 0% / 0.5% |
| WRRRAEDEMDLDRRAYAVWVSEVM | 6% | MNG1 | MNG679 | 0 | | |
| ALK tyrosine kinase receptor (ALK; Q9UM73) | | | | 6% | 1% | 0% / 1% |
| PHNEAAREILLMPTPG | 6% | MNG7 | MNG638 | 0 | | |

**A**

**B**



$R^2 = 0.9477$

$R^2 = 0.9501$

$R^2 = 0.9631$

$R^2 = 0.9772$

$R^2 = 0.9877$

$R^2 = 0.9685$

$R^2 = 0.9652$

$R^2 = 0.9730$

$R^2 = 0.9679$

$R^2 = 0.9848$

**C**

$R^2 = 0.7999$

$R^2 = 0.9702$

$R^2 = 0.9834$
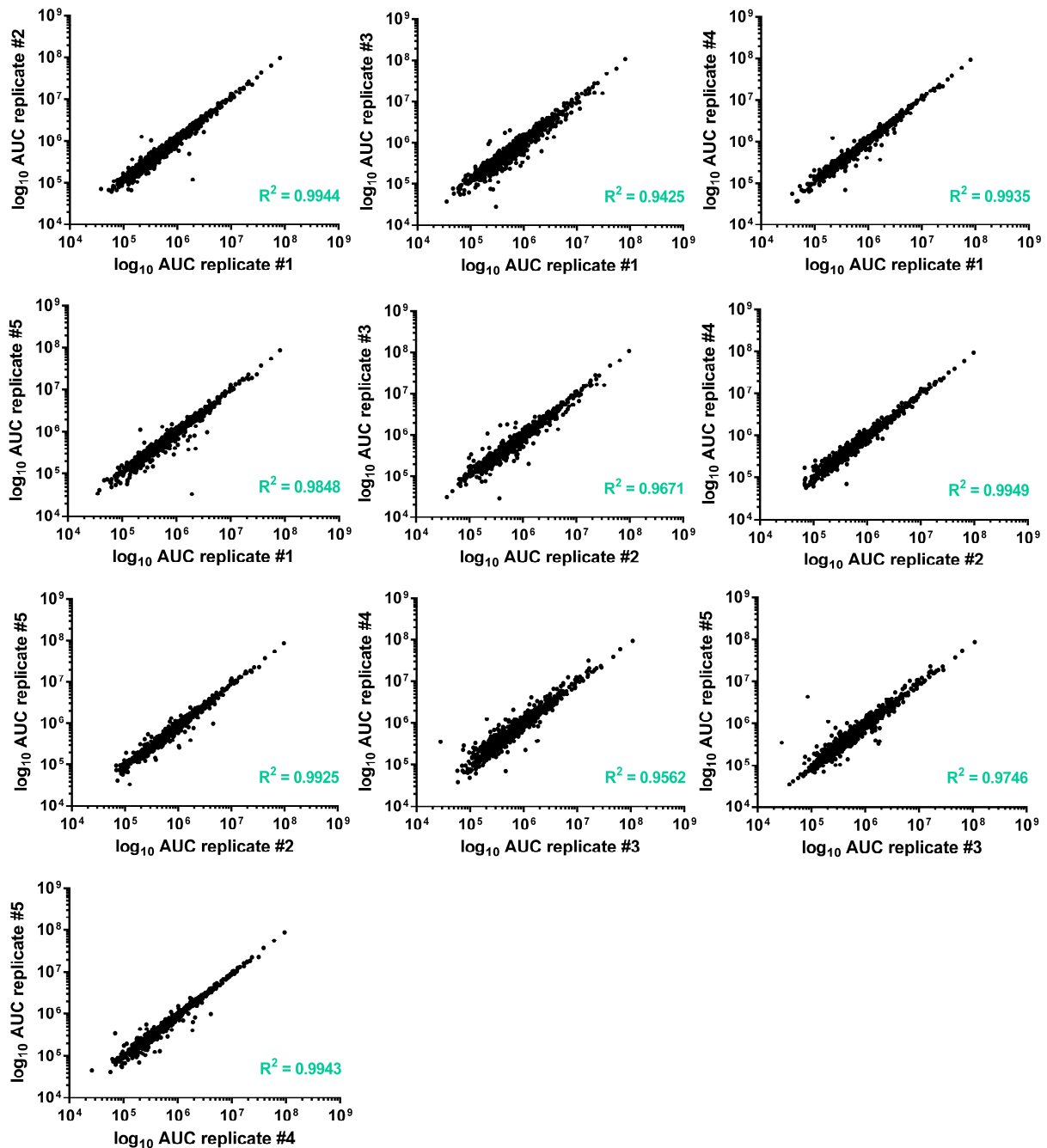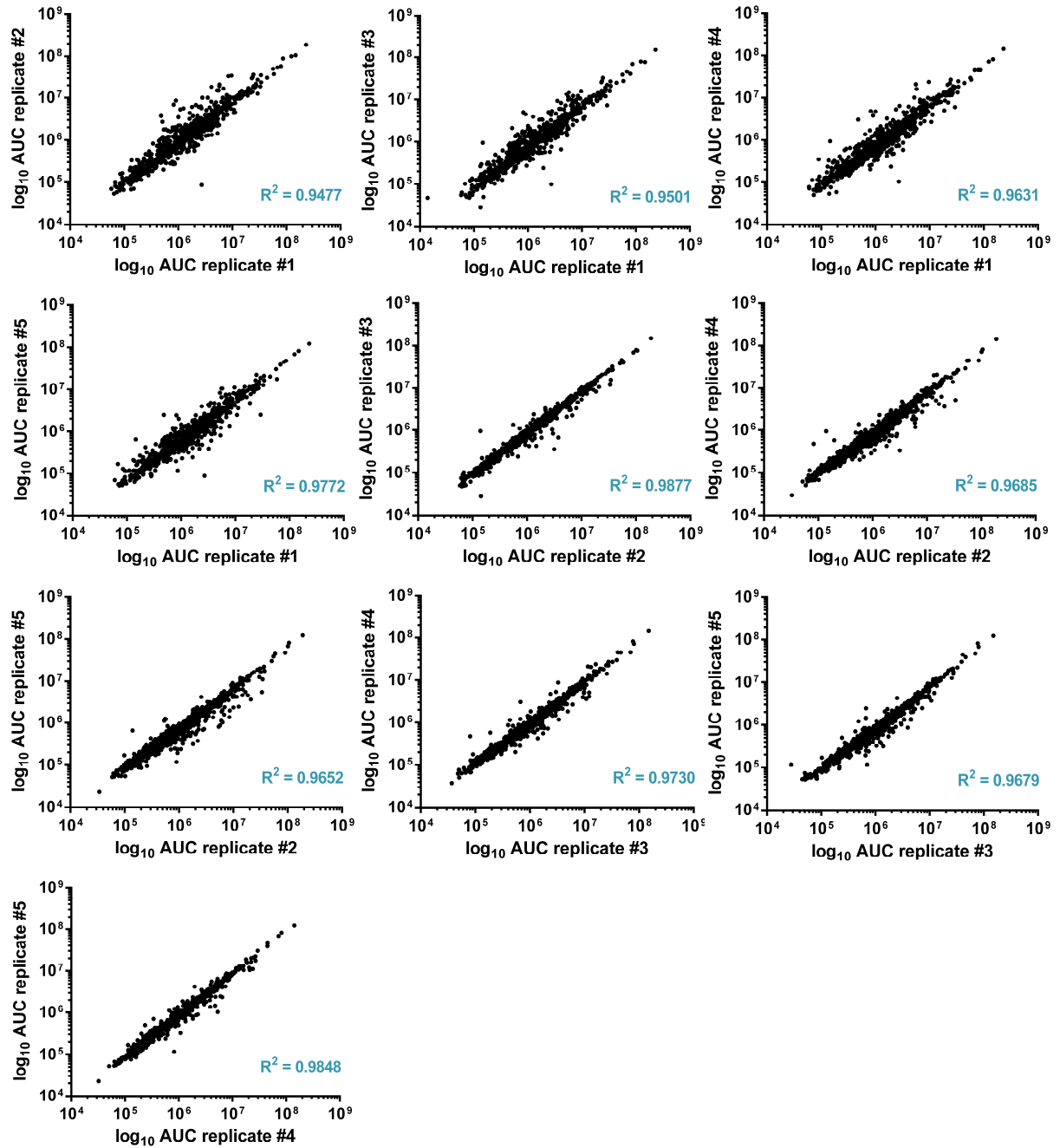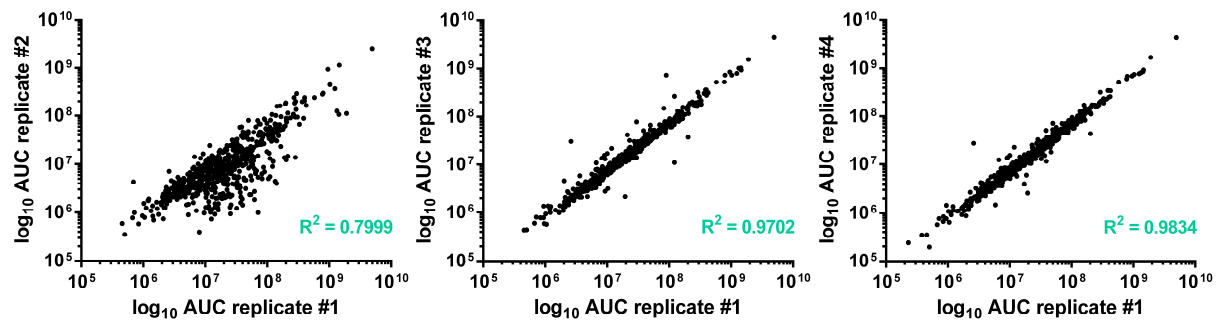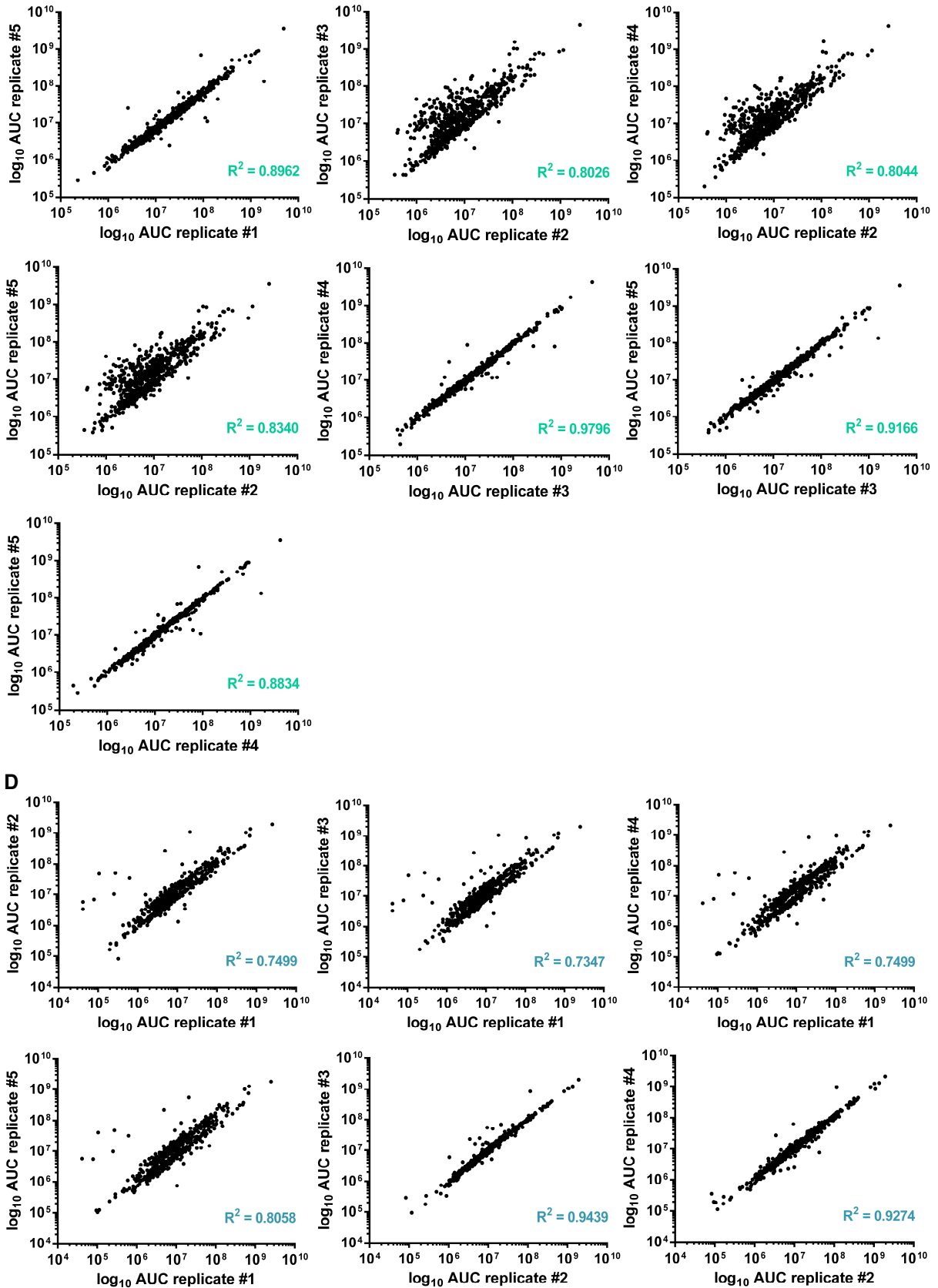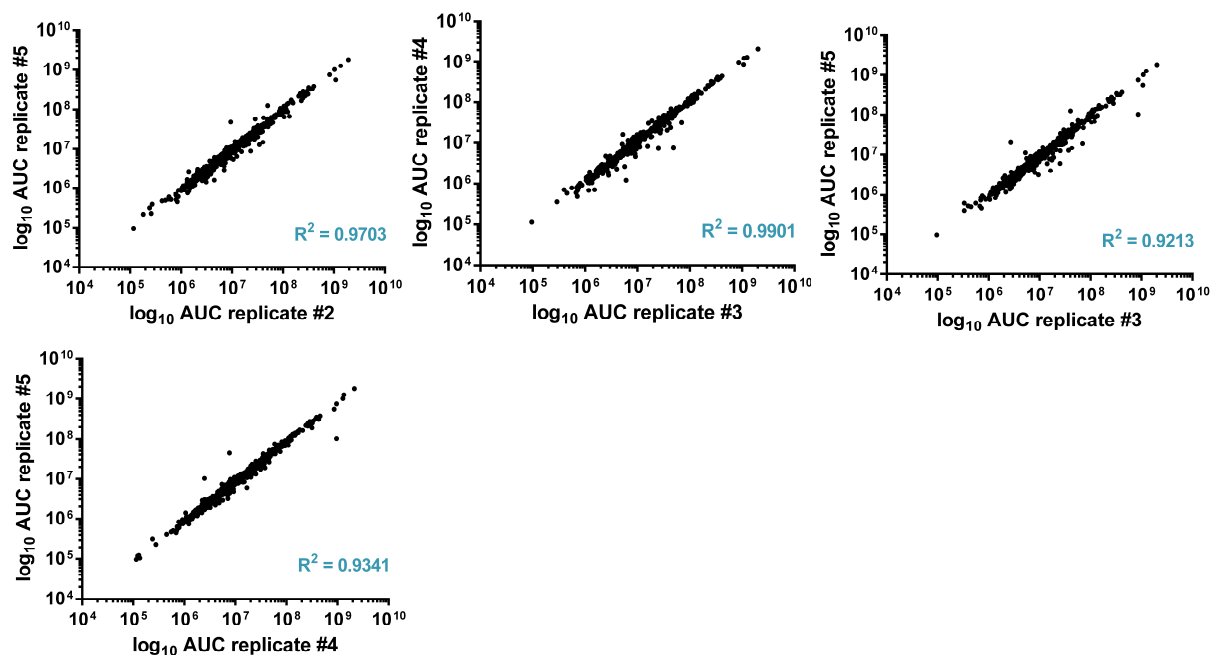
**Supplementary Figure 11. Reproducibility of precursor ion intensities across technical replicates exemplarily shown for MNG679.** Scatter plots depicting raw MS[1] intensities prior to normalization in pairwise comparisons of technical replicates acquired from **(A) HLA class I ligands eluted from tumor-free dura**, **(B) HLA class I ligands identified in meningioma**, **(C) HLA class II-presented peptides eluted from tumor-free dura**, and **(D) HLA class II-presented peptides identified in meningioma.** Correlation coefficients were calculated by linear regression and amounted to > 0.85 except for HLA class II peptide intensity comparisons including replicate #2 acquired from dura and replicate #1 acquired from meningioma. HLA class II peptide intensities were in general slightly less reproducible as compared with HLA class I ligand intensities.

**Supplementary Table 20. Peptide sequences and level of modulation for antigens exclusively represented by up- or down-modulated HLA class I- and II-presented peptides.** Oxidized (m) and reduced (M) methionine were not treated equally in LFQ-MS and are listed separately. Multi-mapping peptides were censored when the second annotated protein diverged in assignment to groups of modulation according to comparative profiling. HLA restrictions not passing manual assessment as quality control are indicated in italic (HLA class I ligands not matching the motif of any of the patient's HLA allotypes; HLA class II-presented proteins neither identified with peptides exceeding a length of twelve AA nor with different sequences across patients). Abbreviation not introduced in the text above: fold change meningioma/dura (fc M/D).

| UniProt accession | Antigen | Patients with significant modulation | Peptide sequence | HLA restric-tion | Fc (M/D) | Corrected p-value |
|---|---|---|---|---|---|---|
| **Antigens exclusively represented by HLA class I ligands up-modulated on meningioma** | | | | | | |
| P05387 | 60S acidic ribosomal protein P2 (RLA2) | 60% | | | | |
| | | MNG679 | MRYVASYLL | *C*04:01* | 67.32 | 0.00093 |
| | | | mRYVASYLL | *C*04:01* | 20.86 | 0.00011 |
| | | MNG702 | MRYVASYLL | C*06:02 | 22.02 | 0.00161 |
| | | | mRYVASYLL | C*06:02 | 60.94 | 0.00014 |
| | | | MRYVASYL | C*06:02 | 68.11 | 0.00005 |
| | | | mRYVASYL | C*06:02 | 7.47 | 0.00014 |
| | | MNG833 | mRYVASYLL | *C*04:01* | 18.48 | 0.00089 |
| P0DJ07 | Protein PET100 homolog, mitochondrial (PT100) | 40% | | | | |
| | | MNG819 | IYLTFPVAmF | A*23:01 | 5.82 | 0.00333 |
| | | MNG833 | IYLTFPVAmF | A*24:02 | 10.58 | 0.00208 |
| P28039 | Acyloxyacyl hydrolase (AOAH) | 40% | | | | |
| | | MNG679 | KFTNFNLFY | A*29:02 | 23.01 | 0.00002 |
| | | MNG702 | SVIEQLAQV | A*02:01 | 18.33 | 0.00112 |
| Q14126 | Desmoglein-2 (DSG2) | 40% | | | | |
| | | MNG679 | RFLDDLGLKF | *C*04:01* | 44.61 | 0.00002 |
| | | | Manual HLA annotation: | A*23:01 | | |
| | | MNG819 | RFLDDLGLKF | A*23:01 | 28.79 | 0.00176 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Q15796 | Mothers against deca-pentaplegic homolog 2 (SMAD2) 1 peptide multi-maps to SMAD1/3/5/9 (Q15797 / P84022 / Q99717 / O15198) | 40% MNG702 MNG679 | LTELPPLDDY FVKGWGAEY | A*01:01 A*29:02 | 10.83 6.52 | 0.00052 0.00049 |
| Q2TAY7 | WD40 repeat-containing protein SMU1 (SMU1) | 40% MNG679 MNG833 | DVIRLIMQY DVIRLImQY DVIRLImQY | A*29:02 A*29:02 B*35:01 | 856.09 209.76 5.15 | 0.00025 0.00256 0.00037 |
| Q68CP4 | Heparan-alpha-glucosaminide N-acetyltransferase (HGNAT) | 40% MNG679 MNG819 | NYFPFQWKL NYFPFQWKL | A*23:01 A*23:01 | 25.65 12.97 | 0.00473 0.00090 |
| Q6KC79 | Nipped-B-like protein (NIPBL) | 40% MNG679 MNG814 | DVIERVIQY EVVAVDPSILAR | A*29:01 A*68:01 | 93.70 4.53 | 0.00012 0.00840 |
| Q6PI26 | Protein SHQ1 homolog (SHQ1) | 40% MNG679 MNG833 | EAIEQILKY NVHDImVSF | A*29:02 B*35:01 | 28.28 6.39 | 0.00002 0.00072 |
| Q6PKG0 | La-related protein 1 (LARP1) | 40% MNG679 MNG819 | YGLEKFWAF YGLEKFWAF | A*23:01 A*23:01 | 45.79 11.98 | 0.00012 0.00169 |
| Q96T76 | MMS19 nucleotide excision repair protein homolog (MMS19) | 40% MNG679 MNG702 | EVVHLILFY VDTLVTKF | A*29:02 B*37:01 | 125.12 21.09 | 0.00043 0.000009 |
| Q9BS91 | Probable UDP-sugar transporter protein SLC35A5 (S35A5) | 40% MNG679 MNG833 | KWSIPAFLY IFIQNSKLYF | A*29:01 A*24:02 | 17.63 10.08 | 0.000005 0.00070 |
| Q9H799 | Ciliogenesis and planar polarity effector 1 (CPLN1) | 40% MNG679 MNG819 | KFLDLFLSY EYIKFLDLF | A*29:01 A*23:01 | 6.92 15.21 | 0.00727 0.00279 |

**Antigens exclusively represented by HLA class I ligands down-modulated on meningioma**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Q9Y520 | Proline-rich and coiled-coil-containing protein 2C (PRC2C) | 60% MNG679 MNG819 MNG833 | FYmDTSHLF FYmDTSHLF FYmDTSHLF | A*23:01 A*23:01 A*24:02 | 0.0491 0.0957 0.1108 | 0.0000002 0.00013 0.00030 |
| Q9UL54 | Serine/threonine-protein kinase TAO2 (TAOK2) 1 peptide multi-maps to TAOK3 (Q9H2K8) | 40% MNG679 MNG702 | SEVVAIKKm QTELGNQLEY | B*44:03 A*01:01 | 0.1283 0.0457 | 0.00119 0.00005 |
| Q9BXC9 | Bardet-Biedl syndrome 2 protein (BBS2) | 40% MNG679 MNG819 | AEQDLIREL IRSNNINTL | B*44:03 C*06:02 | 0.0303 0.0793 | 0.00047 0.00205 |
| Q9BRK3 | Matrix-remodeling-associated protein 8 (MXRA8) | 40% MNG702 MNG814 | RSEDIQLDY ALPSRILLWK | A*01:01 A*03:01 | 0.0470 0.0240 | 0.00002 0.000002 |
| Q99733 | Nucleosome assembly protein 1-like 4 (NP1L4) peptides multi-map to NP1L1 (P55209) | 40% MNG679 MNG833 | EEVHDLERKY QPmSFVLEF | B*44:03 B*35:03 | 0.0603 0.1462 | 0.00027 0.00007 |
| Q8NG06 | Tripartite motif-containing protein 58 (TRI58) | 40% MNG702 MNG819 | GLLEGVRGV GLLEGVRGV | A*02:01 A*02:01 | 0.0168 0.0444 | 0.000006 0.00034 |
| Q8IUQ4 | E3 ubiquitin-protein ligase SIAH1 (SIAH1) | 40% MNG679 MNG814 | VFDTSIAQL ATALPTGTSK | C*04:01 A*03:01 | 0.0425 0.2459 | 0.00030 0.00541 |
| Q6N022 | Teneurin-4 (TEN4) | 40% MNG679 MNG819 | AYSDGHFLF AYSDGHFLF | A*23:01 A*23:01 | 0.0951 0.0517 | 0.00045 0.00163 |
| Q53FT3 | Protein Hikeshi | 40% MNG679 MNG819 | KmLDNFYNF KmLDNFYNF | A*23:01 A*23:01 | 0.0579 0.2422 | 0.00001 0.00099 |
| Q15746 | Myosin light chain kinase, smooth muscle (MYLK) | 40% MNG702 MNG814 | DAFEEKANI SPQQVDFRSVL | B*51:01 B*07:02 | 0.0115 0.1016 | 0.00001 0.00024 |
| Q15005 | Signal peptidase complex subunit 2 (SPCS2) | 40% MNG679 MNG819 | AEFTKSIAKF REAEFTKSIA | B*44:03 B*50:01 | 0.0469 0.0947 | 0.00114 0.00709 |
| Q01459 | Di-N-acetylchitobiase (DIAC) | 40% MNG679 MNG819 | REIEGSQVTF SQITTVATF | B*44:03 B*15:01 | 0.0492 0.1910 | 0.000003 0.00014 |
| P57740 | Nuclear pore complex protein Nup107 (NU107) | 40% MNG679 MNG819 | AEDELFNRY AYLEAHETF | B*44:03 A*23:01 | 0.0275 0.1817 | 0.000001 0.00013 |
| P43243 | Matrin-3 | 40% MNG679 MNG702 | FFGETSHNY RTEEGPTLSY | A*29:02 A*01:01 | 0.2240 0.0696 | 0.00004 0.0000003 |

| P29966 | Myristoylated alanine-rich C-kinase substrate (MARCS) | 40% | | | | |
| | | MNG679 | AESGAKEEL | B*44:03 | 0.0649 | 0.00006 |
| | | MNG819 | AERPGEAAVA | B*50:01 | 0.0866 | 0.00604 |
| P24821 | Tenascin (TN-C) | 40% | | | | |
| | | MNG679 | TYLPAPEGLKF | A*23:01 | 0.0468 | 0.00029 |
| | | MNG819 | TYLPAPEGLKF | A*23:01 | 0.0147 | 0.00017 |
| P12755 | Ski oncogene (SKI) | 40% | | | | |
| | | MNG814 | KPSSWLRTL | B*07:02 | 0.0073 | 0.00008 |
| | | MNG819 | QELEFLRVA | B*50:01 | 0.0619 | 0.00006 |
| O15066 | Kinesin-like protein KIF3B (KIF3B) | 40% | | | | |
| | | MNG679 | VYVKDLSSF | A*23:01 | 0.0474 | 0.00005 |
| | | MNG819 | VYVKDLSSF | A*23:01 | 0.1181 | 0.00039 |

**Antigens exclusively represented by HLA class II-restricted peptides up-modulated on meningioma**

| Q6PCB0 | von Willebrand factor A domain-containing protein 1 (VQA1) | 33% | | | | |
| | | MNG700 | ADSGYYVLELVPSAQPG | Class II | 23.73 | 0.00107 |
| | | | DSGYYVLELVPSAQPG | Class II | 49.63 | 0.00150 |
| | | | SGYYVLELVPSAQPG | Class II | 18.03 | 0.00140 |
| | | MNG819 | ADSGYYVLELVPSAQPG | Class II | 21.16 | 0.00066 |
| | | | DSGYYVLELVPSAQPG | Class II | 10.57 | 0.00112 |
| | | | SGYYVLELVPSAQPG | Class II | 4.18 | 0.00053 |
| O14949 | Cytochrome b-c1 complex subunit 8 (QCR8) | 33% | | | | |
| | | MNG700 | FRVVPQFVVF | *Class II* | 5.25 | 0.00119 |
| | | MNG702 | FRVVPQFVVF | *Class II* | 22.51 | 0.00019 |

**Antigens exclusively represented by HLA class II-restricted peptides down-modulated on meningioma**

| Q9UBX1 | Cathepsin F (CATF) | 33% | | | | |
| | | MNG700 | LPSNAYSAIKNLGGLE | Class II | 0.1075 | 0.00486 |
| | | MNG819 | LPSNAYSAIKNLGGLE | Class II | 0.0996 | 0.00112 |
| Q9GZZ6 | Neuronal acetylcholine receptor subunit alpha-10 (ACH10) | 33% | | | | |
| | | MNG700 | YTSALRPVADTDQTLNV | Class II | 0.0148 | 0.00733 |
| | | MNG819 | YTSALRPVADTDQTLNV | Class II | 0.0014 | 0.00073 |
| Q96NH3 | Protein broad-minded (BROMI) | 33% | | | | |
| | | MNG700 | TLCEKLTVSLSDPDPVF | Class II | 0.0075 | 0.00722 |
| | | MNG819 | TLCEKLTVSLSDPDPVF | Class II | 0.0014 | 0.00073 |
| P46459 | N-ethylmaleimide-sensitive vesicle-fusing ATPase (NSF) | 33% | | | | |
| | | MNG700 | AAEFIQQFNNQAFS | Class II | 0.0573 | 0.00639 |
| | | MNG819 | AAEFIQQFNNQAFS | Class II | 0.0379 | 0.00006 |

**Supplementary Table 21. Functional annotation clustering of source proteins exclusively represented by up- or down-modulated HLA class I- or II-presented peptides on meningioma *versus* autologous tumor-free dura.** Clustering for biological processes was performed on the basis of Gene Ontology (GO) terms using DAVID Bioinformatic Resources 6.8 ('Homo sapiens' as background, medium classification stringency, GO BP_ALL). As recommended by Huang *et al.* (Ref. 419 CHAPTER 1), only clusters with an enrichment score ≥ 1.0 were reported.

| **Biological process** **Gene Ontology IDs** | | | | | **Enrichment score** | |
|---|---|---|---|---|---|---|
| **Antigens exclusively represented by HLA class I ligands up-modulated on meningioma** | | | | | | |
| organelle organization | | | | | 3.50 | |
| GO:0071840 | GO:0016043 | GO:0006996 | | | | |
| ribosome biogenesis | | | | | 2.04 | |
| GO:0034470 | GO:0034660 | GO:0006364 | GO:0016072 | GO:0006396 | GO:0022613 | GO:0042254 |
| cellular protein localization | | | | | 2.03 | |
| GO:0051641 | GO:0008104 | GO:0034613 | GO:0033036 | GO:0070727 | GO:1902580 | GO:0033365 |
| GO:0046907 | GO:1902582 | GO:0015031 | GO:0006605 | GO:0045184 | GO:0051649 | GO:0006886 |
| GO:0051179 | GO:0072594 | GO:0071702 | GO:0051234 | GO:0006810 | GO:1902578 | GO:0044765 |
| DNA-templated transcription | | | | | 1.84 | |
| GO:0032784 | GO:0034243 | GO:0006354 | GO:0032786 | GO:0006368 | | |
| histone modification | | | | | 1.69 | |
| GO:0016570 | GO:0051276 | GO:0016569 | GO:0006325 | GO:0018205 | | |
| protein complex biogenesis | | | | | 1.61 | |
| GO:0043933 | GO:0044085 | GO:0022607 | GO:0065003 | GO:0006461 | GO:0070271 | GO:0071822 |
| GO:0034622 | GO:0043623 | | | | | |
| intracellular protein transport | | | | | 1.57 | |
| GO:1903829 | GO:0090316 | GO:0015031 | GO:0051222 | GO:0032388 | GO:0006605 | GO:1904951 |
| GO:0032880 | GO:0006886 | GO:1903827 | GO:0051223 | GO:0070201 | GO:0033157 | GO:1903533 |
| GO:0032386 | GO:0032386 | GO:0032386 | GO:0032386 | GO:0032386 | GO:0032386 | GO:0032386 |
| cellular component organization | | | | | 1.37 | |
| GO:0051130 | GO:0033043 | GO:0051128 | | | | |
| membrane protein localization | | | | | 1.32 | |
| GO:1902580 | GO:0061024 | GO:0044802 | GO:0072657 | GO:0007009 | GO:0010256 | GO:0072659 |
| GO:1990778 | | | | | | |
| protein oligomerization | | | | | 1.19 | |
| GO:0070206 | GO:0070207 | GO:0051259 | GO:0051260 | | | |
| positive regulation of GTPase activity | | | | | 1.15 | |
| GO:0044093 | GO:0043547 | GO:0043085 | GO:0065009 | GO:0051345 | GO:0043087 | GO:0050790 |
| GO:0051336 | GO:0051338 | GO:0007264 | | | | |

| DNA damage response | | | | | 1.15 | |
|---|---|---|---|---|---|---|
| GO:2001020 | GO:0006282 | GO:0006281 | GO:0006974 | GO:0006259 | GO:2001022 | GO:2000779 |
| GO:0045739 | GO:0051052 | GO:0006302 | GO:0051054 | | | |
| **positive regulation of cell cycle** | | | | | 1.11 | |
| GO:0051983 | GO:0090068 | GO:0045787 | | | | |
| **response to endogenous cyclic compound** | | | | | 1.10 | |
| GO:0009719 | GO:0071363 | GO:0070848 | GO:0071407 | GO:0010033 | GO:0070887 | GO:0071495 |
| GO:0071310 | GO:0042221 | | | | | |
| **amino acid biosynthesis** | | | | | 1.10 | |
| GO:0071265 | GO:0009086 | GO:0006555 | GO:0009067 | GO:0000096 | GO:0046394 | GO:0008652 |
| GO:1901607 | GO:0016053 | GO:0009066 | GO:0006790 | GO:0006520 | GO:1901605 | |
| **nuclear protein import** | | | | | 1.08 | |
| GO:0006913 | GO:0051169 | GO:1902582 | GO:1900182 | GO:0051170 | GO:1903533 | GO:0034504 |
| GO:0046824 | GO:0044744 | GO:0006606 | GO:1902593 | GO:0042991 | GO:0042307 | GO:0042993 |
| GO:1904591 | GO:0017038 | GO:1900180 | GO:0046822 | GO:0042990 | GO:0042306 | GO:1904589 |
| **chromosome segregation** | | | | | 1.07 | |
| GO:0051983 | GO:0007059 | GO:0007062 | GO:0000819 | GO:0033045 | GO:0051304 | GO:0098813 |
| GO:0033044 | GO:0007091 | GO:0010965 | GO:0044784 | GO:0051306 | GO:0051784 | GO:0033047 |
| GO:0000070 | GO:0007094 | GO:0034502 | GO:0071173 | GO:0022402 | GO:2001251 | GO:2000816 |
| GO:0051985 | GO:0031577 | GO:0000280 | GO:1903047 | GO:0010948 | GO:0045786 | GO:0045839 |
| GO:0030071 | GO:1902099 | GO:1901990 | GO:0010639 | GO:0007346 | GO:0007067 | GO:0048285 |
| GO:1901991 | GO:0007049 | GO:0051301 | GO:1901987 | GO:0051783 | GO:1901988 | GO:0010564 |
| GO:0000075 | GO:0007126 | GO:1903046 | GO:0051726 | GO:0044772 | GO:0007088 | GO:0044770 |
| GO:0051129 | GO:0007093 | GO:0051321 | GO:0000086 | GO:1902589 | GO:0044839 | GO:0051129 |
| **regulation of organelle organization** | | | | | 1.05 | |
| GO:0033043 | GO:0010638 | GO:0051493 | | | | |
| **ribonucleoprotein complex assembly** | | | | | 1.05 | |
| GO:0022613 | GO:0001731 | GO:0022618 | GO:0071826 | | | |
| **amide biosynthesis** | | | | | 1.02 | |
| GO:0006412 | GO:0043043 | GO:0043604 | GO:1901564 | GO:1901566 | GO:0006518 | GO:0006417 |
| GO:0010608 | GO:0043603 | GO:0034248 | | | | |
| **Antigens exclusively represented by HLA class I ligands down-modulated on meningioma** | | | | | | |
| antigen processing and presentation | | | | | 3.98 | |
| GO:0002478 | GO:0019884 | GO:0048002 | GO:0019882 | GO:0019886 | GO:0002495 | GO:0002504 |
| GO:0002479 | GO:0042590 | GO:0002474 | | | | |
| **intracellular signal transduction** | | | | | 2.90 | |
| GO:0035556 | GO:0023051 | GO:0010646 | GO:0048583 | GO:0007165 | GO:0044700 | GO:0023052 |
| GO:0007154 | GO:0051716 | GO:0050794 | GO:0009966 | GO:0050789 | GO:0050896 | GO:0065009 |
| GO:0065007 | GO:1902531 | | | | | |
| **negative regulation of signal transduction** | | | | | 2.89 | |
| GO:0023051 | GO:0010646 | GO:0010648 | GO:0023057 | GO:0009966 | GO:0009968 | GO:0048585 |
| **membrane protein localization** | | | | | 2.87 | |
| GO:1902580 | GO:1990778 | GO:0090150 | GO:0072657 | GO:0072659 | GO:0090002 | GO:0010256 |
| GO:0034613 | GO:0070727 | GO:0007009 | GO:0061024 | GO:0044802 | | |
| **viral process** | | | | | 2.60 | |
| GO:0044403 | GO:0044419 | GO:0016032 | GO:0044764 | GO:0044033 | GO:0019080 | GO:0019083 |
| GO:0019058 | GO:0051704 | | | | | |
| **protein complex biogenesis** | | | | | 2.56 | |
| GO:0006996 | GO:0065003 | GO:0043933 | GO:0071840 | GO:0044085 | GO:0016043 | GO:0034622 |
| GO:0070271 | GO:0022607 | GO:0006461 | GO:0071822 | | | |
| **cellular protein localization** | | | | | 2.27 | |
| GO:1902580 | GO:0051641 | GO:0008104 | GO:0045184 | GO:0034613 | GO:0033036 | GO:0070727 |
| GO:1902582 | GO:0051234 | GO:0051179 | GO:0051649 | GO:0015031 | GO:1902578 | GO:0072594 |
| GO:0006810 | GO:0046907 | GO:0033365 | GO:0006605 | GO:0044765 | GO:0006886 | GO:0071702 |
| **apoptosis** | | | | | 2.25 | |
| GO:0051402 | GO:0043523 | GO:0070997 | GO:1901214 | GO:0043524 | GO:1901215 | GO:0006915 |
| GO:0042981 | GO:0010941 | GO:0043067 | GO:0043069 | GO:0008219 | GO:0012501 | GO:0043066 |
| GO:0060548 | GO:0010942 | GO:0043065 | GO:0043068 | | | |
| **inhibition of kinase activity** | | | | | 2.23 | |
| GO:0031400 | GO:0043549 | GO:0010563 | GO:0051338 | GO:0032269 | GO:0051248 | GO:0051348 |
| GO:0033673 | GO:0045859 | GO:0042325 | GO:0031324 | GO:0006469 | GO:0001933 | GO:0042326 |
| GO:0001932 | GO:0043086 | GO:0044092 | GO:0045936 | | | |
| **cellular response to peptide hormone** | | | | | 2.19 | |
| GO:0010033 | GO:0071310 | GO:0009725 | GO:1901700 | GO:0010243 | GO:0071417 | GO:0071495 |
| GO:0032870 | GO:1901698 | GO:0070887 | GO:0009719 | GO:0043434 | GO:1901653 | GO:1901652 |
| GO:0071375 | GO:1901699 | GO:1901701 | GO:0007166 | GO:0042221 | GO:0032868 | GO:0032869 |
| **EGFR signaling** | | | | | 1.98 | |
| GO:0007173 | GO:0038127 | GO:0007169 | | | | |
| **cell development** | | | | | 1.92 | |
| GO:0044767 | GO:0032502 | GO:0048731 | GO:0048856 | GO:0044707 | GO:0007275 | GO:0048869 |
| GO:0030154 | GO:0048513 | GO:0032501 | GO:0007399 | | | |
| **ribonucleoprotein complex assembly** | | | | | 1.73 | |
| GO:0022613 | GO:0022618 | GO:0071826 | | | | |
| **platelet activation** | | | | | 1.63 | |
| GO:0007596 | GO:0042060 | GO:0050817 | GO:0007599 | GO:0009611 | GO:0030168 | GO:0050878 |
| **nucleosome organization** | | | | | 1.61 | |
| GO:0071103 | GO:0006323 | GO:0034728 | GO:0006334 | GO:0006333 | GO:0031497 | GO:0071824 |
| GO:0065004 | | | | | | |

| regulation of MAPK cascade | | | | | 1.50 | |
|---|---|---|---|---|---|---|
| GO:0051246 | GO:0032268 | GO:0031399 | GO:0044267 | GO:0006464 | GO:0036211 | GO:0043549 |
| GO:0006468 | GO:0019538 | GO:0016310 | GO:0043412 | GO:0051338 | GO:0045860 | GO:0033674 |
| GO:0045859 | GO:0050790 | GO:0051347 | GO:0065009 | GO:0032147 | GO:0043406 | GO:0042325 |
| GO:0001932 | GO:0019220 | GO:0051174 | GO:0060255 | GO:0043085 | GO:1902531 | GO:0006796 |
| GO:0006793 | GO:0031401 | GO:0044093 | GO:0001934 | GO:0042327 | GO:0045937 | GO:0010562 |
| GO:0019222 | GO:0071902 | GO:0071900 | GO:0023014 | GO:0000165 | GO:0032270 | GO:0043170 |
| GO:0051247 | GO:0070372 | GO:0043405 | GO:0043410 | GO:0043408 | GO:0044260 | GO:0080090 |
| GO:0000187 | GO:0009893 | GO:0010604 | GO:0048584 | GO:0010647 | GO:0031323 | GO:0023056 |
| GO:0051336 | GO:0070371 | | | | | |
| T-cell activation | | | | | 1.46 | |
| GO:0050852 | GO:0007155 | GO:0022610 | GO:0031295 | GO:0031294 | GO:0050851 | GO:0030155 |
| GO:0098609 | GO:0002429 | GO:0002768 | GO:0050865 | GO:0002376 | GO:0001775 | GO:0022407 |
| GO:0002764 | GO:0002757 | GO:0050863 | GO:1903037 | GO:0002253 | GO:0045321 | GO:0002694 |
| GO:0050870 | GO:0038095 | GO:0070486 | GO:0045785 | GO:0007159 | GO:1903039 | GO:0006952 |
| GO:0046649 | GO:0045087 | GO:0002682 | GO:0002252 | GO:0071593 | GO:0070489 | GO:0042110 |
| GO:0002223 | GO:0051249 | GO:0002220 | GO:0050778 | GO:0022409 | GO:0050867 | GO:0098602 |
| GO:0031349 | GO:0050776 | GO:0006955 | GO:0031347 | GO:0002684 | GO:0016337 | GO:0002696 |
| GO:0002758 | GO:0038093 | GO:0051251 | GO:0002218 | GO:0045089 | GO:0045088 | GO:0002221 |
| GO:0002683 | | | | | | |
| membrane fusion | | | | | 1.41 | |
| GO:0022406 | GO:0006904 | GO:0048278 | GO:0061025 | GO:0048284 | GO:0022406 | GO:0006904 |
| microtubule-associated transport | | | | | 1.37 | |
| GO:0030705 | GO:0072386 | GO:0072383 | GO:0072384 | GO:0007018 | GO:0010970 | GO:0047496 |
| GO:0099518 | GO:0051656 | GO:0051640 | GO:0007017 | GO:0051648 | GO:0051650 | |
| stress-activated MAPK cascade | | | | | 1.32 | |
| GO:0080135 | GO:0032872 | GO:0070302 | GO:0046328 | GO:0023014 | GO:0000165 | GO:0031098 |
| GO:0051403 | GO:0046330 | GO:0043408 | GO:0043410 | GO:0032874 | GO:0070304 | GO:0043507 |
| GO:0007254 | GO:0043506 | | | | | |
| respiratory system development | | | | | 1.30 | |
| GO:0060541 | GO:0030324 | GO:0030323 | GO:0060425 | GO:0035295 | | |
| regulation of cell proliferation | | | | | 1.29 | |
| GO:0008283 | GO:0008285 | GO:0042127 | GO:0008284 | | | |
| cell physiology | | | | | 1.28 | |
| GO:0065007 | GO:0009987 | GO:0044763 | GO:0044699 | | | |
| response to vitamin | | | | | 1.26 | |
| GO:0071305 | GO:0031670 | GO:0031669 | GO:0071295 | GO:0031668 | GO:0033280 | GO:0071496 |
| GO:0031667 | GO:0009991 | GO:0033273 | GO:0007584 | GO:0042594 | | |
| negative regulation of TGF-β / bone morphogenetic protein signaling | | | | | 1.22 | |
| GO:0071560 | GO:0071559 | GO:0090101 | GO:0007179 | GO:1903845 | GO:0030512 | GO:0007167 |
| GO:0090288 | GO:1903844 | GO:0017015 | GO:0007178 | GO:0070848 | GO:0071363 | GO:0060021 |
| GO:0090092 | GO:0030514 | GO:0090287 | GO:0030509 | GO:0071773 | GO:0071772 | GO:0030510 |
| neuron differentiation | | | | | 1.21 | |
| GO:0048468 | GO:0048666 | GO:0022008 | GO:0030154 | GO:0030182 | GO:0031175 | GO:0048699 |
| GO:0061564 | GO:0048667 | GO:0007399 | GO:0000902 | GO:0007409 | GO:0000904 | GO:0032989 |
| GO:0048812 | GO:0030030 | GO:0048858 | GO:0032990 | | | |
| viral genome replication | | | | | 1.21 | |
| GO:0019079 | GO:0039703 | GO:0039694 | | | | |
| cell-cell and cell-substrate adhesion | | | | | 1.18 | |
| GO:0045216 | GO:0034332 | GO:0034330 | GO:0051017 | GO:0032231 | GO:0061572 | GO:0048010 |
| GO:0001952 | GO:0051492 | GO:0007160 | GO:0048041 | GO:0007045 | GO:0007044 | GO:0034333 |
| GO:2000114 | GO:0001954 | GO:0032878 | GO:0031589 | GO:1901888 | GO:0034329 | GO:0010810 |
| GO:0090109 | GO:0051893 | GO:1903391 | GO:0010811 | | | |
| response to steroid hormone | | | | | 1.17 | |
| GO:0009725 | GO:0014070 | GO:0071407 | GO:0033993 | GO:0030522 | GO:0071396 | GO:0048545 |
| GO:0071383 | GO:0043401 | GO:0009755 | GO:0030518 | | | |
| nuclear protein import | | | | | 1.09 | |
| GO:1902580 | GO:0034613 | GO:0070727 | GO:1902582 | GO:0051649 | GO:0072594 | GO:0046907 |
| GO:0033365 | GO:0006606 | GO:0044744 | GO:1902593 | GO:0017038 | GO:0034504 | GO:0051170 |
| GO:0006605 | GO:0006913 | GO:0051169 | GO:0006886 | GO:0042306 | GO:1904589 | GO:0046822 |
| GO:0043433 | GO:0042990 | GO:0042991 | GO:1900180 | GO:1903827 | GO:0032386 | GO:1903533 |
| GO:0033157 | GO:1903829 | GO:0090316 | GO:0032388 | | | |
| histone acetylation | | | | | 1.04 | |
| GO:0051276 | GO:0006325 | GO:0016570 | GO:0043970 | GO:0018205 | GO:0006475 | GO:0016569 |
| GO:2000615 | GO:0016573 | GO:0061647 | GO:0018393 | GO:0006473 | GO:0018394 | GO:0035065 |
| GO:0035066 | GO:0043543 | GO:2000758 | GO:2000756 | GO:0043967 | GO:1901983 | GO:1901985 |
| GO:0031056 | GO:0031058 | GO:1902275 | GO:1905269 | GO:0033044 | GO:0043966 | GO:2001252 |
| establishment or maintenance of cell polarity | | | | | 1.04 | |
| GO:0006939 | GO:0007163 | GO:0070830 | GO:0043297 | GO:0034329 | GO:0030866 | GO:0030865 |
| GO:0007043 | | | | | | |
| cytokine-mediated signaling | | | | | 1.04 | |
| GO:0045087 | GO:0019221 | GO:0071345 | GO:0034097 | GO:0002684 | | |
| actin organization | | | | | 1.02 | |
| GO:0051017 | GO:0030036 | GO:0032231 | GO:0061572 | GO:1902589 | GO:0051493 | GO:0010638 |
| GO:0051492 | GO:0007010 | GO:0032233 | GO:0051130 | GO:0044087 | GO:0030029 | GO:0032956 |
| GO:0032970 | GO:0031032 | GO:0043254 | GO:0051496 | GO:0030038 | GO:0043149 | GO:0007015 |
| GO:0051130 | GO:0051130 | GO:0051130 | GO:0051130 | GO:0051130 | GO:0051130 | |
| iron ion homeostasis | | | | | 1.02 | |
| GO:0006880 | GO:0097577 | GO:0051651 | GO:0006879 | GO:0046916 | GO:0051238 | GO:0055072 |
| GO:0055076 | GO:0006826 | GO:0000041 | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| ribosome biogenesis | | | | | 1.02 | |
| GO:0022613 | GO:0042254 | GO:0006364 | GO:0016072 | GO:0034660 | GO:0006396 | GO:0042274 |
| negative regulation of RNA transcription | | | | | 1.01 | |
| GO:0048519 | GO:0048523 | GO:0010605 | GO:0051246 | GO:0009892 | GO:0032268 | GO:0009058 |
| GO:1901576 | GO:0044267 | GO:0043412 | GO:0019538 | GO:0031324 | GO:0060255 | GO:0019222 |
| GO:0000122 | GO:0043170 | GO:0010629 | GO:0080090 | GO:0010468 | GO:0009890 | GO:0044260 |
| GO:0045934 | GO:0031323 | GO:0006366 | GO:0010558 | GO:1902679 | GO:0031327 | GO:0010467 |
| GO:1903507 | GO:0051171 | GO:0051172 | GO:0051253 | GO:2000113 | GO:0090304 | GO:0034654 |
| GO:0045892 | GO:0006139 | GO:0008152 | GO:0018130 | GO:0034641 | GO:0071704 | GO:0019438 |
| GO:0044238 | GO:0016070 | GO:0006357 | GO:0019219 | GO:0046483 | GO:0006725 | GO:1901362 |
| GO:0044271 | GO:0010556 | GO:0032774 | GO:1901360 | GO:0051252 | GO:0009889 | GO:0006807 |
| GO:0031326 | GO:0097659 | GO:2001141 | GO:2000112 | GO:0044237 | GO:1903506 | GO:0006355 |
| GO:0009059 | GO:0034645 | GO:0044249 | GO:0006351 | | | |

**Antigens exclusively represented by HLA class II-restricted peptides up-modulated on meningioma**

| | | | | | | |
|---|---|---|---|---|---|---|
| cell-cell adhesion | | | | | 3.94 | |
| GO:0007156 | GO:0098742 | GO:0098609 | GO:0007155 | GO:0022610 | GO:0007399 | |
| ribonucleoside metabolism | | | | | 2.16 | |
| GO:0033275 | GO:0030049 | GO:0030048 | GO:0006936 | GO:0070252 | GO:0003012 | GO:0003009 |
| GO:0006928 | GO:0046034 | GO:0050881 | GO:0050879 | GO:0009205 | GO:0009199 | GO:0009144 |
| GO:0009167 | GO:0009126 | GO:0009161 | GO:0009141 | GO:0009123 | GO:0003008 | GO:0046128 |
| GO:0042278 | GO:0006941 | GO:0009119 | GO:0009116 | GO:0030029 | GO:1901657 | GO:0007517 |
| GO:0009150 | GO:0009259 | GO:0006163 | GO:0019693 | GO:0072521 | GO:0061061 | GO:0060537 |
| GO:0009117 | GO:0006753 | GO:0055086 | GO:0019637 | GO:0014706 | GO:1901135 | GO:0006470 |
| GO:0006796 | GO:0006793 | GO:0009888 | GO:0044281 | GO:0016311 | GO:1901564 | GO:0044710 |
| actin organization | | | | | 2.00 | |
| GO:0033275 | GO:0030049 | GO:0030048 | GO:0070252 | GO:0006941 | GO:0030029 | GO:0030239 |
| GO:0031032 | GO:0061061 | GO:0055002 | GO:0055001 | GO:0010927 | GO:0070925 | GO:0030036 |
| GO:0048646 | GO:0051146 | GO:1902589 | GO:0032989 | GO:0042692 | GO:0007010 | |

**Antigens exclusively represented by HLA class II-restricted peptides down-modulated on meningioma**

| | | | | | | |
|---|---|---|---|---|---|---|
| response to oxidative stress | | | | | 1.74 | |
| GO:0098869 | GO:1990748 | GO:0098754 | GO:0009636 | GO:0055114 | GO:0000302 | GO:0006979 |
| GO:1901700 | | | | | | |
| protein catabolism | | | | | 1.14 | |
| GO:0044248 | GO:0009056 | GO:1901575 | GO:0009057 | GO:0044265 | GO:0009894 | GO:0042176 |
| GO:0031329 | GO:1903050 | GO:1903362 | GO:0030163 | GO:0051603 | GO:0044257 | GO:0006508 |
| GO:0043161 | GO:0006511 | GO:0019941 | GO:0043632 | GO:0010498 | GO:0042787 | GO:0030162 |
| GO:0016567 | GO:0032446 | GO:0051246 | GO:0032268 | GO:0070647 | | |

## *Supplement of CHAPTER 6*

**Supplementary Table 22. Peptide yields with PROCAL or heavy isotope-labeled RT peptides spiked into a complex matrix of HLA class I peptides eluted from JY cells.** PROCAL peptides were spiked at 2.5 / 5 / 10 / 20 fmol/peptide/μl into JY17#3 and LC-MS/MS measurements were performed back-to-back on an Orbitrap Fusion Lumos. A selection of ten in-house produced heavy isotope-labeled RT peptides were diluted in JY17#3 (Orbitrap Fusion Lumos) or in JY17#4 (LTQ Orbitrap XL) to a concentration of 0.01 / 0.25 / 0.05 / 0.1 / 0.25 / 0.5 / 1 / 5 fmol/peptide/μl and LC-MS/MS data acquisition was performed in the order of ascending synthetic peptide concentration. Of note, neither PROCAL nor RT peptides had an apparent influence on the total number of unique peptide identifications at any of the evaluated concentrations. RT peptides were spiked at determined concentration into the newly produced LC-MS/MS standard JY19#1.

| LC-MS/MS measurement | Concentration [fmol/ peptide/μl] | Unique peptides | Total PSMs | Total intensity |
|---|---|---|---|---|
| **PROCAL peptides** | | | | |
| **Orbitrap Fusion Lumos** | | | | |
| 180628_LF_jpt_RTPeptides_12-5fmol-5ul_2-5fmol-ul_ JY**17#3**_4-1diluted_thawed20180629_DDA#1_400-650mz_msms67 | 2.5 | 1057 | 3435 | 3.504E+10 |
| 180628_LF_jpt_RTPeptides_25fmol-5ul_5fmol-ul_ JY**17#3**_4-1diluted_thawed20180629_DDA#1_400-650mz_msms66 | 5 | 1094 | 3936 | 6.012E+10 |
| 180628_LF_jpt_RTPeptides_50fmol-5ul_10fmol-ul_ JY**17#3**_4-1diluted_thawed20180629_DDA#1_400-650mz_msms65 | 10 | 1080 | 3833 | 1.064E+11 |
| 180628_LF_jpt_RTPeptides_100fmol-5ul_20fmol-ul_ JY**17#3**_4-1diluted_thawed20180629_DDA#1_400-650mz_msms64 | 20 | 1029 | 4404 | 2.333E+11 |
| **In-house heavy isotope-labeled RT peptides** | | | | |
| **Orbitrap Fusion Lumos** | | | | |
| 190605_LF_10_RTPeptides_in-house_0-fmol-ul_Aload_1-10_ in_JY**17#3**_ thawed20190604_DDA#1_400-650mz_Lumos_msms1 | 0 | 1254 | 2785 | 2.452E+10 |
| 190605_LF_10_RTPeptides_in-house_0-01fmol-ul_in_ JY**17#3**_thawed20190604_DDA#1_400-650mz_Lumos_msms2 | 0.01 | 1201 | 2670 | 2.035E+10 |
| 190605_LF_10_RTPeptides_in-house_0-025fmol-ul_in_ JY**17#3**_thawed20190604_DDA#1_400-650mz_Lumos_msms3 | 0.025 | 988 | 2197 | 1.718E+10 |
| 190605_LF_10_RTPeptides_in-house_0-05fmol-ul_in_ JY**17#3**_thawed20190604_DDA#1_400-650mz_Lumos_msms4 | 0.05 | 1393 | 3014 | 2.621E+10 |
| 190605_LF_10_RTPeptides_in-house_0-1fmol-ul_in_ JY**17#3**_thawed20190604_DDA#1_400-650mz_Lumos_msms5 | 0.1 | 1430 | 3106 | 2.807E+10 |
| 190605_LF_10_RTPeptides_in-house_0-25fmol-ul_in_ JY**17#3**_thawed20190604_DDA#1_400-650mz_Lumos_msms6 | 0.25 | 1441 | 3269 | 2.817E+10 |
| 190605_LF_10_RTPeptides_in-house_0-5fmol-ul_in_ JY**17#3**_thawed20190604_DDA#1_400-650mz_Lumos_msms7 | 0.5 | 1419 | 3182 | 3.003E+10 |
| 190605_LF_10_RTPeptides_in-house_1fmol-ul_in_ JY**17#3**_thawed20190604_DDA#1_400-650mz_Lumos_msms8 | 1 | 1358 | 3201 | 3.150E+10 |
| 190913_AN_JY_Standard_**19#1**_RT_ aufgetaut190913_DDA#1_400-650mz_msms7 | 0.05-1 | 2429 | 5453 | 5.347E+10 |
| **LTQ Orbitrap XL** | | | | |
| 190604_LF_10_RTPeptides_in-house_0fmol-ul_Aload_1-10_ inJY**17#4**_thawed20190604_Rep#1_400-650mz_XL_msms11 | 0 | 923 | 2525 | 9.267E+09 |
| 190604_LF_10_RTPeptides_in-house_0-025fmol-ul_ inJY**17#4**_thawed20190604_Rep#1_400-650mz_XL_msms12 | 0.025 | 1004 | 2545 | 8.724E+09 |
| 190604_LF_10_RTPeptides_in-house_0-05fmol-ul_ inJY**17#4**_thawed20190604_Rep#1_400-650mz_XL_msms13 | 0.05 | 915 | 2356 | 8.654E+09 |
| 190604_LF_10_RTPeptides_in-house_0-1fmol-ul_ inJY**17#4**_thawed20190604_Rep#1_400-650mz_XL_msms14 | 0.1 | 947 | 2454 | 7.730E+09 |
| 190604_LF_10_RTPeptides_in-house_0-25fmol-ul_ inJY**17#4**_thawed20190604_Rep#1_400-650mz_XL_msms15 | 0.25 | 974 | 2477 | 9.842E+09 |
| 190604_LF_10_RTPeptides_in-house_0-5fmol-ul_ inJY**17#4**_thawed20190604_Rep#1_400-650mz_XL_msms16 | 0.5 | 976 | 2565 | 1.014E+10 |
| 190604_LF_10_RTPeptides_in-house_1fmol-ul_ inJY**17#4**_thawed20190604_Rep#1_400-650mz_XL_msms17.msf | 1 | 839 | 2227 | 7.066E+09 |
| 190604_LF_10_RTPeptides_in-house_5fmol-ul_ inJY**17#4**_thawed20190604_Rep#1_400-650mz_XL_msms18.msf | 5 | 912 | 2656 | 1.126E+10 |
| 191125_LM_Standard_JY**19#1**_W_10RTpeptides_ 20191112_Rep#4_400-650mz_XL_PerformanceCheck_msms25.msf | 0.1-0.5 | 1350 | 3347 | 1.623E+10 |