Aus der

Universitäts-Hautklinik Tübingen

Sektion Dermatologische Onkologie

Clinicopathologic nomogram of stage IV melanoma patients treated with anti-PD-1 based immunotherapy and study of patients who achieved a complete response

Inaugural-Dissertation
zur Erlangung des Doktorgrades
der Medizin

der Medizinischen Fakultät
der Eberhard Karls Universität
zu Tübingen

vorgelegt von

Chatziioannou, Eftychia

2026

Dekanin:	Professorin Dr. S. Y. Brucker
1. Berichterstatter:	Professor Dr. L. Flatz
2. Berichterstatter:	Privatdozentin Dr. I. Bonzheim

Tag der Disputation: 13.04.2026

Dedication

For my Family

In Gratitude

Table of contents

List of Abbreviations……v

1. Introduction 1
   1.1. Melanoma background 1
   1.2. Staging Melanoma 7
   1.3. Therapy of melanoma 8
      1.3.1. Excision 9
      1.3.2. Sentinel lymph node biopsy 9
   1.4. Systemic Therapy 10
      1.4.1. Response RECIST criteria 16
      1.4.2. Adverse effects 17
      1.4.3. Biomarkers 19
   1.5. Aims and objectives 22
2. Results and discussion 23
   2.1. Publication 1: Features and long-term outcomes of stage IV melanoma patients achieving complete response under anti-PD-1-based immunotherapy 23
   2.2. Publication 2: Nomogram for predicting survival after first-line anti-PD-1-based immunotherapy in unresectable stage IV melanoma: a multicenter international study 39
3. Discussion 50
4. Summary 62
5. German summary -Zusammenfassung 63
6. Bibliography / List of References 66
7. Declaration and contributions 91
   7.1. Declaration 91
   7.2. Contributions of other authors 92
8. Acknowledgements 95

Abbreviations

AJCC	American Joint Committee on Cancer
ALM	Acral Lentiginous Melanoma
BRAF	V-Raf Murine Sarcoma Viral Oncogene Homolog B
BRAF-WT	*BRAF* Wild Type
BRAFi	BRAF Inhibitor
BMI	Body Mass Index
CD274	Cluster of Differentiation 274 Molecule (PD-L1)
cKIT	Proto-Oncogene Receptor Tyrosine Kinase
CLND	Complete Lymph Node Dissection
CM	Cutaneous Melanoma
CMMR	German Central Malignant Melanoma Registry
CR	Complete Response
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
ctDNA	Circulating Tumor DNA
CVD	Cisplatin, Vinblastine, and Dacarbazine
DC	Disease Control
DNA	Deoxyribonucleic Acid
DTIC	Dacarbazine

ECOG	Eastern Cooperative Oncology Group
ESMO	European Society of Medical Oncology
FUP	Follow-Up Time
HLA	Human Leukocyte Antigen
HR	Hazard Ratio
ICI	Immune Checkpoint Inhibitor
IFNGR1	Interferon Gamma Receptor 1
IL	Interleukin
iRECIST	Immune Response Evaluation Criteria in Solid Tumors
irAE	Immune-Related Adverse Event
IQR	Interquartile Range
IT	Immunotherapy
Jak	Janus Kinase
LAG3	Lymphocyte Activation Gene-3
LDH	Lactate Dehydrogenase
LMM	Lentigo-Maligna Melanoma
MAPK	Mitogen-Activated Protein Kinase
MAPKi	MAPK Pathway Inhibitor
MEK	Mitogen-Activated Protein Kinase Kinase
MM	Malignant Melanoma
mOS	Median Overall Survival

mPFS	Median Progression-Free Survival
NA	Not Applicable
NF-1	Neurofibromin 1
NLR	Neutrophil-to-Lymphocyte Ratio
NM	Nodular Melanoma
NRAS	Neuroblastoma RAS Viral Oncogene Homolog
OR	Odds Ratio
ORR	Objective Response Rate
OS	Overall Survival
PD	Progressive Disease
PD-1	Programmed Cell Death Protein 1
PD-L1	Programmed Cell Death Ligand 1
PFS	Progression-Free Survival
PLR	Platelet-to-Lymphocyte Ratio
PR	Partial Response
RAS	Rat Sarcoma
RECIST	Response Evaluation Criteria in Solid Tumors
S100	S100 Calcium-Binding Protein
SD	Stable Disease
SLNB	Sentinel Lymph Node Biopsy
SPSS	Statistical Package for the Social Sciences

SSM	Superficial Spreading Melanoma
STAT	Signal Transducer and Activator of Transcription
T	Tumor
TAP1	Transporter Associated with Antigen Processing 1
TERT	Telomerase Reverse Transcriptase
TIGIT	T Cell Immunoreceptor with Ig and ITIM Domains
TIL	Tumor-Infiltrating Lymphocyte
TIM3	T Cell Immunoglobulin and Mucin Domain-Containing Protein 3
TMB	Tumor Mutational Burden
TCR	T Cell Receptor
TNM	Tumor Node Metastasis
TT	Targeted Therapy
UM	Uveal Melanoma
UV	Ultraviolet
WT	Wild Type

1. Introduction

1.1. Melanoma background

MM is a malignant tumor that arises from cells that produce melanin, called melanocytes (Snyman et al. 2024).

From an embryological perspective, melanocytes originate from the neural crest during development (Dupin and Le Douarin 2003). Melanoblasts differentiate and colonize various sites, including the epidermis, hair follicles (bulb), uveal tract, leptomeninges, ears, and certain mucosal surfaces (Dupin and Le Douarin 2003; Mort, Jackson, and Patton 2015). This developmental pathway explains the fact that MM arises not only in sun-exposed skin from the epidermis but also in the eye, mucosal surfaces, and within the nervous system (Brombin and Patton 2024).

CM represents only a small percentage of cutaneous cancers, which mainly are basal and squamous cell carcinomas. Despite the lower incidence of CM than other cutaneous cancers, it is the main cause of death from cutaneous malignancies due to its aggressiveness and significant metastatic potential as compared to basal cell and squamous cell carcinoma. Non-acral CM has the most favorable prognosis, while acral, mucosal, and uveal melanomas carry a more dismal prognosis (Arnold et al. 2022; Sadeq et al. 2023).

A persistent upward trend in melanoma incidence has been observed in the past decades, especially in the younger population (Bosetti et al. 2004). Australia, New Zealand, the United States, and parts of Europe have the highest incidence rates, representing a significant healthcare burden (L. Zhou et al. 2025; Garbe et al. 2024; Conforti and Zalaudek 2021; Sneyd and Cox 2013). The main risk factor of non-acral CM is UV radiation, while genetic predisposition, sunburns, phototype, number of nevi, color of hair, and eyes can have an additive and modifying effect.

There are several histological variants of CM, each exhibiting unique morphological features, preferred anatomical localizations, epidemiological associations, and clinical characteristics. Identification of these subtypes is important, as they exhibit distinct

behavior that can influence prognosis and therapeutic planning (Whiteman, Pavan, and Bastian 2011; Druskovich et al. 2025).

SSM is the most common histological subtype and represents approximately 60-70% of CM cases. SSM is commonly found on intermittently sun-exposed sites, for example, due to sunburns, such as the trunk in male and the lower extremities in female population. Microscopically, it demonstrates a radial growth pattern, along with the spread of atypical melanocytes within the epidermis. This histological subtype is associated with *BRAF* mutations (R. A. Scolyer, Long, and Thompson 2011; Trindade, de Freitas, and Bittencourt 2021; Singh, Kim, and Schwartz 2016; Whiteman, Pavan, and Bastian 2011).

NM comprises about 15-20% of cases and is characterised by its aggressive vertical growth pattern, resulting in rapid tumor growth. Clinically, NM appears as a pigmented, dome-shaped nodule, often ulcerated. Due to its minimal radial component, NM often is thicker and therefore has a worse prognosis (García-Lozano et al. 2020; Myers and Hyde 2021; Susok et al. 2021).

LMM represents approximately 5-10% of CM and typically develops on chronically sun-damaged skin. Therefore, it is most commonly found in older individuals, who also have solar elastosis and other non-melanoma skin cancers. It evolves from lentigo maligna, an in-situ precursor that may persist for years in a purely radial phase before becoming invasive. LMM is found on the face, scalp, and neck and has atypical melanocytes that extend in a lentiginous pattern along an atrophic epidermis (Tsatsou et al. 2012; Kasprzak and Xu 2015).

ALM is rare in Caucasians and whites and is much more common in patients with dark skin. ALM arises on palms, soles, or subungual regions as an irregular pigmented patch or as longitudinal melanonychia. ALM represents a diagnostic challenge, is often diagnosed late and at a more advanced stage (Holman et al. 2023; Bradford et al. 2009; Boriani et al. 2014; Nakamura and Fujisawa 2018).

Mucosal melanoma is rare and represents 1-2% of all melanoma cases and arises in the mucous membranes of the respiratory (nasal, paranasal cavities), gastrointestinal (from oral, oropharynx, larynx, stomach, esophagus, small intestine to anorectal), and genitourinary (vulva, vagina, cervix) tract, with the head and neck being the most frequent one. This subtype has not been linked to UV radiation, and its pathogenesis remains unknown. It manifests with vague or site-specific symptoms and, due to its difficult localisation, is diagnosed at an advanced stage. These tumors typically are aggressive with higher recurrence rates and worse OS outcomes than CM, necessitating specialized surgical and systemic management (Hahn et al. 2019; Lazarev et al. 2014; Sergi et al. 2023). Also, it is unresponsive to ICI, while its genomic landscape is distinct.

Desmoplastic melanoma is 1-4% of melanomas and mainly affects older adults with chronically sun-damaged skin. It is characterized by spindle cells within a dense stroma, often displaying a scar-like appearance. This subtype is frequently amelanotic, and therefore also difficult to diagnose. Histology can also be a challenge, and special stains are usually needed, like immunohistochemical confirmation with SOX10. It often shows neurotropism and is associated with a high risk of local relapse, though regional nodal or distant spreading are relatively uncommon compared to other subtypes. Moreover, it carries *NF1* mutations, high TMB, and increased T cell infiltration and appears to respond better to ICI than other subtypes (Bailey, Thompson, and Scolyer 2020; Shannon, Zager, and Perez 2024; J.Y. Chen et al. 2008; L.L. Chen et al. 2013; Kendra et al. 2023).

Nevoid Melanoma is a rare variant that histologically resembles benign melanocytic nevi and is also a significant diagnostic challenge. Despite its benign appearance, it demonstrates histologically atypia (Valentini, Quinn, and Murphy 2025; Zembowicz et al. 2001; Idriss et al. 2015).

Spitzoid and Spitz melanoma mimics benign Spitz nevi but exhibits cytological atypia and asymmetry. Distinguishing spitzoid melanoma from atypical Spitz tumors and

benign Spitz nevi is challenging and often requires additional molecular diagnostic tools. Loss of p16 has been used (Zedek and McCalmont 2011; Cheng, Ahern, and Giubellino 2022; Harms et al. 2016).

Animal-Type, or pigment-synthesizing melanoma, is a very rare melanoma type and is characterized by increased melanin production, which gives it its characteristic appearance, making it resemble melanomas found in certain animals. It can appear like blue nevi and affects younger individuals. Although it has a more indolent course than other types, local aggressiveness and recurrence are a risk (Vyas et al. 2015; Antony et al. 2006; Crowson, Magro, and Mihm 1999).

Primary Central Nervous System Melanoma is also very rare and originates from leptomeningeal melanocytes, and it should be distinguished from secondary metastatic spread. This more commonly affects children and young patients with neurocutaneous melanosis or congenital nevi. Intracranial localisation is often associated with a worse prognosis than spinal, and *NRAS* mutations are common. Its diagnosis requires differentiation from other melanotic lesions and metastatic melanoma, which is more common in the CNS (Man and Wang 2019; P. Guo et al. 2024; Pellerino et al. 2024; Wadasadawala et al. 2010; Liubinas, Maartens, and Drummond 2010).

As previously discussed, UV radiation is the main risk factor for melanoma, but its impact depends on the pattern and duration of exposure. CM like SSM are strongly linked to intermittent, high-dose UV exposure, typically after severe sunburns. These lesions commonly arise on intermittently exposed areas like the trunk and limbs (Raimondi, Suppa, and Gandini 2020; Gandini et al. 2005; Elwood et al. 1985; Chang et al. 2022). In contrast, LMM is associated with prolonged, cumulative chronic UV exposure over a lifetime. It usually affects sun-damaged sites like the face, ears, and scalp, showing histological signs of chronic sun exposure like solar elastosis (Linos et al. 2017; Tsatsou et al. 2012; DeWane et al. 2019). Meanwhile, subtypes such as ALM and mucosal melanoma develop independently of UV radiation. Their occurrence highlights alternative pathogenetic factors, including genetic predisposition, somatic

mutations unrelated to UV-induced DNA damage, and chronic irritation or inflammation in mucosal sites. Acral and mucosal melanomas have a distinct genomic profile characterised by a lack of *BRAF* and *NRAS* mutations, while higher frequency of *KIT* mutations and *CDK4* amplifications, has lower TMB and no UV signature (Fortuna and Amaral 2024; Indini et al. 2022; M. Wang et al. 2022; Mao et al. 2021).

Other factors that contribute to increased melanoma risk include genetic susceptibility. Around 10% of CM have a familial background. Germline mutations in cell cycle genes like *CDKN2A, CDK4*, in telomere-related genes like *TERT, POT1, ACD, TERF2IP,* and in other genes like *BAP1* have been implicated in germline susceptibility (Aoude et al. 2014; Baptista Freitas et al. 2024; Potrony et al. 2015).

Other risk factors include use of tanning beds, phototype with Fitzpatrick I and II being most at risk, light eye and hair color, presence of nevi (common, congenital, atypical), and immunosuppression. The mean age at diagnosis is 65 years (Conforti and Zalaudek 2021).

From a molecular standpoint, a hallmark of melanoma biology is the heterogeneity of its molecular alterations. Mutations in the MAPK pathway are very common, and *BRAF* mutations are the most common ones in melanoma. The *BRAF* V600E mutation is the most prevalent driver, occurring in approximately 40-50% of CMs, predominantly on intermittently sun-exposed skin. This mutation activates the MAPK pathway, which is considered the first step of nevogenesis and oncogene-driven senescence (I. Vanni, Tanda, Spagnolo, et al. 2020; Greaves et al. 2013; Rubió-Casadevall et al. 2021; Spathis et al. 2019). This mutation is most common in non chronically sun damaged skin, especially in SSM, and is more commonly found on the trunk and in younger patients. At codon V600, other mutations are also found, although rarer (Vredeveld et al. 2012).

NRAS mutations are in about 15-30% of CM and similarly activate the MAPK pathway as well as the PI3K-AKT pathways. The most common mutated codon is Q61, whereas also at 12 and 13 positions, rarer mutations are found. The Q61 mutations are mutually exclusive with *BRAF* mutations and are often associated with aggressive behavior and

resistance to TT. MEK inhibitors, although inhibiting the pathway downstream, have not exhibited promising results. They are most commonly found in thicker tumors, NM, in extremities, and more commonly found in congenital nevi, leptomeningeal melanosis, and primary CNS melanoma (Awada and Neyns 2022; Burd et al. 2014).

HRAS mutations and amplifications have been found in Spitz nevi, and *KRAS* amplifications have been associated with worse prognosis (Irene Vanni, Tanda, Dalmasso, et al. 2020; Zhao et al. 2021; Heppt et al. 2017; Jenkins and Sullivan 2016; Rabbie et al. 2021).

Melanomas on chronically sun-damaged skin, like LMM, as well as other types not associated with UV, including acral and mucosal melanomas, have a distinct molecular and genomic landscape.

KIT mutations are more common in these subtypes, around 10-15%, than in other subtypes and involve gain-of-function alterations that activate receptor tyrosine kinase, promoting melanocyte proliferation and survival. Mutations in exons 11,13, and 17 of *KIT* are most commonly found, as well as amplification of *KIT* (Nassar and Tan 2020; Beadling et al. 2008; Yun et al. 2011; M. Wang et al. 2022; F. Newell, Johansson, et al. 2022; Felicity Newell et al. 2020). For these mutations, inhibitors also exist (Akbani et al. 2015; Awada and Neyns 2022; Beadling et al. 2008; Y.-j. Cai et al. 2021).

NF1 loss of function is also a driver in melanoma, primarily in tumors lacking *BRAF* or *NRAS* mutations. These mutations are more commonly observed in older patients with chronic UV exposure and activate the MAPK pathway. They are found in tumors with high TMB and are found in a higher percentage of desmoplastic melanoma (Irene Vanni, Tanda, Dalmasso, et al. 2020; Krauthammer et al. 2015; Nissan et al. 2014; Cancer Genome Atlas 2015; Wiesner et al. 2015). These mutations have been associated with a favorable response to ICI. Traditionally, CM is classified as *BRAF, NRAS, NF1* mutated versus triple WT (Cancer Genome Atlas 2015).

TERT promoter mutations, mainly C228T and C250T at -124 and -146 hotspots, occur mainly in melanomas, especially in SSM and NM. These mutations are associated with

UV signature and lead to increased telomerase activity (Y. Guo, Chen, et al. 2022; F.W. Huang et al. 2013; Cancer Genome Atlas 2015; Griewank et al. 2014; Broseghini et al. 2025).

In addition to common drivers, melanoma harbors a range of rarer somatic genomic alterations contributing to its heterogeneity, metastatic potential, and therapeutic resistance.

Mutations in *MAP2K1, KRAS, RAC1,* and *SPRED1* can also activate the MAPK or other related pathways, while alterations in *PIK3CA, AKT1, TSC1/TSC2,* and *PTEN* activate the PI3K-AKT-mTOR axis. Mutations in *CTNNB1* or *APC* disrupt the β-catenin pathway and promote metastasis and immune evasion (J. J. Luke et al. 2019). Cell cycle deregulation arises from mutations or genomic copy number alterations in cell cycle genes, including *CDKN2A, CDK4, CCND1, RB1*, and *MDM2*. Epigenetic modifiers like *ARID1A, DNMT1, ARID2, EZH2, KMT2C,* and *KMT2D* affect chromatin structure, remodeling and gene expression, while rare alterations in *IDH1/2, FBXW7,* and *MET* pathways influence metabolism and metastatic behavior (Ye et al. 2022; Dreier and de la Serna 2022; Ramani, Morani, and Zhang 2022; Redmer et al. 2024).

1.2. Staging Melanoma

The AJCC 8th is the mainstay for CM classification, integrating the TNM to stratify patients according to the risk of melanoma progression to guide prognosis and therapeutic management. This edition refined definitions in 2017 to better reflect survival outcomes derived from large international datasets, also after the introduction of anti-PD-1-based IT. It has better prognostic accuracy than AJCC-7 (2010) and better stratifies high-risk patients. Stages I-II are localized disease, stratified into risk groups using the primary tumor thickness and presence or absence of ulceration. Stage III encompasses regional spread to sentinel lymph nodes or clinically or radiologically affected lymph nodes, and in-transit, satellite, or microsatellite cutaneous/subcutaneous metastases. Stage IV includes metastatic melanomas to distant organs (Keung and Gershenwald 2018; Hynes et al. 2022; Tjokrowidjaja, Browne, and Soudy 2022; Barreiro-Capurro et al. 2021; Gershenwald and Scolyer 2018).

Tumor (T) classification is primarily determined by Breslow tumor thickness and ulceration of the primary tumor. Tumor thickness is grouped into four categories, those below or equal to 1 mm, those between 1.01 and 2 mm, those between 2.01 and 4 mm, and those above >4 mm, with each T group subdivided using the presence or absence of ulceration. T1 distinguishes lesions ≤0.8 mm without ulceration T1a, from those ≤0.8 mm with ulceration or with a thickness between 0.8 and 1 mm, T1b, reflecting evidence that these T1b tumors carry higher metastatic risk (Gershenwald and Scolyer 2018; Ferguson, Gershenwald, and Scolyer 2018; Richard A. Scolyer et al. 2020). This was a new incorporation in AJCC edition 8.

Nodal (N) staging considers the number of affected lymph nodes. Nodal metastases are considered either the microscopic ones, termed micrometastases, identified by SLNB, or macroscopic, clinically or radiologically evident, termed macrometastases. The N category also stratifies according to the presence of in-transit, satellite, or microsatellite metastases. These locoregional cutaneous or lymphonodal metastases significantly affect the prognosis, and incorporating their presence enhances the accuracy of the classification of AJCC edition 8 (Keung and Gershenwald 2018; Bartlett et al. 2014; Shaikh et al. 2005).

Distant metastasis (M) is categorized by site of distant metastasis and by serum LDH status. The inclusion of CNS metastasis as a distinct category (M1d) with the worst prognosis reflects its unique clinical behavior and poorer outcomes (Gershenwald and Scolyer 2018; Diem et al. 2016; Janka et al. 2021).

1.3. Therapy of melanoma

The treatment of CM is determined by disease stage and relies on a multidisciplinary strategy that integrates surgical excision, nodal staging, systemic therapy, and, when indicated, regional or radiotherapeutic approaches. The principal aim is complete local control of the primary tumor, assessment of regional metastasis, and optimal management of high-risk or advanced disease to prolong survival and reduce

recurrence risk. In this context, the use of biomarkers to predict and monitor disease is of high importance (Gamboa et al. 2020; Eljilany, Castellano, and Tarhini 2023; Seth et al. 2023; McKean and Amaria 2018; A. M. M. Eggermont et al. 2022).

1.3.1. Excision

Surgical excision remains the front-line therapeutic option for localized primary melanoma, and the primary tumor is resected with surgical margins. The surgical margins are determined according to the Breslow tumor thickness of primary tumor, with international consensus recommending margins ranging from 0.5 to 1 cm for melanoma in situ, 1 cm for tumors up to 1 mm thick, 1 to 2 cm for 1 to 2 mm thick, and 2 cm for thicker lesions exceeding 2 mm, as 4cm margins did not show a benefit in prognosis. For lentigo maligna, a precursor of LMM, Mohs can be considered as an alternative, and for melanomas in specific localizations, Mohs is also under research. For UM, the current therapeutic strategy for the primary tumor is proton beam, while past methods, including brachytherapy and enucleation, have been abandoned (Utjés et al. 2019; Thomas et al. 2004; Nacchiero et al. 2024; Khayat et al. 2003; R.N. Hussain, Chiu, et al. 2023; Sharma et al. 2021).

1.3.2. Sentinel lymph node biopsy

Regional nodal staging is essential for prognostication and thus therapeutic decisions, mainly in the era of neoadjuvant and adjuvant therapy. SLNB is recommended for melanoma patients at initial diagnosis when no macroscopic metastasis is clinically or radiologically found. Eligible patients are those with melanomas that are thicker than 0.8 mm or exhibiting ulceration, as well as some younger patients in daily clinical practice. SLNB identifies the first lymph nodes to which the tumor drains, and detects microscopic nodal metastasis that cannot be clinically or radiologically found (Morton et al. 2006; Y. Hu et al. 2020; Holmberg et al. 2023; Venna et al. 2013). The presence of micrometastases identified in the sentinel node with SLNB automatically upgrades the staging to stage III and predicts a worse melanoma-specific survival as compared to those without localized disease.

However, the exact stratification and prognosis are more complex. SLNB not only detects micrometastases but also offers valuable additional prognostic information based on the extent and distribution of tumor cells within the node, parameters that influence prognosis. Research indicates that factors such as the size of metastatic as

tumor burden, their anatomical location within the node, and the density of disseminated cancer cells per million nodal cells, provide a more accurate prediction of patient outcomes than nodal positivity alone (van Akkooi et al. 2008; Z. Hussain, Heaton, et al. 2023; Rhodin et al. 2022; Ulmer et al. 2018; van der Ploeg et al. 2011; Teresa Amaral, Nanz, et al. 2024).

Traditionally, a positive SLNB was subsequently followed by CLND; however, trials demonstrated that immediate CLND does not prolong OS compared to nodal surveillance, like with ultrasonography, while also increasing the risk of complications like lymphedema and seroma. As a result, now with the use of adjuvant therapies, immediate CLND has been largely abandoned in favor of observation and surveillance. This is true for all micrometastases where adjuvant therapy can be given, reserving further surgical intervention for clinically evident nodal progression. Especially for patients with micrometastases below 0.1 mm and subscapular localisation, the CLND has been abandoned, although the benefit that adjuvant therapy can give is yet unclear (Faries et al. 2017; Leiter et al. 2019; Jiminez et al. 2025; Kretschmer et al. 2021; Farrow et al. 2020).

Emerging technologies, including RNA-based molecular assays, have demonstrated potential for noninvasive prediction of nodal metastasis risk, which may potentially reduce the need for surgical SLNB. Such molecular assays hold promise for better patient selection (Yousaf et al. 2021; Kriza et al. 2024; Teresa Amaral et al. 2025; T. Amaral, Chatziioannou, et al. 2024; J. Sun, Karasaki, and Farma 2024; Whitman et al. 2021; Garg et al. 2021).

1.4. Systemic Therapy

Before the emergence of MAPK inhibition with BRAF/MEK inhibition and ICI, chemotherapy was the principal systemic option for advanced melanoma. DTIC was the standard of care, and as an alkylating agent, it induced DNA damage to promote tumor cell apoptosis. Despite its use, DTIC monotherapy achieved ORR of only 10-20%, with CR being rare. Median PFS generally did not exceed 2-3 months, and OS benefit was minimal, therefore, DTIC is now largely considered ineffective (Velho 2012; Rydén et al. 2024; Ascierto et al. 2019).

Other cytotoxic agents, including temozolomide, platinum compounds (cisplatin, carboplatin), taxanes (paclitaxel, nab-paclitaxel, docetaxel), have demonstrated only slight improvements in ORR and survival. Combinatorial chemotherapeutic regimens, like CVD yielded slightly higher ORRs but have not demonstrated prolongation of OS and are associated with increased toxicity (Bhatia, Tykodi, and Thompson 2009; Atkins et al. 2008; Chapman et al. 1999; T. Hu et al. 2021).

Today, conventional chemotherapy has only a minor role, typically as palliative therapy, reserved for patients who progress after IT and TT. However, studies also show that in this context, no significant survival benefit can be seen. Therefore, it is given only as a last line palliative portion, reserved for control of the symptoms after TT, ICI, and trial enrollment. Only some trials are investigating whether the addition of some chemotherapeutic agents, like temozolomide, can provide benefit when combined with anti-PD-1-based IT (Goldinger et al. 2022; Gaughan and Horton 2022; Pham et al. 2023; T. Hu et al. 2021).

In light of this, the use of ICI has revolutionized the therapeutic management of melanoma, particularly in cases of advanced disease and high-risk resected tumors (stage IIB to IV), which before use of ICI they carried a dismal prognosis with OS of 20%-30% at 24 months for patients with macroscopic metastases (Cascinelli et al. 1998). CM is considered a highly immunogenic tumor, which makes it eligible for ICI. This intrinsic immunogenicity of melanoma is due to the high TMB induced by UV, which in turn increases the neoantigen presentation, making tumor cells more visible to the immune system (Conway et al. 2018; Kim et al. 2019; A.M. Goodman et al. 2017). On the other hand, subtypes that are not associated with UV, like mucosal, uveal and acral melanoma, do not respond to ICI as they have lower TMB (Andrews et al. 2024; A.M. Goodman et al. 2017).

Immune checkpoints include a variety of molecules, like CTLA-4, PD-1, TIM-3, TIGIT, VISTA, and CD276, which are molecules found on many cells that regulate the immune system, and some of them maintain immune tolerance, suppress the T cells, and therefore prevent autoimmunity. CTLA-4, found on T cells, binds and inhibits or prevents the activation of B7 ligands, which are CD80/CD86 on antigen-presenting

cells, especially in lymphoid tissues. PD-1 within peripheral tissues and the tumor microenvironment on T cells binds to PD-L1, which can be found on tumor cells, and this axis leads to suppression of the T cells. Melanoma and other tumor cells frequently upregulate PD-L1 as an immune resistance mechanism, suppressing T-cell and evading immune surveillance. The primary immune checkpoints targeted in melanoma IT include CTLA-4 and PD-1 along with its PD-L1, while recently, anti-LAG3 has been approved (Seidel, Otsuka, and Kabashima 2018; A.C. Huang and Zappasodi 2022; Mandalà, Merelli, and Massi 2016; Ellebaek et al. 2024). The use of PDL1 expression on tumor cells has been used in various tumors as a biomarker to predict response towards anti-PD-1-based IT, although it is not included in the AJCC classification of melanoma (Alves Costa Silva et al. 2020; L. Cai et al. 2023; G. Chen et al. 2024; Ellebaek et al. 2024; Khunger et al. 2017).

The first type of immune inhibitor approved for melanoma was ipilimumab, an anti-CTLA-4 antibody, in 2011. Subsequent studies, however, showed the survival benefit and lower toxicities with the use of anti-PD1 antibodies. Therefore, clinical practice has changed toward the preferential use of PD-1 inhibitors, mainly nivolumab and pembrolizumab, or anti-PD-1-based combination IT with ipilimumab and nivolumab. Combination ICI has better ORR and survival outcomes; however, it also demonstrates more irAEs, necessitating careful patient selection and optimal IT duration, especially for those who will receive monotherapy and those who will receive combination IT (James Larkin et al. 2019; Postow et al. 2015; Wolchok et al. 2017; Robert et al. 2019; Robert et al. 2015). In daily clinical practice, stage IV patients, especially with unfavorable melanoma subtypes, start with combination ICI.

Further progress in melanoma IT has been made by the approval of relatlimab, an inhibitor targeting LAG-3. Relatlimab, administered together with nivolumab, has demonstrated better survival outcomes than nivolumab alone in advanced stage III and IV melanoma patients. The advantage of this combination IT is that it has been observed in clinical practice to have better tolerability and fewer immune adverse events than the ipilimumab and nivolumab regimen (Tawbi et al. 2022; Tawbi et al. 2025; J. Su et al. 2023; Lipson et al. 2025; Rutkowski and Jagodzińska-Mucha 2023).

As far as adjuvant therapy is concerned, patients with R0-resected stage III disease benefit significantly from ICI or, in *BRAF*-mutant cases, from combined BRAF/MEK inhibition that targets the activated MAPK pathway (dabrafenib plus trametinib, vemurafenib plus cobimetinib). Recent guidelines now extend the indication for adjuvant ICI to patients with SLNB-negative resected stage IIB and IIC melanomas, reflecting the substantial risk of recurrence in these stages that is similar to some substages III. Stage IIB and IIC have worse survival outcomes than stage IIIA (James Larkin et al. 2019; Jason J. Luke et al. 2024; Kirkwood et al. 2023; Schadendorf et al. 2024; Long et al. 2024; Weber et al. 2023). In stage III, a new study showed that front-line ICI is better than BRAF/MEK inhibition for the adjuvant setting, as front-line MAPK inhibition could lead to genomic and phenotypic alterations within the tumor and tumor environment, rendering it resistant to subsequent ICI. In *BRAF*-mutant melanoma, starting with ICI and then switching to MAPK inhibition gives the possibility to exploit that dependence of melanoma growth on melanoma after potential ICI resistance (Schumann et al. 2025).

For unresectable metastatic stage III and IV disease, systemic treatment aims to achieve durable DC. ICI, as monotherapy or in combination (nivolumab plus ipilimumab or relatlimab), is the mainstay of therapy. In *BRAF* V600 mutant tumors, TT with BRAF/MEK inhibition remains an important option, producing high response rates despite eventual resistance in many cases, which, as discussed above, can also cause cross-resistance to ICI (S. Liu et al. 2023). Recent evidence suggests that optimal first-line is ICI over TT also in *BRAF*-mutated non-adjuvant unresectable patients. Even though promising data were seen for triple combination with ICI and TT on mice, in clinical trials, this endpoint was not met (James Larkin et al. 2019; Long et al. 2017; Switzer et al. 2022; van Breeschoten et al. 2021; C. Sun et al. 2024; Biermann et al. 2022; Atkins et al. 2019; Trojaniello et al. 2023).

The neoadjuvant and perioperative use of ICI represents an emerging therapeutic option for managing resectable macroscopic stage III melanoma. ICI is delivered before surgery rather than after, allowing the immune system to produce an antitumor response targeted specifically against the tumor antigens and neoantigens while the tumor remains present, making it a more personalized strategy. This approach can increase systemic immunity, target microscopic disease early, and enable assessment

of treatment effectiveness through pathological analysis of the surgical specimen. Achieving a pathological CR has been linked to better long-term outcomes while making the surgery redundant (Blank et al. 2024; Sussman and Ott 2024; Versluis et al. 2023; Bushara et al. 2023; Hieken et al. 2023; Song et al. 2020).

Current research is focused on addressing primary and acquired secondary resistance to ICI, which is a challenge affecting a substantial proportion of patients. Research is investigating targeting alternative checkpoint molecules such as TIGIT, TIM-3, and CD276 to further potentiate antitumor immune response. Clinical trials are evaluating combination strategies that combine ICIs with TT, cancer vaccines, oncolytic viruses like talimogene laherparepvec, and radiation therapy, with the aim to increase therapeutic efficacy through multimodal immune activation (L. Cai et al. 2023; Nandini, Catherine, and Ana Carrizosa 2020; Khan, Arooj, and Wang 2020; Tang et al. 2023; Kreidieh and Tawbi 2023; Ledderose, Ledderose, and Ledderose 2024; Sachin et al. 2023).

The duration of ICI therapy in tumors and especially in melanoma is determined by factors including disease stage, therapeutic response, and clinical evidence. In the adjuvant setting for high-risk stage II (IIB and IIC) or III R0-resected melanomas, treatment is commonly administered for up to one year, then followed up and monitored to document a potential relapse. For patients with advanced or metastatic unresectable disease, therapy typically continues until disease progression, high-grade toxicity, or individual decision to discontinue. A subset of patients demonstrates durable clinical remission, allowing cessation of treatment after approximately two years, particularly those achieving complete remission, who can discontinue treatment after 2 years, with real-world data showing sustained remissions. This length of ICI duration was due to the design of clinical trials; however, it has not been investigated. Current research aims to define optimal treatment duration that maximizes efficacy while minimizing toxicity, including investigations into biomarker-guided strategies to personalize therapy duration (J. Larkin et al. 2023; Alexander M.M. Eggermont et al. 2018; A. M. M. Eggermont et al. 2020; Czarnecka et al. 2025; L. Warburton et al. 2020).

From a financial perspective, the cost associated with ICI therapy represents a significant burden on healthcare systems globally, which is associated with the cost of the therapy as well as the cost of management of toxicities. Individuals with prolonged therapy duration exhibit psychological distress and lose many working hours. This burden underscores the necessity for precision medicine approaches that incorporate predictive biomarkers and treatment algorithms to optimize patient selection and therapy duration, ultimately improving the cost-effectiveness of ICI but in parallel retaining clinical benefit (Verma et al. 2018; Bregman et al. 2020; Oh et al. 2017; Quon et al. 2019).

For selected patients, especially those with injectable cutaneous or nodal metastases, intralesional therapies such as oncolytic viral therapy, including T-VEC and other HSV1-related vaccines (RP1) and IL2 injections, can induce local and systemic immune responses that can complement systemic IT. These demonstrate local responses and local control of the cutaneous metastases, while also promoting systemic immune effects and regression of other untreated metastases, a phenomenon known as the abscopal effect. Therefore, these can be used to complement systemic IT and improve outcomes (Danielli et al. 2015; Ray et al. 2016; Radny et al. 2003; Khoury et al. 2021; Agarwala 2015; D.Y. Wang and Johnson 2016).

Many different vaccines, including dendritic, protein, and RNA-based, are also under investigation (Bhatia, Tykodi, and Thompson 2009; Forchhammer et al. 2024).

TIL therapy, is a personalized type of IT using adoptive cell transfer of a patient's tumor-reactive T cells obtained from the tumor (and therefore infiltrate the tumor) to induce tumor arrest. This approach involves the acquisition and isolation of T cells that infiltrate the resected melanoma tissue, their ex vivo expansion, and reinfusion to the patient following a lymphodepletion (Rohaan et al. 2022; Besser et al. 2013). As it is based on the use of the TILs of the patients, these are directed against the tumor antigens that the patient has, making them more effective.

Melanoma is among the first solid cancers to demonstrate the efficacy of TIL therapy, with early-phase clinical trials reporting ORRs of 35-50% in advanced, refractory melanoma patients that exhibited progression following ICI or TT. Responses can be durable, as in other types of IT, where an immune equilibrium can be achieved,

including complete remission in a subset of patients that relapsed and thereby are resistant to previous immune checkpoint and MAPK inhibitors, a phenomenon that can be due to the targeting of cancer antigen, rendering it a personalized therapy (Besser et al. 2013; Rohaan et al. 2022; Chesney et al. 2022; Allison Betof Warner, Corrie, and Hamid 2023; Pillai et al. 2022).

Tebentafusp is a first-in-class immunotherapeutic agent specifically approved for HLA-A02:01-positive metastatic UM. It has been recently approved in UM as first-line therapy, as in this type of melanoma, ICI only showed minimal benefit, and due to its distinct genomic background, it is intrinsically resistant to anti-PD-1 IT. Tebentafusp is a bispecific fusion protein of a soluble T-cell receptor that has one arm targeting the gp100 peptide presented by HLA-A02:01 on the cell membrane and the second arm being an anti-CD3 fragment, which binds CD3 on T cells. GP100 is a melanocyte-specific protein involved in melanin synthesis; therefore, it is found on melanocytes. This redirects and activates patient T cells to selectively target and eliminate melanoma cells expressing gp100 and presented on HLA-A02:01. The disadvantage of this type of therapy is that the activation of T cells can cause cytokine storm. Clinical trials and other cohort studies have demonstrated that tebentafusp significantly improves survival outcomes when compared to ICI in HLA-A02:01-positive metastatic UM. Clinical trials also exist for HLA-A02:01-positive CM patients who have progressed on ICI and TT (Hassel et al. 2023; Sacco et al. 2024; Piulats et al. 2024; Dian et al. 2024).

1.4.1. Response RECIST criteria

In clinical research and practice, response to ICI in melanoma is most commonly evaluated using the RECIST, version 1.1.

According to RECIST 1.1, measurable target lesions are selected and their longest diameters recorded at baseline with imaging techniques including computed tomography, magnetic resonance, or PET. Follow-up imaging is performed to compare tumor measurements with baseline dimensions, allowing responses to be classified into four categories (Kataoka and Hirano 2018), CR, PR, PD, and SD. The ORR is calculated as the ratio of those patients who have either a CR or PR by RECIST 1.1, while DC is defined as those who achieve CR, PR, or SD. BOR is the best response documented during follow-up imaging. In advanced melanoma, treatment with ICIs, particularly anti-PD-1 monotherapy or combination CTLA-4 and PD-1 inhibition, has

demonstrated ORR ranging from approximately 30% to over 50% (Eisenhauer et al. 2009).

IT can induce atypical response patterns that were uncommon in chemotherapy and TT. Specifically, pseudoprogression is a phenomenon where initial increases in lesion size are documented as progression, although later response is seen. This is because IT, by activating the immune system, increases the T cell infiltration in the metastases rather than true tumor growth. To address this, immune-adapted criteria such as iRECIST have been proposed to refine response assessment for IT trials, as well as imaging techniques to quantify the T cells. Despite this, RECIST 1.1 remains the main criteria used in studies evaluating ICIs in melanoma (Khoja et al. 2016; Amrane et al. 2019; Park et al. 2021; Yirgin et al. 2021).

1.4.2. Adverse effects

The use of ICI has significantly prolonged survival outcomes for melanoma patients; however, this therapeutic strategy is inherently associated with the emergence of irAEs as it inhibits the immune breaks that are responsible for tolerance and autoimmunity control. The toxicity profile of ICIs reflects an activation of immune responses against healthy host tissues (Fahey, Gracie, and Johnson 2023; C. Xu et al. 2018; Puzanov et al. 2017).

IrAEs result from the loss of peripheral tolerance due to blockade of inhibitory pathways such as CTLA-4 and PD-1, which normally restrain and suppress autoreactive T cells. The activation of the immune system can provoke a range of inflammatory processes across multiple organ systems, like the skin, lungs, colon, and hypophysis. Proposed mechanisms include the expansion of self-reactive T-cell clones, cross-reactivity with shared antigens between healthy tissue and cancer tissue, increased cytokine production, B cell activation, and the induction of autoantibodies (S.J. Wang, Dougan, and Dougan 2023; Casagrande et al. 2024; Mor and Strazza 2022; Buchbinder and Desai 2016; Taylor et al. 2023).

The clinical spectrum of irAEs is broad, with dermatologic manifestations, including rash, pruritus, and vitiligo, being the most frequent and typically mild. Endocrinopathies, including thyroid dysfunction, hypophysitis, and adrenal

insufficiency, are also common and may be irreversible, requiring lifelong hormone replacement. Hypophysitis can also be fatal if not diagnosed early. Gastrointestinal toxicity, particularly colitis and hepatitis, and less frequent but serious toxicities such as pneumonitis, myocarditis, or neurologic syndromes, can be life-threatening if not recognized and managed early. The incidence and severity of irAEs vary between agents, CTLA-4 inhibitors exhibit a higher frequency of severe irAEs compared to PD-1 inhibitors, while combination regimens further elevate this risk profile (Martins et al. 2019; Shivaji et al. 2019; Ball et al. 2019; Johnson et al. 2016; Bastacky et al. 2021).

IrAEs are a well-recognized effect of ICI and often arise when the immune system mistakenly targets healthy tissues that share antigens with the tumor. In melanoma, skin-related toxicities such as rash, itching, vitiligo, and bullous pemphigoid or other immune blistering diseases are among the most frequent irAEs. This is believed to result from immune responses against melanocyte antigens, like gp100, MART-1, and tyrosinase, which are found in both melanoma cells and normal pigment-producing skin cells. A similar pattern is seen in other cancers, for example, lung cancer patients treated with ICIs more commonly experience pneumonitis, possibly due to immune targeting of lung-specific antigens or underlying inflammation in damaged lung tissue. These organ-specific toxicities suggest that irAEs may be influenced by the tumor's tissue of origin and the presence of shared antigens between cancer cells and normal tissues, emphasizing the importance of tailoring irAE monitoring to each cancer type (Teng and Yu 2023; Hassel 2022; F. Berner and Flatz 2023).

An interesting aspect of irAEs is their correlation with therapeutic efficacy. Multiple retrospective studies have reported that patients developing irAEs may achieve higher ORRs and prolonged survival, suggesting that increased systemic immune activation parallels a more effective antitumor response, especially when toxicity is developed against tissue with more shared antigens, like skin and melanoma. Retrospective studies show that patients who develop irAEs, particularly mild-to-moderate dermatologic or endocrine toxicities, often have better outcomes. This association is more complex and likely depends on the nature and severity of the irAE. Severe toxicities often necessitate immunosuppressive interventions, including systemic corticosteroids and, in steroid-refractory cases, other options such as TNF inhibitors, methotrexate, mycophenolate mofetil, some of which can be linked to poorer survival

(Wu et al. 2020; Das and Johnson 2019; Fujisawa et al. 2018; Kuzmanovszki et al. 2022; Jurlander et al. 2024; Fukushima, Kobayashi, and Ueno 2024; Zhu et al. 2020; Möhn et al. 2019; Barroso-Sousa et al. 2018).

The relationship between irAEs and the duration of ICI is an area of active investigation. Durable remissions have been observed in some patients even after early discontinuation due to severe toxicity, indicating that sustained benefit may be possible once an adequate antitumor immune response has been triggered (Swami et al. 2019; Mayer et al. 2025; Perez, Samlowski, and Lopez-Flores 2022; Warner et al. 2020; Lydia Warburton et al. 2023; Asher et al. 2021). Some irAEs can be developed after prolonged ICI or even after discontinuation, although the majority appear early in therapy.

1.4.3. Biomarkers

Biomarker identification for ICI in melanoma, which is the current first-line therapy for the cutaneous subtypes, encompasses a broad array of molecular, circulating, and clinical parameters that collectively inform prognosis, predict therapeutic response, and guide personalized treatment strategies. These biomarkers are under investigation, although not routinely used in clinical practice, with the exception of LDH, which has been incorporated in the AJCC classification.

Among molecular biomarkers, TMB and neoantigen load have emerged as critical determinants of immunogenicity; higher TMB correlates with increased neoantigen presentation, enhancing tumor recognition by cytotoxic T cells and improving responsiveness to ICIs. Higher numbers of presented neoantigens are associated with better outcomes (Andrews et al. 2024; A.M. Goodman et al. 2017).

PD-L1 expression, which is the immune checkpoint, on tumor cells and infiltrating immune cells, assessed by immunohistochemistry, is used as a predictive marker, although its heterogeneous expression and variable predictive value limit its utility, and it is not used in routine practice for melanoma from all centers (Ellebaek et al. 2024).

Specific gene expression profiles associated with an inflamed tumor microenvironment, such as upregulation of interferon-γ signaling pathways, are associated with favorable responses (M. Lee, Samstein, et al. 2020; Andrews et al. 2024; Morrison et al. 2018; Khunger et al. 2017; Teixidó et al. 2015; Espinosa et al.

2017). IFN-γ is a cytokine from immune cells that has diverse antitumor effects. By activating the JAK/STAT signalling pathway, it promotes tumor cell cycle arrest, senescence, increased antigen presentation, and apoptosis (H. Braumüller et al. 2013a; Brenner et al. 2020).

Alterations in genes involved in antigen presentation, including β2-microglobulin, *TAP1/2,* or in pathways regulating immune evasion, like *JAK1/2, STAT1/2, IFNGR1,* leading to interferon resistance, which are involved in the IFN-γ signalling or cell cycle and apoptosis, have been implicated in primary or acquired resistance to ICI. Molecular profiling may thus identify tumors with immune escape mechanisms (Sade-Feldman et al. 2017; Shin et al. 2017; Zaretsky et al. 2016; Kalbasi et al. 2020). Primary resistance refers to the fact that tumors are inherently resistant to ICI and no response can be achieved, while acquired resistance is an adaptive process where genomic and phenotypic switch leads to resistance following previous therapy with ICI or TT (S. Liu et al. 2023).

Serum biomarkers, including LDH and S100B protein, remain important prognostic tools. Elevated LDH reflects high tumor burden and aggressive disease, correlating with poorer ICI outcomes, while higher S100B levels are associated with unfavorable outcomes. LDH is incorporated for stage IV classification in AJCC edition 8. Systemic inflammatory markers such as increased NLR and CRP have been linked to poorer survival outcomes, suggesting that these can indicate a baseline immunosuppressive environment that may diminish therapeutic efficacy (Knispel et al. 2021; Soumoy et al. 2024; Janka et al. 2021; Jialin Su et al. 2025; Y. Guo, Xiang, et al. 2022; Y. Xu et al. 2023).

Clinical parameters, including ECOG and presence of brain metastases, contribute to patient stratification and prognosis. Patients with comorbidities have a worse prognosis.

IrAEs occurring during IT often indicate robust systemic immune activation and have been correlated with improved tumor control and survival; however, their management necessitates careful immunosuppression to balance efficacy and toxicity (van Not et

al. 2022; Placke et al. 2025; Sperduto, Salama, and Anders 2023; Pedersen et al. 2025; Fager et al. 2025; Watson et al. 2022). Skin toxicities have been associated with better responses.

CtDNA offers a minimally invasive biomarker for dynamic and longitudinal monitoring, meaning follow-up of tumor burden and early response assessment. As a predictive biomarker, decreasing ctDNA levels following ICI initiation predict favorable clinical outcomes, whereas persistent or rising levels may show therapeutic resistance or progression (L. Liu et al. 2024; Burkey et al. 2025; Schroeder et al. 2024; Costantini et al. 2025).

1.5. Aims and objectives

This thesis aims to contribute to the personalization of ICI in unresectable stage III/IV melanoma by addressing two key aspects: the clinical outcomes (survival and response) and management of those patients achieving a CR following an anti-PD-1-based IT, and the development of a baseline model to predict PFS prior to treatment initiation. This is important for the duration of ICI, closer FUP and monitoring, as well as switching to other therapies like MAKP inhibition or prioritization of other regimens, combinatorial strategies, and enrollment in clinical trials. By retrospective analysis, the work investigates the characteristics associated with CR, the long-term outcomes after therapy discontinuation, and the optimal duration of treatment in this subgroup or whether there is an association with the length of therapy. It also explores the role of longitudinal monitoring of blood and therefore easily accessible biomarkers such as LDH, S100, and NLR in monitoring disease status and relapse by comparing them at baseline and at the time of best overall response. We aim to examine the incidence and clinical relevance of irAEs, particularly regarding the durability of response and late toxicity. Another objective of our work was to develop a predictive nomogram, using commonly available baseline clinical and laboratory parameters, to estimate individual PFS probabilities. This tool will enable patient stratification into distinct risk groups, which will help provide more informed decisions regarding therapy, follow-up schedules, and early referral to clinical trials. Finally, the ultimate goal is to improve outcomes for patients while reducing overtreatment and minimizing toxicity.

2. Results and discussion

2.1. Publication 1: Features and long-term outcomes of stage IV melanoma patients achieving complete response under anti-PD-1-based immunotherapy

Authors: Chatziioannou Eftychia, Leiter Ulrike, Thomas Ioannis, Keim Ulrike, Seeber Olivia, Meiwes Andreas, Boessenecker Isabell, Gonzalez Stephanie Sanchez, Torres Francisco Merraz and Niessner, Heike, Sinnberg Tobias, Forschner Andrea, Flatz Lukas, Amaral Teresa

Year: 2023

Journal: American Journal of Clinical Dermatology

Volume: 24

Issue: 3

Pages: 453-467

ISSN: 1175-0561

DOI:10.1007/s40257-023-00775-7

American Journal of Clinical Dermatology (2023) 24:453–467
https://doi.org/10.1007/s40257-023-00775-7

ORIGINAL RESEARCH ARTICLE

Features and Long-Term Outcomes of Stage IV Melanoma Patients Achieving Complete Response Under Anti-PD-1-Based Immunotherapy

Eftychia Chatziioannou[1] · Ulrike Leiter[1] · Ioannis Thomas[1] · Ulrike Keim[1] · Olivia Seeber[1] · Andreas Meiwes[1] · Isabell Boessenecker[1] · Stephanie Sanchez Gonzalez[1] · Francisco Merraz Torres[1] · Heike Niessner[1,2] · Tobias Sinnberg[1,2,4] · Andrea Forschner[1] · Lukas Flatz[1,3] · Teresa Amaral[1,2]

Accepted: 25 March 2023 / Published online: 4 May 2023

Abstract

Background Immune checkpoint inhibition (ICI) has changed the melanoma treatment spectrum. Few studies have examined the characteristics and long-term outcomes of patients achieving complete response (CR) under ICI.

Materials and methods We evaluated patients with unresectable stage IV melanoma treated with first-line ICI. The characteristics of those achieving CR were compared with those not achieving CR. Progression-free survival (PFS) and overall survival (OS) were assessed. Late-onset toxicities, response to second-line treatment, the prognostic value of clinicopathologic features, and blood markers were examined.

Results A total of 265 patients were included; 41 (15.5%) achieved CR, while 224 (84.5%) had progressive disease, stable disease, or partial response. At the therapy start, those who had CR were more likely to be older than 65 years of age ($p = 0.013$), have a platelet-to-lymphocyte ratio below 213 ($p = 0.036$), and have lower lactate dehydrogenase levels ($p = 0.008$) than those not achieving a CR. For those who discontinued therapy after CR, the median follow-up time after CR was 56 months (interquartile range [IQR] 52–58) and the median time from CR to therapy end was 10 months (IQR 1–17). Five-year PFS after CR was 79% and 5-year OS was 83%. Most complete responders had a normalization of S100 at the time of CR ($p < 0.001$). In simple Cox regression analysis, age below 77 years at CR ($p = 0.04$) was associated with better prognosis after CR. Eight patients received second-line ICI; disease control was seen in 63%. Late immune-related toxicities occurred in 25% of patients, most being cutaneous immune-related toxicities.

Conclusions Response, according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria, is, until now, the most important prognostic factor, and CR is a valid surrogate marker for long-term survival in patients treated with ICI. Our results highlight the importance of investigating the optimal therapy duration in complete responders.

✉ Teresa Amaral
teresa.amaral@med.uni-tuebingen.de

1 Center for Dermato-Oncology, Department of Dermatology, Eberhard Karls University of Tuebingen, Liebermeisterstrasse 25, 72076 Tuebingen, Germany

2 Cluster of Excellence iFIT (EXC 2180), Tuebingen, Germany

3 Institute for Immunobiology, Kantonsspital St. Gallen, St. Gallen, Switzerland

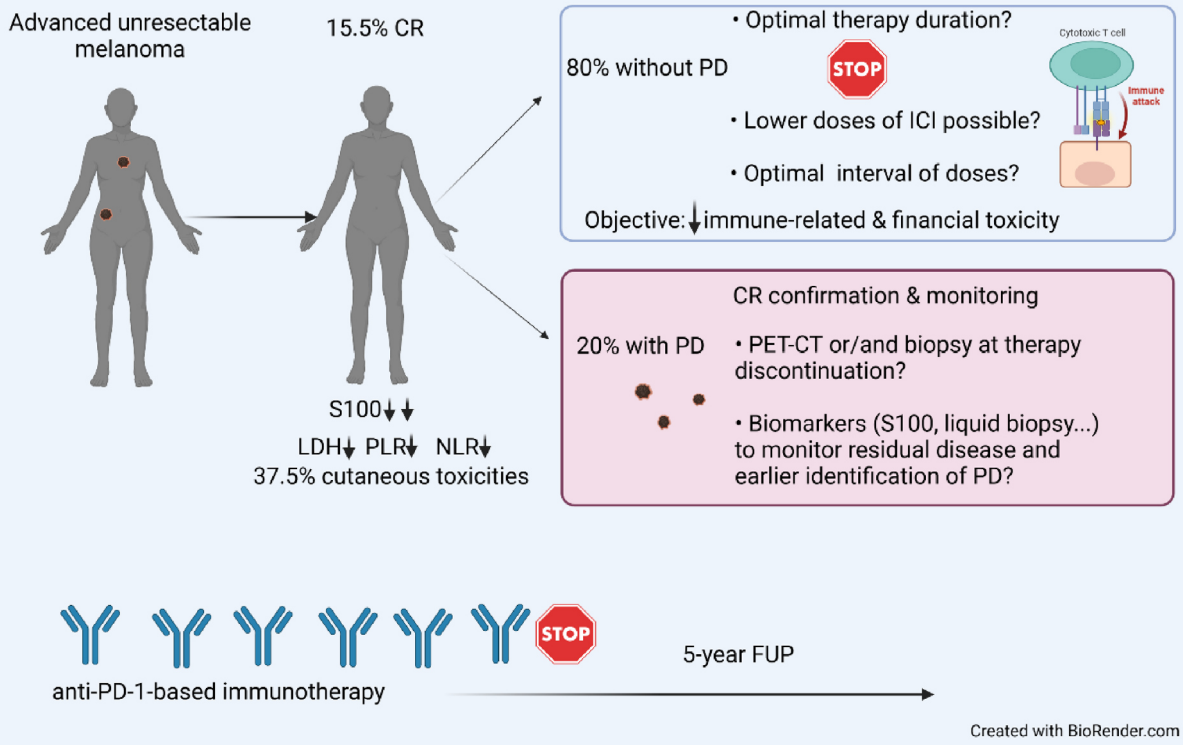
4 Department of Dermatology, Venereology and Allergology, Charité-Universitätsmedizin Berlin, Berlin, Germany

Graphical Abstract

FEATURES AND LONG-TERM OUTCOMES OF STAGE IV MELANOMA PATIENTS ACHIEVING COMPLETE RESPONSE UNDER ANTI-PD-1-BASED IMMUNOTHERAPY

Eftychia Chatziioannou · Ulrike Leiter · Ioannis Thomas · Ulrike Keim · Olivia Seeber · Andreas Meiwes · Isabell Boessenecker · Stephanie Sanchez Gonzalez · Francisco Merraz Torres · Heike Niessner · Tobias Sinnberg · Andrea Forschner · Lukas Flatz · Teresa Amaral

American Journal of Clinical Dermatology



PEER-REVIEWED FEATURE

Adis

CR; complete response, FUP; follow-up, PD; progressive disease, ICI; immune checkpoint inhibition, LDH; lactate dehydrogenase, PLR; platelet-to-lymphocyte ratio, NLR; neutrophil-to-lymphocyte-ratio

This graphical abstract represents the opinion of the authors. For a full list of declarations, including funding and author disclosure statements, and copyright information, please see the full text online.

Key Points

Response, according to the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, is prognostic for long-term survival, and complete response (CR) is a valid surrogate marker in patients treated with immune checkpoint inhibition.

Higher CR rates were seen in patients older than 65 years and in those with lower baseline LDH and platelet/lymphocyte ratio.

Among complete responders, 5-year progression-free survival and overall survival after CR were 79% and 83%, respectively.

Currently, the optimal duration of anti-PD-1-based therapy in patients achieving complete response is unknown.

Better tools are needed to identify patients at higher risk of progression, despite achieving a complete response

1 Introduction

Anti-PD-1-based immunotherapy has improved survival in advanced melanoma patients. Therapeutic options in this setting include immune checkpoint inhibitors (ICI) and targeted therapy (TT) with BRAF and MEK inhibitors. One advantage of immunotherapy compared with TT is the sustained response off-treatment [1–4].

Prolonged treatment for up to 24 months affects the quality of life due to potential immune-related adverse events (irAEs) and psychological burden [5]. Longer therapy duration directly impacts the healthcare system through the direct therapy costs, the costs associated with managing irAEs, and the loss of working hours from the patients' side. Therefore, there is a great need to determine the optimal duration of anti-PD-1-based therapy; however, there is currently no consensus on this matter.

Outside clinical trials, the standard total duration of ICI in advanced unresectable melanoma is 2 years if no progression or severe toxicities are documented. The standard therapy duration is based on the previous clinical trials for melanoma, where immunotherapy was given continuously for up to 2 years. Shorter therapy courses have been associated with less response durability [6]. Other studies showed that the type of response is the most important prognostic factor for survival and that patients without CR should be treated longer [7–10]. This implies that the ideal therapy duration should depend on the type of response and that patients with complete response (CR), partial response (PR), or stable disease (SD) might need different therapy durations. Moreover, this raises the question of whether additional markers, besides the type of response, could more accurately predict progression.

Around 15–25% of patients receiving ICI achieve a CR. A pooled analysis of data from stage III and IV melanoma patients treated with first-line nivolumab or nivolumab plus ipilimumab in the CheckMate 066, CheckMate 067, and CheckMate 069 studies showed that 19% of patients achieved CR under nivolumab and 23% under combined immunotherapy [11]. Another publication showed that 16% of patients with advanced melanoma treated with pembrolizumab achieved a CR and 87.6% exhibited ongoing responses 30 months after achieving CR [12].

The long-term outcomes of patients with CR treated outside clinical trials have not been systematically evaluated, although some publications have addressed this topic [8, 13–15]. Predictors of progression in complete responders have not been broadly examined. Likewise, biomarkers to identify drug-related toxicities associated with prolonged treatment in these patients are missing. Indeed, shorter therapy duration that induces the same type of response could potentially minimize these toxicities [16].

In this study, we assess the characteristics and long-term survival outcomes of a real-world cohort of patients who achieved CR under ICI. We also analyzed potential prognostic biomarkers and immune-related toxicities.

2 Methods

2.1 Study Design and Population

This retrospective, monocentric study includes unresectable stage IV melanoma patients who received ICI (anti-PD1 monotherapy or combination therapy with anti-PD-1 and anti-CTLA-4) as first-line systemic therapy. Patients were treated at the Dermato-Oncology Center of the University of Tuebingen between 2014 and 2018. Additional eligibility criteria were (1) patients who provided informed consent to be enrolled in the Central Malignant Melanoma Registry (CMMR); (2) age above 18 years; (3) patients achieving CR, PR, SD, or progressive disease (PD) under ICI as the best overall response (BOR); and (4) minimum follow-up (FUP) of 2 months after BOR. The exclusion criteria were (1) patients treated in an adjuvant setting; (2) patients treated with anti-CTLA-4 monotherapy as first-line therapy; (3) participation in a clinical trial; (4) patients receiving fewer than two cycles of therapy and not achieving CR or PR; and (5) diagnosis of uveal melanoma (electronic supplementary material [ESM] Fig. 1). Staging was based on the 8th edition of the American Joint Committee on Cancer (AJCC) staging

system [17]. The response was assessed using the Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1 [18] approximately 3 months after starting therapy and every 3 months thereafter. The severity of irAEs was documented using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

2.2 Statistical Analysis

Descriptive statistics were reported with median and interquartile range (IQR) for continuous variables, and counts (*n*) and percentages (%) for categorical variables. Patient characteristics of those who achieved CR were documented at the time of treatment start and compared with those who had PD, SD, or PR. The difference in CR rates between the groups was evaluated using the Chi-square test. The Mann–Whitney *U* test was used to compare differences in the continuous variables between the two groups.

Age, sex, body mass index (BMI), *BRAF* variants, histologic subtype, presence of brain or liver metastases, type of ICI received, reason for treatment discontinuation, diagnosis of irAEs, and blood markers were documented and evaluated for their prognostic value. Blood markers included lactate dehydrogenase (LDH), serum protein S100, serum neutrophil-to-lymphocyte ratio (NLR), and serum platelet-to-lymphocyte ratio (PLR). Blood biomarkers were documented at therapy start (T0), at CR (T1), and at the time of progress when applicable (T2). Their relative change was calculated to assess their prognostic significance and investigate their variation over time. Using the median, upper, and lower quartiles or the reference value of our center, we evaluated continuous variables as continuous and categorical. For PLR, the optimal cut-off was determined using the Youden index.

Late-onset irAEs were defined as irAEs occurring 6 months after treatment started. Response to second-line treatment was also documented.

Survival analysis was performed using endpoints of PD for PFS and death for OS, and patients were censored at the last FUP date. The median survival and 5-year survival rates were calculated using the Kaplan–Meier estimator, and hazard ratios (HRs) were estimated using the simple Cox proportional hazards model.

All statistical analyses and data visualization were performed using R (survival, survminer, and swimplot) version 4.1. Reported *p* values were two-sided and a p value < 0.05 was considered statistically significant.

3 Results

3.1 Patient and Disease Characteristics

Our study included 265 patients; 41 (15.5%) achieved a CR, while 224 (84.5%) had PD, SD or PR. The entire cohort had a median age of 67 years (IQR 56–77) at the therapy start, and 62% were male; 60% received anti-PD-1 monotherapy, while 40% received combination immunotherapy as first-line systemic therapy. At the therapy start, the median LDH was 217 U/L (IQR 185–275) and the median S100 was 0.1 µg/L (IQR 0.06–0.22) [ESM Table 1].

Among those achieving CR and discontinuing therapy thereafter ($n = 40$), most (70%) were men and the median age at CR was 74 (IQR 66–77). Of these patients, 31 (77.5%) had cutaneous melanoma, 8 (20%) had occult melanoma, and 1 (2.5%) had mucosal melanoma. Twenty-six (65%) patients received anti-PD-1 monotherapy (15% nivolumab, 50% pembrolizumab), while 14 (35%) patients received ipilimumab plus nivolumab.

The median treatment duration was 22 months (IQR 17–24), the median time from therapy start to CR was 6 months (95% confidence interval [CI] 4–8), and the median time from CR to therapy stop was 10 months (IQR 1–17). Five (12%) patients were treated for <6 months, 2 (5%) between 6 and 12 months, 5 (12%) between 12 and 18 months, 20 (50%) between 18 and 24 months, and 8 (20%) for more than 24 months. The reasons for therapy discontinuation were PD ($n = 1$, 2.5%), severe irAEs ($n = 8$, 20%), patient/physician decision ($n = 17$, 42.5%), and therapy discontinuation 2 years after therapy start without evidence of PD ($n = 14$, 35%). Of these 14 patients, 8 received therapy for more than 2 years and 6 received therapy for 2 years (ESM Table 2). In one patient, therapy is ongoing without evidence of PD.

After achieving CR, PD occurred in 8 (20%) patients within 5 years after CR. Only one patient had disease progression after CR while still receiving therapy, whereas the other seven had disease progression after therapy cessation. One more patient (patient 31) had PD 5 years after achieving CR. Three patients died during the FUP period after PD.

3.2 Survival Analysis of Complete Responders

In patients achieving CR and discontinuing therapy thereafter ($n = 40$), the median FUP (mFUP) time after CR was 56 months (95% CI 52–58), and 47 months (95% CI 38–51) after treatment discontinuation (Fig. 1a, b). Median progression-free survival (mPFS) and median overall survival (mOS) after CR were not reached (NR) [95% CI

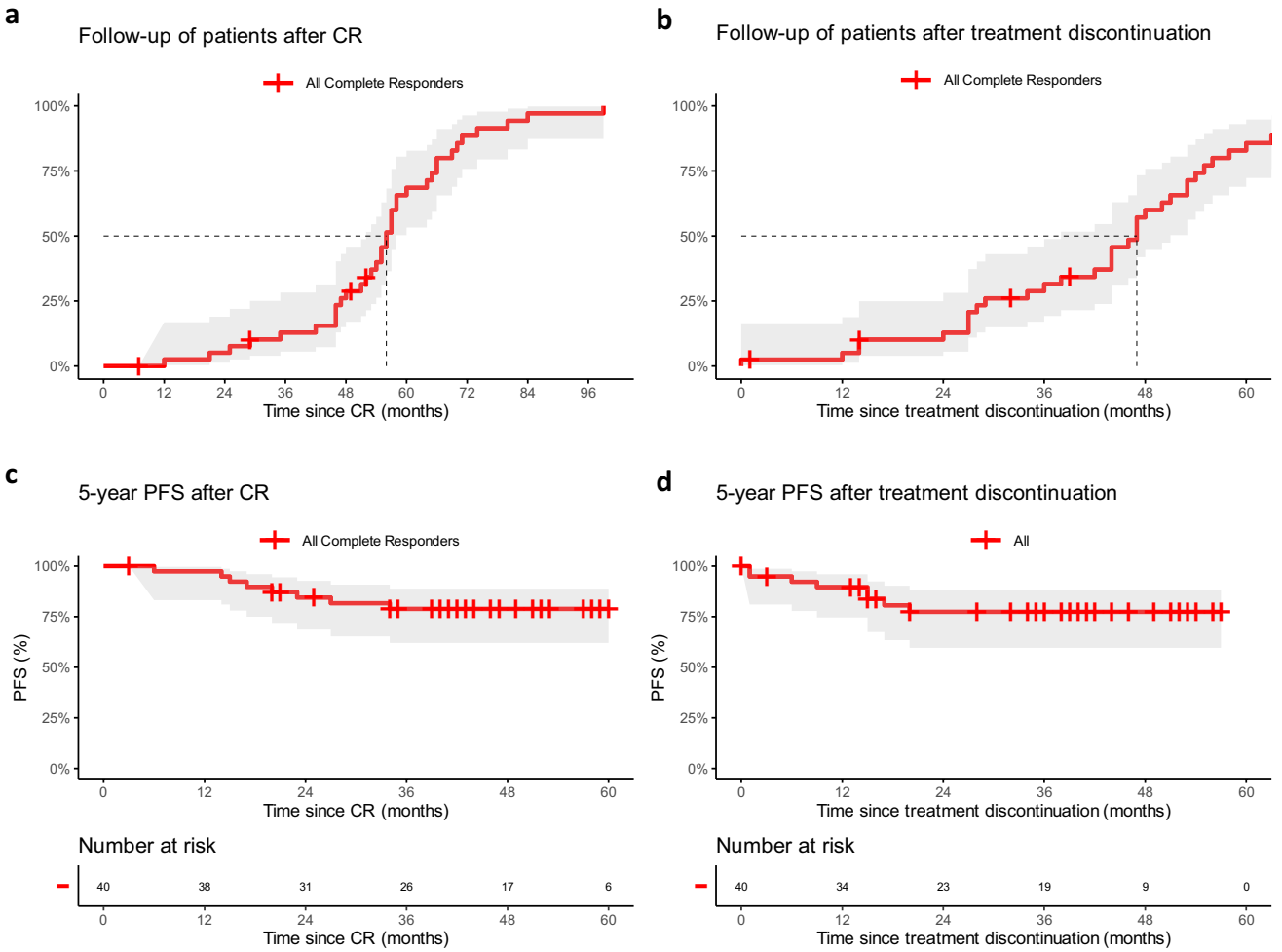


Fig. 1 **a** Patient FUP using the reverse Kaplan–Meier method of those who achieved a CR under ICI. Time is calculated since CR. The mFUP time after CR was 56 months (95% CI 52–58). **b** Patient FUP using the reverse Kaplan–Meier method after treatment discontinuation. mFUP time after treatment discontinuation was 47 months (95% CI 38–51). **c** Five-year PFS analysis of patients ($n = 40$) who achieved a CR under ICI and subsequently discontinued therapy. The Kaplan–Meier curve is displayed, and time is calculated since achieving CR. The 5-year PFS was 79% (95% CI 62–89%). **d** Five-year PFS analysis of patients ($n = 40$) who achieved a CR under ICI and subsequently discontinued therapy. The Kaplan–Meier curve is displayed, and time is calculated since treatment discontinuation. *ICI* immune checkpoint inhibition, *CR* complete response, *FUP* follow-up, *mFUP* median follow-up, *CI* confidence interval, *PFS* progression-free survival

NR–NR]. The median time to PD after CR was 17 months (95% CI 10–24). After CR, the 5-year PFS rate was 79% (95% CI 62–89%) and the 5-year OS rate was 83% (95% CI 60–93%) (Fig. 1c, d).

Among those achieving a CR, we saw that patients with CR within 3 months of therapy start did not develop PD, suggesting that a shorter time to CR since therapy start could potentially be associated with a more favorable prognosis (ESM Fig. 1a). However, these differences were not statistically significant, and the number of patients was small to draw any definitive conclusions.

No relation was found between total treatment duration ($p = 0.754$) or treatment duration after CR ($p = 0.398$) and survival. Furthermore, the type of immunotherapy, i.e. monotherapy versus combination therapy, was not significantly associated with response durability after CR ($p = 0.551$) and after therapy cessation ($p = 0.663$) [ESM Fig. 1b, c]. In the simple Cox regression analysis performed for the different clinicopathologic variables evaluated at the time of CR, age below 77 years (the upper quartile of the cohort) at CR was associated with a better PFS after CR (HR 0.24, 95% CI 0.06–0.95; $p = 0.043$)

Table 1 Patients and disease characteristics of patients with stage IV melanoma who achieved a complete response compared with those with progressive disease, stable disease or partial response

	Complete responders [$n = 41$][a]	Non-complete responders [$n = 224$][a]	CR rate (%)	p value[b]
Age, years				
Median (IQR)	73 (65–77)	66 (55–77)		0.13
Age at therapy start, years				
≤ 65	11 (27)	107 (48)	9.3	**0.013**
> 65	30 (73)	117 (52)	20.4	
Sex				
Female	12 (29)	88 (39)	12	0.224
Male	29 (71)	136 (61)	17.6	
First-line systemic therapy				
Anti-PD-1 monotherapy	27 (66)	132 (59)	17	0.405
Combination immunotherapy	14 (34)	92 (41)	13.2	
Brain metastases at therapy start				
Metastases	6 (15)	57 (25)	9.5	0.135
No metastases	35 (85)	167 (75)	17.3	
Liver metastases at therapy start				
Metastases	7 (17)	76 (34)	8.4	**0.032**
No metastases	34 (83)	148 (66)	18.7	
BRAF mutation				
BRAF mut	12 (31)	58 (30)	17.1	0.898
BRAF wt	27 (69)	137 (70)	16.4	
Missing (*n*)	2	29		
LDH at therapy start (U/L)				
Median (IQR)	197 (172–226)	221 (189–279)		**0.008**
Missing (*n*)	1	20		
LDH at therapy start (U/L)				
≤ 250	32 (80)	134 (66)	19.3	0.076
> 250	8 (20)	70 (34)	10.3	
Missing (*n*)	1	20		
S100 at therapy start (μg/L)				
Median (IQR)	0.1 (0.06–0.16)	0.1 (0.06–0.24)		0.6
Missing (*n*)	3	21		
S100 at therapy start (μg/L)				
≤ 0.1	18 (47)	102 (50)	15	0.745
> 0.1	20 (53)	101 (50)	16.5	
Missing (*n*)	3	21		
BMI, kg/m^2				
Median (IQR)	25.7 (22.6–28.5)	26.3 (23.5–29.7)		0.4
Missing (*n*)	2	47		
PLR at therapy start				
Median (IQR)	165 (132–211)	195 (145–251)		0.095
Missing (*n*)	9	53		
PLR at therapy start				
≤ 213	25 (78)	100 (58)	20	**0.036**
> 213	7 (22)	71 (42)	8.9	
Missing (*n*)	9	53		
NLR at therapy start				
Median (IQR)	3.2 (2.2–4.2)	3.6 (2.5–5.3)		0.2
Missing (*n*)	9	53		

BMI body mass index, *CR* complete response, *IQR* interquartile range, *LDH* lactate dehydrogenase, *mut* mutant, *NLR* neutrophil-to-lymphocyte ratio, *PD-1* programmed death-1, *PLR* platelet-to-lymphocyte ratio, *wt* wild-type

[a]Values are reported as counts (*n*) and percentages (%) for discrete values, and as median and IQR for continuous values. For comparisons between categorical variables, Pearson's Chi-square test was used, and for comparisons between continuous and categorical values, the Mann–Whitney *U* test was used

[b]The Chi-square test was used and tested only for patients with information about this certain characteristic; the percentages do not include the patients for whom information was not available. Significant values are reported in bold

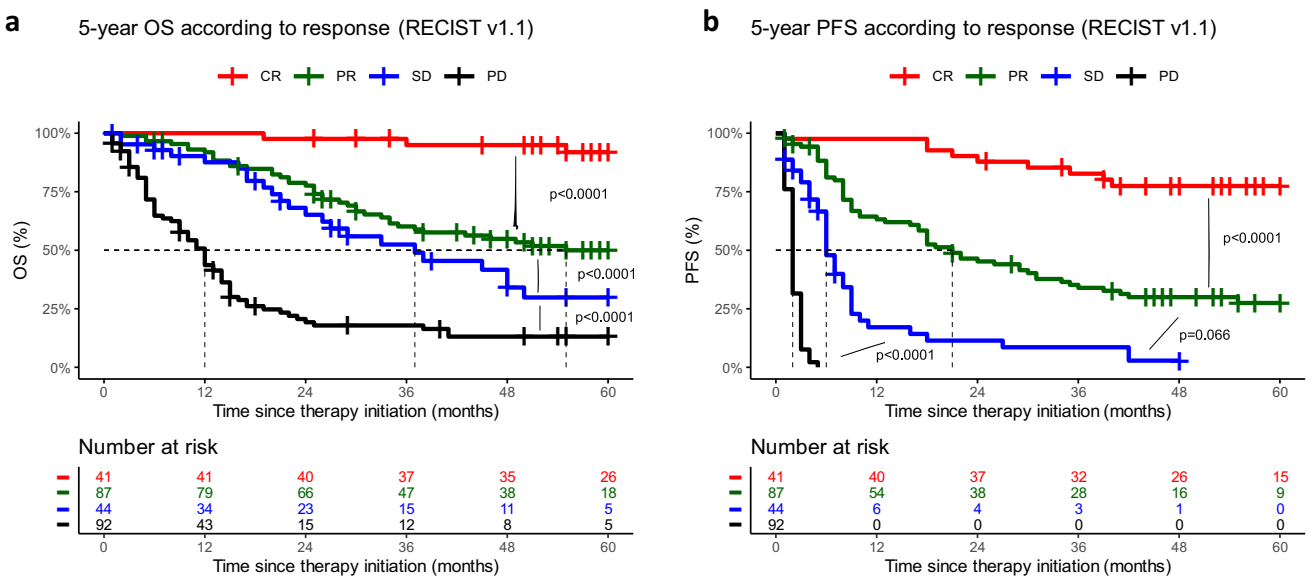


Fig. 2 **a** The 5-year OS analysis of patients according to the response. The Kaplan–Meier curve is displayed, and time is calculated since therapy initiation. **b** The 5-year PFS analysis of patients according to the response. The Kaplan–Meier curve is displayed, and time is calculated since treatment initiation. *OS* overall survival, *PFS* progression-free survival, *RECIST* Response Evaluation Criteria in Solid Tumors, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

and after treatment discontinuation (HR 0.17, 95% CI 0.04–0.73; $p = 0.022$).

Of the 40 patients who discontinued therapy after CR, 11 had a fluorodeoxyglucose-positron emission tomography/computed tomography (FDG-PET/CT) scan to validate CR, among whom 2 (18%) had PD during FUP.

3.3 Comparison between Complete Responders and Non-Complete Responders

Patients who achieved a CR had a median age of 73 years (IQR 65–77), while those who did not achieve CR had a median age of 66 years (IQR 55–77). Complete responders were more likely to be over 65 years of age (Chi-square test, $p = 0.013$) and less likely to have liver metastasis (Chi-square test, $p = 0.032$). At treatment start, the median LDH in complete responders was lower than in non-complete responders (Mann–Whitney U test, $p = 0.008$), and the baseline PLR was more likely to be below 213 (Chi-square test, $p = 0.036$). The presence of brain metastases was not significantly different between those who had CR and those who did not ($p = 0.135$) (Table 1).

When comparing the OS of patients with CR and patients with PR, SD and PD, a statistically significant difference was observed between all subgroups (Fig. 2a). Of note, PFS and OS in patients with PR and CR differed significantly. Patients with PR had a 5-year PFS and OS since therapy initiation of 27% (95% CI 18–38%) and 50% (95% CI 38–61%), respectively, while those achieving CR had a 5-year PFS and 5-year OS of 78% (95% CI 61–88%) and 92% (95% CI 77–97%), respectively (Fig. 2a, b). The mPFS and mOS in patients with PR were 21 months (IQR 16–31) and 55 months (IQR 35–NR), respectively, while both were NR in those having CR.

3.4 Blood Biomarkers

Using the Wilcoxon signed-rank test for matched pairs, we saw a statistically significant decrease in serum S100 (Wilcoxon matched-pairs signed rank test, $p = 0.043$) from T0 (therapy start) to T1 (time of CR) (Fig. 3a). At the time of CR, there was serum S100 normalization (Fisher test, $p < 0.001$). Moreover, although not statistically significant, a decrease in serum LDH (median difference = −3, mean difference T1–T0 = −44), PLR and NLR, and an increase in relative lymphocyte count from T0 (therapy start) to T1 (time of CR) was seen (Fig. 3a–g). We subsequently compared the S100 and LDH values at the time of CR (T1) and at the time of progression (T2) when these were available at both time points ($n = 7$). We saw a significant increase in S100 ($p = 0.015$) (Fig. 3c), and, although not significant, an increase in LDH was also seen (Fig. 3d).

Besides this, we saw that among patients with CR who later had PD, there were patients ($n = 3$) who had high baseline NLR (above 7), PLR (above 340), and a low baseline relative lymphocyte count. The other patients who progressed ($n = 4$) and did not have a low baseline relative

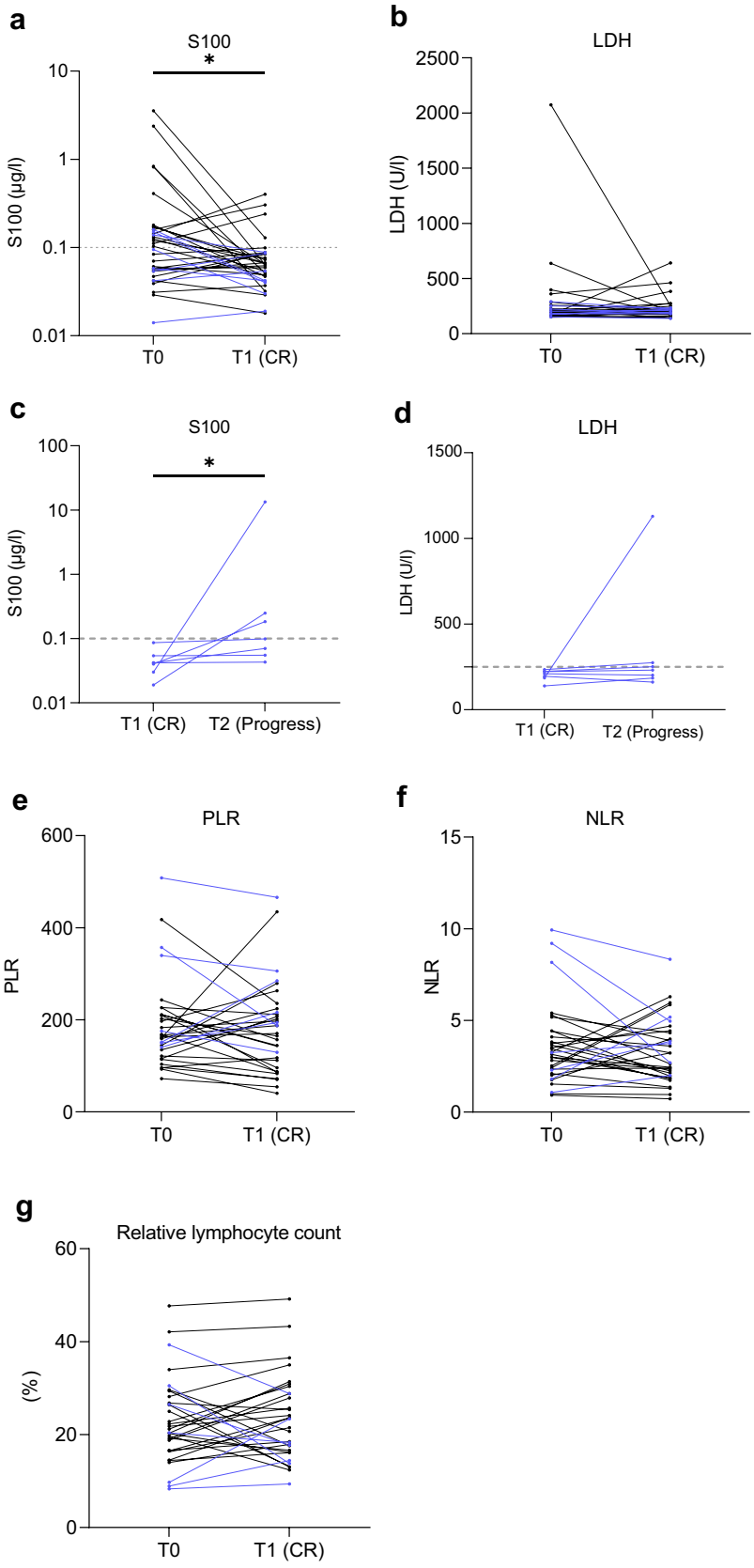


Fig. 3 **a**–**g** Dot plots showing the variation of blood biomarkers (LDH, S100, PLR, NLR, and relative lymphocyte count) in patients who achieved a CR from baseline value at the time of therapy start (T0) to the time of complete response (T1) and time of progression (T2). Each line represents one patient, and the plots show only patients whose values were reported at both time points. *LDH* lactate dehydrogenase, *PLR* platelet-to-lymphocyte ratio, *NLR* neutrophil-to-lymphocyte ratio, *CR* complete response

lymphocyte count did exhibit a decrease in the relative number of lymphocytes and an increase in NLR (Fig. 3a–g).

3.5 Rechallenge with Second-Line Immune Checkpoint Inhibitors

Rechallenge with ICIs was offered to the eight patients with PD after CR; seven (87.5%) patients received anti-PD-1-based immunotherapy and one patient received ipilimumab. Disease control (CR, PR, or SD) was observed in five (63%) patients, while three (37%) patients experienced PD (Fig. 4a).

3.6 Immune-Related Adverse Events

Overall, 28 of 40 (70%) patients had an irAE, 15 (37.5%) were cutaneous, and 15 (37.5%) were endocrinological; 8 patients had hepatitis, 7 had gastroenterological irAEs, 5 had respiratory irAEs, and 2 had neurological irAEs. Toxicities that led to therapy discontinuation were as follows: one patient was diagnosed with neuritis, one with meningitis, one with pneumonitis, two with colitis, one with hypophysitis, and one patient with pancreatitis.

Late-onset toxicities occurred in 10 (25%) patients. Nine were diagnosed during treatment, while one developed toxicity after the therapy was stopped. Drug-related adverse events included cutaneous toxicities ($n = 7$), hepatitis ($n = 1$), arthritis ($n = 1$), neuritis ($n = 1$), and pancreatitis ($n = 1$). The cutaneous late-onset toxicities identified were bullous pemphigoid ($n = 2$), vitiligo ($n = 2$), Grover's disease ($n = 1$), rhagade formation ($n = 1$), and mucositis ($n = 1$) (Table 2 and Fig. 4b). Four late-onset cutaneous toxicities were identified at or before the time of CR.

4 Discussion

In our study, with an mFUP of 56 months after CR, patients with CR, according to RECIST 1.1, displayed a survival benefit sustained at the landmark analysis of 5 years. Therefore, we can assume that CR is a surrogate marker for long-term survival in our cohort. Most patients (80%) experienced a durable response after treatment cessation, and neither the overall therapy duration nor the therapy duration after CR were related to survival. These data suggest that in most cases, it is reasonable to discontinue treatment after a CR.

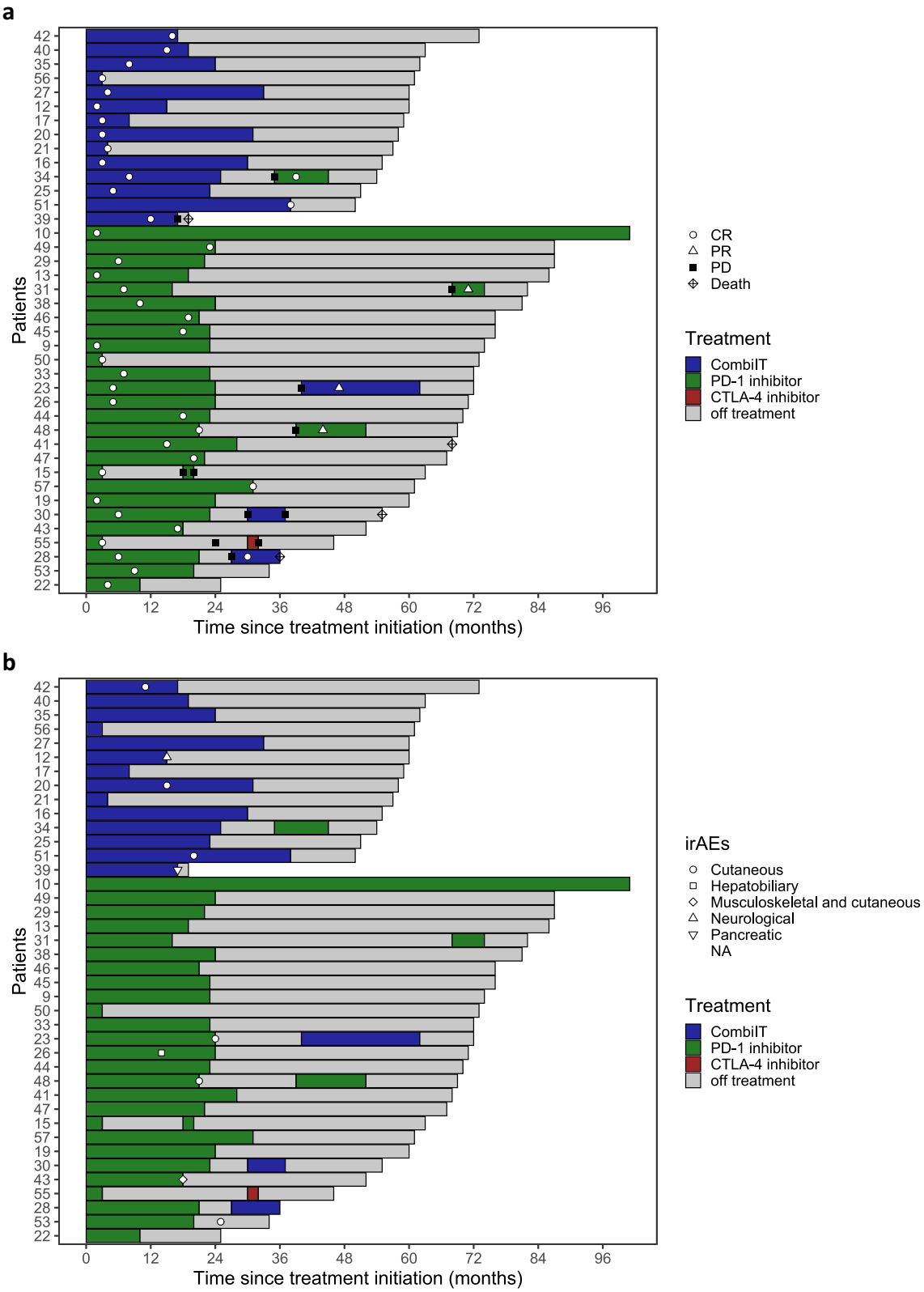
In patients who achieved CR, the median time from therapy start to CR was 6 months, which coincides with the second radiological assessment. Patients with CR within 3 months of therapy initiation ($n = 5$) did not develop PD, suggesting that an early CR could be more favorable than CR achieved later in the course of therapy; however, the number of patients was low, therefore no definitive conclusions could be drawn.

Robert et al. showed that CR was more common in patients over 65 years of age, and other studies have demonstrated that older age might be associated with response [12, 19]. In mouse experiments, young mice had more T regulatory cells than older mice injected with the same tumors [19]. Moreover, older age has been linked to increased tumor mutation burden and a proinflammatory gut microbiome [20]. In our study, patients achieving CR were more likely to be older than 65 years of age, but patients older than 77 years of age (upper quantile) had a worse survival outcome than younger patients who also achieved CR. Our findings are in line with the meta-analysis of Wu et al., which shows that although people over 65 years of age had a more significant benefit from immunotherapy, no difference in survival was seen for those over 75 years of age [21].

Besides age, our data showed that elevated serum LDH, higher PLR, and liver metastases at the time of treatment start were associated with lower CR rates. LDH depicts the tumor burden, is an already known prognostic marker associated with worse therapy outcomes, and is incorporated into the M category of the AJCC classification of melanoma [22–24]. PLR is a marker of systemic inflammation. NLR, PLR, and C-reactive protein have been associated with a worse prognosis in cancer, including melanoma [25–28]. In our cohort, patients with liver metastases were less likely to have a CR under ICI, which aligns with other working groups showing that different metastatic sites impact therapy outcomes differently. Lee et al. reviewed that the liver microenvironment causes local immunosuppression due to its different types of antigen-presenting cells leading to an unconventional T-cell tolerance, showing that the tumor microenvironment plays a role in melanoma progression [29, 30]. Finally, Pires da Silva et al. showed that liver melanoma metastases had the lowest response rate, and melanoma patients having liver metastases had low objective response rates and worse survival outcomes [31].

In our study, the majority of patients were men, and this overrepresentation is probably due to a selection bias that cannot be overcome due to the retrospective nature of this study.

In our analysis, 20% of patients developed PD—14% received combination ICI and 6% received monotherapy. In Checkmate 066, 067, and 069, up to 30% of patients receiving nivolumab had PD after CR [12]. Therefore, it is critical to find other tools and biomarkers besides response, according to RECIST 1.1, to identify those at risk of progression [32]. It is also important to compare how well the various imaging systems (RECIST, mRECIST, irRC, and iRECIST) identify complete responders in melanoma. Combining these with PET/CT (PET Response Criteria in Solid Tumors [PERCIST] and immunotherapy-modified PERCIST



[imPERCIST]), which assesses the tumors metabolically, or performing a pathological assessment of the radiographic remnants, may increase accuracy in predicting long-term survival [33–36]. Dimitriou et al. used PET/CT to evaluate patients with PR according to RECIST 1.1. In this analysis, the authors showed that patients with PR according to

◂**Fig. 4** **a** Patient swimmer plot showing the time patients achieved a CR under first-line immunotherapy. The type of ICI, therapy duration, off-treatment duration, response to rechallenge with second-line immunotherapy, disease progression, and death as events are also depicted. Type of treatment includes PD-1 inhibitor monotherapy (PD-1 inhibitor), CTLA-4 inhibitor monotherapy (CTLA-4 inhibitor), and combined CTLA-4 and PD-1 inhibitor (CombiIT). **b** Patient swimmer plot showing the late-onset irAEs related to first-line immunotherapy. The time of onset and late-occurring immune-related toxicities are depicted. Type of treatment includes PD-1 inhibitor monotherapy (PD-1 inhibitor), CTLA-4 inhibitor monotherapy (CTLA-4 inhibitor), and combined CTLA-4 and PD-1 inhibitor (CombiIT). *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *CombiIT* combination immunotherapy, *irAEs* immune-related adverse events, *ICI* immune checkpoint inhibition, *PD-1* programmed death-1, *CTLA-4* cytotoxic T-lymphocyte-associated protein 4

RECIST 1.1 and complete metabolic response in PET/CT had a better outcome [34]. Ellebaek et al. also demonstrated that in patients with CR or PR, according to RECIST 1.1, the presence of FDG-avid lesions at the time of therapy discontinuation was an independent predictor of unfavorable survival [36].

The PET STOP trial (NCT04462406) is currently evaluating the use of PET/CT and biopsy of the tumor lesions to decide on the optimal therapy duration. Specifically, patients in this study with a negative FDG-PET/CT scan or a negative biopsy from an avid FDG-PET/CT lesion will discontinue anti-PD-1 therapy (Table 3). Trials evaluating the optimal treatment duration, lower doses of ICI, and the ideal interval between doses in different solid cancers, including melanoma, are ongoing [37–39]. These are expected to shed light on the topic (Table 3).

Furthermore, as we see that 20% of the patients with CR progress after therapy discontinuation, monitoring them to detect minimal residual disease and progression is essential. Our study shows that serum S100 varied greatly from baseline level to time of CR and time of PD, making S100 a good monitoring marker for serial FUP after therapy discontinuation. We showed a significant decrease in S100 at the time of CR and a significant increase when PD was documented. In the future, the use of technics such as liquid biopsy for the detection of circulating tumor DNA (ctDNA), cell-free DNA (cfDNA), circulating tumor cells (CTCs), microRNAs, extracellular vesicles, antibodies against tumor antigens, and tumor-educated platelets (TEP) could help clinicians when deciding to stop or start therapy [40–46].

Regarding safety, up to 37.5% of patients with CR presented cutaneous toxicities. Previous publications have shown that cutaneous toxicities are associated with improved survival outcomes [47]. Melanoma-associated vitiligo is associated with a good prognosis and shows increased melanoma-specific CD8+ T cells and a high interferon-γ signature, while anti-skin immunoglobulin (Ig) G has been associated with favorable outcomes [48–51]. Specifically, in a prospective observational study of patients developing vitiligo under pembrolizumab, Hua et al. showed that 18% achieved a CR and 53% achieved a PR [52]. Late-onset toxicity was documented in 25% of patients; in most, the irAEs were mild, in line with previous publications [53].

The advantages of our study include its long FUP time of up to 5 years after CR and the inclusion of patients receiving treatment outside of clinical trials, which have restricted the inclusion and exclusion criteria. We focused on the subgroup of patients achieving CR to shed light on the long-term outcomes of this patient subgroup. We included only patients receiving first-line anti-PD-1 monotherapy or combined immunotherapy, as prior treatment lines can influence response durability.

Table 2 Late-onset immune-related adverse events appearing after 6 months of therapy initiation

ID	Toxicity	Type	Start after therapy (months)	Start after CR (months)	Grade
51	Rhagades	Cutaneous	20	−17	1
42	Bullous pemphigoid	Cutaneous	11	−4	2
43	Arthritis and mucositis	Arthritis and cutaneous	18	0	1
48	Vitiligo	Cutaneous	21	0	1
39	Pancreatitis[a]	Pancreatic	17	5	4
12	Neuritis[a]	Neurological	15	12	4
20	Grover's disease	Cutaneous	15	12	2
26	Hepatitis	Hepatobiliary	14	12	2
53	Bullous pemphigoid	Cutaneous	25	16	2
23	Vitiligo	Cutaneous	24	22	1

CR complete response

[a]These immune-related adverse events led to therapy discontinuation

Table 3 Clinical trials assessing the optimal duration of immunotherapy in advanced unresectable melanoma patients. All clinical trials are currently recruiting, except the Safe-Stop IPI-NIVO, which has not yet begun to recruit

Trial	Responsible party	Study type	No. of participants	Intervention		Primary outcome measure	Identifier
SAFE-STOP	Netherlands	Multicenter, single-arm, prospective, interventional	200	Early discontinuation of therapy	6 weeks after CR or PR	Ongoing response rate at 2 years after therapy discontinuation	NL7293
Safe-Stop IPI-NIVO	Erasmus Medical Center (Netherlands)	Single-arm, prospective, interventional	80	Early discontinuation of therapy	Upon achieving CR or PR	Ongoing response rate at 1 year after therapy start	NCT05652673
DANTE	UK	Multicenter, randomized, phase III, interventional	1208	Early discontinuation of therapy (Arm A) vs. SOC (Arm B)	12 months after therapy start (arm A)	Progression-free survival at 3, 6, 9, and 12 months after therapy discontinuation	ISRCTN15837212
STOP-GAP	Canadian Clinical Trials Group	Multicenter, randomized, phase III, interventional	614	Intermittent therapy when tumor regression (arm A) vs. SOC (Arm B)		Overall survival	NCT02821013
PET-STOP	Eastern Cooperative Oncology Group/ ACRIN group	Multicenter, two-arm, non-randomized, phase II, interventional	150	Discontinuation of therapy in patients with disease control and negative PET/CT or biopsy (arm A)	12 months after therapy start (arm A)	Event-free survival at 1 year after therapy discontinuation	NCT04462406

SOC standard of care, *CR* complete response, *PR* partial response, *PET* positron emission tomography, *CT* computed tomography

The study's limitations include its single-center retrospective design and CR being assessed using only RECIST 1.1 criteria.

5 Conclusions

CR using RECIST 1.1 is a surrogate marker for long-term survival in patients receiving ICI. CR rates seem to be higher in patients older than 65 years of age and those with lower LDH and PLR before therapy started. The optimal therapy duration after achieving CR is unclear and needs to be investigated. Among complete responders, tools are still needed to identify patients at higher risk of PD.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40257-023-00775-7.

Declarations

Funding Open Access funding enabled and organized by Projekt DEAL.

Conflicts of interest/competing interest Ulrike Leiter reports research support from MSD; consulting fees and honoraria from Sun Pharma, Sanofi (personal and institutional), MSD (personal and institutional), Novartis, Roche, and Almirall Hermal; support for attending meetings from Sun Pharma; and participation on a Data Safety Monitoring Board or Advisory Board from Sun Pharma, Sanofi, MSD, Novartis, Roche, and Almirall Hermal, outside the submitted work. Ioannis Thomas reports honoraria for talks or advisory boards from BMS and Pierre Fabre; and research funding (institutional) from BMS, Pfizer, MSD, Amgen, ARGN-X, LEO, Novartis, UCB, 4SC, AstraZeneca, BioNTech, Genentech, Roche, Biotech, CureVac, HUYA, Incyte, Idera, Iovance, InflaRx, Cerpass, Kartos, Nektar, Philogen, Pierre Fabre, Regeneron, Replimune, and Sanofi. Heike Niessner reports institutional funding from Novartis and Pierre Fabre outside the submitted work. Tobias Sinnberg reports institutional funding from Novartis and Pierre Fabre outside the submitted work. Andrea Forschner served as a consultant to Roche, Novartis, MSD, BMS, and Pierre Fabre; received travel support from Roche, Novartis, BMS, and Pierre Fabre; received speaker fees from Roche, Novartis, BMS, MSD, and CeGaT; and reports institutional research grants from BMS Stiftung Immunonkologie. Lukas Flatz reports grants or contracts from Hookipa Pharma, SAKK/Immunophotonics, DFG Grant (Deutsche Forschungsgemeinschaft), Philogen, and Mundipharma; consulting fees from Philogen, Sanofi, Novartis, and BMS; participation on the Data Safety Board, University of Basel; and stocks or stock options from Hookipa Pharma, outside the submitted work. Teresa Amaral reports personal honoraria from BMS, CeCaVa, Novartis, and Pierre Fabre; institutional financial support from iFIT, NeraCare, Novartis, Sanofi, and SkylineDx, and an institutional research grant from Novartis, outside the submitted work. Eftychia Chatziioannou, Ulrike Keim, Olivia Seeber, Andreas Meiwes, Isabell Boessenecker, Stephanie Sanchez Gonzalez, and Francisco Merraz Torres have no relationships to disclose.

Ethics approval The Ethics Commission of the Eberhard Karls University Tuebingen approved this retrospective single-center study (number 676/2016BO2).

Consent to participate Patients provided informed consent to be enrolled in the CMMR and for the use of their clinical data.

Consent for publication Not applicable.

Data availability The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Author contributions Study concept: EC, TA. Data collection: EC, TS, HN, TA. Data analysis: EC, TA. Data interpretation: All authors. Writing: All authors. Final approval: All authors. All authors agree to be accountable for all aspects of this work.

References

1. McDermott D, Lebbé C, Hodi FS, Maio M, Weber JS, Wolchok JD, et al. Durable benefit and the potential for long-term survival with immunotherapy in advanced melanoma. Cancer Treat Rev. 2014;40(9):1056–64.
2. Ribas A, Hodi FS, Kefford R, Hamid O, Daud A, Wolchok JD, et al. Efficacy and safety of the anti-PD-1 monoclonal antibody MK-3475 in 411 patients (pts) with melanoma (MEL). J Clin Oncol. 2014;32(15 Suppl):LBA9000-LBA.
3. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. J Clin Oncol. 2015;33(17):1889–94.
4. van Zeijl MCT, van den Eertwegh AJM, Wouters M, de Wreede LC, Aarts MJB, van den Berkmortel F, et al. Discontinuation of anti-PD-1 monotherapy in advanced melanoma-outcomes of daily clinical practice. Int J Cancer. 2022;150(2):317–26.
5. Marron TU, Ryan AE, Reddy SM, Kaczanowska S, Younis RH, Thakkar D, et al. Considerations for treatment duration in responders to immune checkpoint inhibitors. J Immunother Cancer. 2021;9(3): e001901.
6. Mäkelä S, Kohtamäki L, Laukka M, Juteau S, Hernberg M. Limited-duration anti-PD-1 therapy for patients with metastatic melanoma. Acta Oncol. 2020;59(4):438–43.
7. Asher N, Israeli-Weller N, Shapira-Frommer R, Ben-Betzalel G, Schachter J, Meirson T, et al. Immunotherapy discontinuation in metastatic melanoma: lessons from real-life clinical experience. Cancers (Basel). 2021;13(12):3074.
8. Betof Warner A, Palmer JS, Shoushtari AN, Goldman DA, Panageas KS, Hayes SA, et al. Long-term outcomes and responses to retreatment in patients with melanoma treated with PD-1 blockade. J Clin Oncol. 2020;38(15):1655–63.
9. Jansen YJL, Rozeman EA, Mason R, Goldinger SM, GeukesFoppen MH, Hoejberg L, et al. Discontinuation of anti-PD-1 antibody therapy in the absence of disease progression

or treatment limiting toxicity: clinical outcomes in advanced melanoma. Ann Oncol. 2019;30(7):1154–61.
10. Jansen Y, van der Veldt AAM, Awada G, Neyns B. Anti-PD-1: when to stop treatment. Curr Oncol Rep. 2022;24(7):905–15.
11. Robert C, Long G, Larkin J, Wolchok JD, Hassel J, Schadendorf D, et al. 1082MO 5-year characterization of complete responses in patients with advanced melanoma who received nivolumab plus ipilimumab (NIVO+IPI) or NIVO alone. Ann Oncol. 2020;31:S734–5.
12. Robert C, Ribas A, Hamid O, Daud A, Wolchok JD, Joshua AM, et al. Durable complete response after discontinuation of pembrolizumab in patients with metastatic melanoma. J Clin Oncol. 2018;36(17):1668–74.
13. Dimitriou F, Zaremba A, Allayous C, Kähler KC, Gerard CL, Festino L, et al. Sustainable responses in metastatic melanoma patients with and without brain metastases after elective discontinuation of anti-PD1-based immunotherapy due to complete response. Eur J Cancer. 2021;149:37–48.
14. Saiag P, AitMehdi R, Blom A, Longvert C, Emile J-F, Boru B, et al. Discontinuation of anti-PD-1 mAb after complete response in advanced melanoma pts. J Clin Oncol. 2018;36(15 Suppl): e21549.
15. De Risi I, Sciacovelli AM, Guida M. Checkpoint inhibitors immunotherapy in metastatic melanoma: when to stop treatment? Biomedicines. 2022;10(10):2424.
16. Dutheil A, Belkadi D, Antigny M, Roy S, Lupu J, Vallet A, et al. Complete responders to checkpoint inhibitors in advanced melanoma: relapse risk factors, and patients' outcomes. J Clin Oncol. 2021;39(15 Suppl):9550.
17. Gershenwald JE, Scolyer RA, Hess KR, Sondak VK, Long GV, Ross MI, et al. Melanoma staging: Evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin. 2017;67(6):472–92.
18. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228–47.
19. Kugel CH III, Douglass SM, Webster MR, Kaur A, Liu Q, Yin X, et al. Age correlates with response to anti-PD1, reflecting age-related differences in intratumoral effector and regulatory T-cell populations. Clin Cancer Res. 2018;24(21):5347–56.
20. Machiraju D, Schäfer S, Hassel JC. Potential reasons for unresponsiveness to anti-PD1 immunotherapy in young patients with advanced melanoma. Life (Basel). 2021;11(12):1318.
21. Wu Q, Wang Q, Tang X, Xu R, Zhang L, Chen X, et al. Correlation between patients' age and cancer immunotherapy efficacy. Oncoimmunology. 2019;8(4): e1568810.
22. Espinosa E, Márquez-Rodas I, Soria A, Berrocal A, Manzano JL, Gonzalez-Cao M, et al. Predictive factors of response to immunotherapy-a review from the Spanish Melanoma Group (GEM). Ann Transl Med. 2017;5(19):389.
23. Weide B, Elsässer M, Büttner P, Pflugfelder A, Leiter U, Eigentler TK, et al. Serum markers lactate dehydrogenase and S100B predict independently disease outcome in melanoma patients with distant metastasis. Br J Cancer. 2012;107(3):422–8.
24. Amaral T, Seeber O, Mersi E, Sanchez S, Thomas I, Meiwes A, et al. Primary resistance to PD-1-based immunotherapy: a study in 319 patients with stage IV melanoma. Cancers. 2020;12(4):1027.
25. Qi Y, Zhang Y, Fu X, Wang A, Yang Y, Shang Y, et al. Platelet-to-lymphocyte ratio in peripheral blood: a novel independent prognostic factor in patients with melanoma. Int Immunopharmacol. 2018;56:143–7.
26. Zhou X, Du Y, Huang Z, Xu J, Qiu T, Wang J, et al. Prognostic value of PLR in various cancers: a meta-analysis. PLoS ONE. 2014;9(6): e101119.
27. Kim J-Y, Jung EJ, Kim J-M, Lee HS, Kwag S-J, Park J-H, et al. Dynamic changes of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio predicts breast cancer prognosis. BMC Cancer. 2020;20(1):1206.
28. Guida M, Bartolomeo N, Quaresmini D, Quaglino P, Madonna G, Pigozzo J, et al. Basal and one-month differed neutrophil, lymphocyte and platelet values and their ratios strongly predict the efficacy of checkpoint inhibitors immunotherapy in patients with advanced BRAF wild-type melanoma. J Transl Med. 2022;20(1):159.
29. Lee JC, Green MD, Huppert LA, Chow C, Pierce RH, Daud AI. The liver-immunity nexus and cancer immunotherapy. Clin Cancer Res. 2022;28(1):5–12.
30. Chatziioannou E, Aydin SA, Forchhammer S, Sinnberg T, Eigentler T. Makrophagen im Melanom—von molekularen Signalen zur therapeutischen Anwendung. Die Dermatologie. 2022;73(12):915–28.
31. Pires da Silva I, Lo S, Quek C, Gonzalez M, Carlino MS, Long GV, et al. Site-specific response patterns, pseudoprogression, and acquired resistance in patients with melanoma treated with ipilimumab combined with anti-PD-1 therapy. Cancer. 2020;126(1):86–97.
32. Stein JE, Soni A, Danilova L, Cottrell TR, Gajewski TF, Hodi FS, et al. Major pathologic response on biopsy (MPRbx) in patients with advanced melanoma treated with anti-PD-1: evidence for an early, on-therapy biomarker of response. Ann Oncol. 2019;30(4):589–96.
33. Borm FJ, Smit J, Oprea-Lager DE, Wondergem M, Haanen J, Smit EF, et al. Response prediction and evaluation using PET in patients with solid tumors treated with immunotherapy. Cancers (Basel). 2021;13(12):3083.
34. Dimitriou F, Lo SN, Tan AC, Emmett L, Kapoor R, Carlino MS, et al. FDG-PET to predict long-term outcome from anti-PD-1 therapy in metastatic melanoma. Ann Oncol. 2022;33(1):99–106.
35. Schank TE, Forschner A, Sachse MM, Dimitrakopoulou-Strauss A, Sachpekidis C, Stenzinger A, et al. Complete metabolic response in FDG-PET-CT scan before discontinuation of immune checkpoint inhibitors correlates with long progression-free survival. Cancers (Basel). 2021;13(11):2616.
36. Ellebaek E, Schina A, Andersen R, Hendel HW, Svane IM, Donia M. Clinical value of routine [18F]2-fluoro-2-deoxy-d-glucose positron emission tomography scans as a decision tool for early immunotherapy discontinuation in advanced melanoma. Int J Cancer. 2022;150(11):1870–8.
37. Coen O, Corrie P, Marshall H, Plummer R, Ottensmeier C, Hook J, et al. The DANTE trial protocol: a randomised phase III trial to evaluate the Duration of ANti-PD-1 monoclonal antibody Treatment in patients with metastatic mElanoma. BMC Cancer. 2021;21(1):761.
38. Mulder E, de Joode K, Litière S, Ten Tije AJ, Suijkerbuijk KPM, Boers-Sonderen MJ, et al. Early discontinuation of PD-1 blockade upon achieving a complete or partial response in patients with advanced melanoma: the multicentre prospective Safe Stop trial. BMC Cancer. 2021;21(1):323.
39. Hirsch I, Goldstein DA, Tannock IF, Butler MO, Gilbert DC. Optimizing the dose and schedule of immune checkpoint inhibitors in cancer to allow global access. Nat Med. 2022;28(11):2236–7.
40. Amrane K, Le Goupil D, Quere G, Delcroix O, Gouva S, Schick U, et al. Prediction of response to immune checkpoint inhibitor therapy using 18F-FDG PET/CT in patients with melanoma. Medicine (Baltimore). 2019;98(29): e16417.
41. Kitajima K, Watabe T, Nakajo M, Ishibashi M, Daisaki H, Soeda F, et al. Tumor response evaluation in patients with malignant melanoma undergoing immune checkpoint inhibitor therapy and prognosis prediction using (18)F-FDG PET/CT: multicenter study

for comparison of EORTC, PERCIST, and imPERCIST. Jpn J Radiol. 2022;40(1):75–85.
42. Tan L, Sandhu S, Lee RJ, Li J, Callahan J, Ftouni S, et al. Prediction and monitoring of relapse in stage III melanoma using circulating tumor DNA. Ann Oncol. 2019;30(5):804–14.
43. Vanni I, Tanda ET, Spagnolo F, Andreotti V, Bruno W, Ghiorzo P. The current state of molecular testing in the BRAF-mutated melanoma landscape. Front Mol Biosci. 2020. https://doi.org/10.3389/fmolb.2020.00113.
44. Boyer M, Cayrefourcq L, Dereure O, Meunier L, Becquart O, Alix-Panabières C. Clinical relevance of liquid biopsy in melanoma and merkel cell carcinoma. Cancers. 2020;12:960.
45. Kamińska P, Buszka K, Zabel M, Nowicki M, Alix-Panabières C, Budna-Tukan J. Liquid biopsy in melanoma: significance in diagnostics, prediction and treatment monitoring. Int J Mol Sci 2021;22(18).
46. Pitcovski J, Shahar E, Aizenshtein E, Gorodetsky R. Melanoma antigens and related immunological markers. Crit Rev Oncol Hematol. 2017;115:36–49.
47. Hasan Ali O, Diem S, Markert E, Jochum W, Kerl K, French LE, et al. Characterization of nivolumab-associated skin reactions in patients with metastatic non-small cell lung cancer. OncoImmunology. 2016;5(11): e1231292.
48. Failla CM, Carbone ML, Fortes C, Pagnanelli G, Datri S. Melanoma and vitiligo: in good company. Int J Mol Sci. 2019;20(22):5731.
49. Byrne KT, Turk MJ. New perspectives on the role of vitiligo in immune responses to melanoma. Oncotarget. 2011;2(9):684–94.
50. Zhao J-J, Wen X-Z, Ding Y, Li D-D, Zhu B-Y, Li J-J, et al. Association between immune-related adverse events and efficacy of PD-1 inhibitors in Chinese patients with advanced melanoma. Aging. 2020;12(11):10663–75.
51. Wu C-E, Yang C-K, Peng M-T, Huang P-W, Chang C-F, Yeh K-Y, et al. The association between immune-related adverse events and survival outcomes in Asian patients with advanced melanoma receiving anti-PD-1 antibodies. BMC Cancer. 2020;20:1018.
52. Hua C, Boussemart L, Mateus C, Routier E, Boutros C, Cazenave H, et al. Association of vitiligo with tumor response in patients with metastatic melanoma treated with pembrolizumab. JAMA Dermatol. 2016;152(1):45–51.
53. Carlet C, Dalle S, Leccia M-T, Mortier L, Dalac-Rat S, Dutriaux C, et al. Late-onset adverse events of anti-PD1 therapy in melanoma patients: an observational study from MELBASE, a nationwide prospective cohort. J Am Acad Dermatol. 2022;86(2):345–52.

2.2. Publication 2: Nomogram for predicting survival after first-line anti-PD-1-based immunotherapy in unresectable stage IV melanoma: a multicenter international study

Authors: Chatziioannou Eftychia, Serna-Higuita Lina Maria, Kreft Sophia, Kandolf Lidija, Dujovic Branko, Reinhardt Lydia, Tamara Etterlin, Márquez-Rodas Ivan, Fortuna Ana Raquel Ferraz Pereira, Nübling Alica, Niessner Heike, Forschner Andrea, Garbe Claus, Popovic Aleksandar, Mirjana Balić, Meier Friedegund, Eigentler Thomas, Leiter Ulrike, Flatz Lukas, Sinnberg Tobias, Amaral Teresa

Year: 2024

Journal: ESMO Open,

Volume: 9,

Issue: 8, 103661

DOI:10.1016/j.esmoop.2024.103661

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ESMO OPEN SCIENCE FOR OPTIMAL CANCER CARE

ORIGINAL RESEARCH

Nomogram for predicting survival after first-line anti-PD-1-based immunotherapy in unresectable stage IV melanoma: a multicenter international study

E. Chatziioannou[1,2†], L. M. Serna Higuita[3†], S. Kreft[4], L. Kandolf[5], B. Dujovic[5], L. Reinhardt[6,7], E. Tamara[8], I. Marquez-Rodas[9], A. R. F. P. Fortuna[10], A. Nübling[1], H. Niessner[1], A. Forschner[1], C. Garbe[1], A. Popovic[11], B. Mirjana[11], F. Meier[6,7], T. Eigentler[4], U. Leiter[1], L. Flatz[1,8,12], T. Sinnberg[1,2,4] & T. Amaral[1,2*]

[1]Center for Dermato-oncology, Department of Dermatology, Eberhard Karls University of Tübingen, Tübingen; [2]Cluster of Excellence iFIT (EXC 2180) 'Image-Guided and Functionally Instructed Tumor Therapies', Tübingen; [3]Department of Clinical Epidemiology and Applied Biostatistics, Eberhard Karls University of Tübingen, Tübingen; [4]Department of Dermatology, Venereology and Allergology, Charité-Universitätsmedizin Berlin, Berlin, Germany; [5]Department of Dermatology, Faculty of Medicine, Military Medical Academy, Belgrade, Serbia; [6]Skin Cancer Center at the University Cancer Center and National Center for Tumor Diseases, Dresden; [7]Department of Dermatology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany; [8]Department of Dermatology and Allergology, Kantonsspital St. Gallen, St. Gallen, Switzerland; [9]Department of Medical Oncology, Hospital General Universitario Gregorio Marañón, Madrid, Spain; [10]Department of Medical Oncology, University Hospital Center of Algarve, Faro, Portugal; [11]Clinic for Medical Oncology, Clinical Center Nis, Niš, Serbia; [12]Institute for Immunobiology, Kantonsspital St. Gallen, St. Gallen, Switzerland

Available online 2 August 2024

Background: The introduction of anti-programmed cell death protein 1 (PD-1) immunotherapy has revolutionized the treatment landscape for melanoma, enhancing both response rates and survival outcomes in patients with advanced stages of the disease. Despite these remarkable advances, a noteworthy subset of patients (40%-60%) does not derive advantage from this therapeutic approach. This study aims to identify key predictive factors and create a user-friendly predictive nomogram for stage IV melanoma patients receiving first-line anti-PD-1-based immunotherapy, improving treatment decisions.

Materials and methods: In this retrospective study, we included patients with unresectable stage IV melanoma who received first-line treatment with either anti-PD-1 monotherapy or anti-PD-1 plus anti-cytotoxic T-lymphocyte associated protein 4 between 2014 and 2018. We documented clinicopathological features and blood markers upon therapy initiation. By employing the random survival forest model and backward variable selection of the Cox model, we identified variables associated with progression-free survival (PFS) after the first-line anti-PD-1-based treatment. We developed and validated a predictive nomogram for PFS utilizing the identified variables. We assessed calibration and discrimination performance metrics as part of the evaluation process.

Results: The study involved 719 patients, divided into a training cohort of 405 (56%) patients and a validation cohort of 314 (44%) patients. We combined findings from the random survival forest and the Cox model to create a nomogram that incorporates the following factors: lactate dehydrogenase (LDH), S100, melanoma subtype, neutrophil-to-lymphocyte ratio (NLR), body mass index, type of immune checkpoint inhibitor, and presence of liver or brain metastasis. The resultant model had a C-index of 0.67 in the training cohort and 0.66 in the validation cohort. Performance remained in different patient subgroups. Calibration analysis revealed a favorable correlation between predicted and actual PFS rates.

Conclusions: We developed and validated a predictive nomogram for long-term PFS in patients with unresectable stage IV melanoma undergoing first-line anti-PD-1-based immunotherapy.

Key words: nomogram, stage IV melanoma, anti-PD-1-based immunotherapy, progression-free survival, biomarkers, immune checkpoint inhibition

INTRODUCTION

The established treatment for unresectable melanoma includes anti-PD-1-based immunotherapy with pembrolizumab or nivolumab as monotherapy or combined with anti-cytotoxic T-lymphocyte associated protein 4 (CTLA-4) or anti-lymphocyte-activation gene 3.[1,2] For melanomas harboring *BRAF* oncogenic variant, BRAF and MEK inhibition may also be

*Correspondence to: Dr Teresa Amaral, Center for Dermato-oncology, Department of Dermatology, Eberhard Karls University, Liebermeisterstrasse 25, 72076, Tübingen, Germany. Tel: +49-7071-298-84555
E-mail: teresa.amaral@med.uni-tuebingen.de (T. Amaral).

†These authors contributed equally.

considered.[3] Recent clinical trials advocate prioritizing anti-PD-1-based therapy as the first-line option also in this subgroup.[4,5] However, a significant proportion of patients, ranging from 40% to 60%, do not benefit from this treatment.[6,7] Furthermore, immune checkpoint inhibitors (ICIs) may induce severe and irreversible immune-related toxicities.[8] Hence, predictive biomarkers upon therapy start hold significant relevance in daily clinical application and research areas. This is important as novel therapeutic approaches are emerging, such as tumor-infiltrating lymphocytes therapy and bispecific drugs.[9]

The progression-free survival (PFS) and overall survival probabilities after initiating anti-PD-1 therapy for melanoma are mostly based on the RECIST criteria.[7,10-13] Response evaluation is carried out during therapy, underscoring the gap for upfront biomarkers potentially guiding treatment decisions.

Certain biomarkers have been evaluated for their predictive potential. These include tumor-infiltrating cells, the programmed death-ligand 1 status, and gene copy number alterations.[14] Nonetheless, literature reports inconsistent findings, impeding their validation as a reliable biomarker.

The American Joint Committee on Cancer (AJCC) classification eighth edition lacks substages for stage IV melanoma in contrast to the substages for stages I, II, and III, which stratify patients into groups with different survival outcomes. Within stage IV, M category is reported according to metastatic sites and lactate dehydrogenase (LDH) level.[15] Still, there is considerable diversity in survival outcomes among patients with stage IV melanoma, highlighting the need for a more detailed stratification system.

We identified key factors associated with progression-free survival in stage IV melanoma patients upon starting first-line ICI and integrated into an online nomogram.

MATERIALS AND METHODS

Study population

This international multicenter study included all inoperable stage IV melanoma patients receiving first-line ICI therapy, either anti-PD-1 monotherapy or in combination with anti-CTLA-4, and met the inclusion criteria: age >18 years, informed consent, and response to be assessed based on RECIST version 1.1.[16] Patients undergoing adjuvant therapy or anti-CTLA-4 monotherapy as first-line treatment were excluded.

The study period ranged from 2014 to 2018. Patients were divided into two cohorts: the training cohort, treated at the skin centers of Tuebingen (Germany), Belgrade (Serbia), and at the Clinic for Medical Oncology in Nis (Serbia); and the validation cohort, treated at the skin centers of Berlin and Dresden (Germany), St Gallen (Switzerland), Madrid (Spain), and Faro (Portugal) (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2024.103661).

The sample size calculation was based on an exponential maximum likelihood test of equality of survival curves test.[17]

This study was approved by the ethics committees of the institutions involved and was conducted in accordance with consensus ethical principles derived from international ethical guidelines, including the Declaration of Helsinki. This study was approved by the Ethics Commission of the Eberhard Karls University Tuebingen with the number 774/2022BO2. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Statistical analysis

Descriptive statistics included counts (*n*) and percentages (%) for categorical variables and mean and standard deviation or medians and interquartile range (IQR) for continuous variables. Input variables comprised sex, age, melanoma subtype (acral cutaneous, non-acral cutaneous, occult, mucosal, uveal), tumor thickness, ulceration, *BRAF* and *NRAS* mutational status, LDH level, protein S100 level, neutrophil-to-lymphocyte ratio (NLR), brain or liver metastasis at therapy initiation, body mass index (BMI), and ICI therapy type. To mitigate extreme value influence and ensure interpretability, values below the fifth percentile were set to the fifth percentile. LDH and S100 were capped at 1000 and 1, respectively.

Primary endpoint was PFS, defined as the time between therapy start and disease progression. For patients not experiencing progressive disease (PD), the last follow-up date was considered the censoring point.

Kaplan—Meier curves were employed for survival analysis. Candidate factors were selected by using the random survival forest (RSF) analysis,[18-20] operating as a decision trees ensemble for right censored survival data. We employed 500 trees and log-rank splitting criteria. The backward approach of the Cox proportional hazards model with the Breslow method, select criterion of significant level, and entry significance level of 0.15 was used to select the final model.[21]

The linear relationship between continuous predictors and the logarithm of hazard was evaluated by plotting martingale residual plots, while the Schoenfeld test confirmed the proportionality assumption requisite. The bootstrapping sampling method (1000 resamples) was used for internal validation.

A nomogram was developed based on selected features. Based on the calculated score, the patients were divided into quartiles and log-rank tests were used for comparisons between groups. Discrimination was evaluated using Harrell's concordance index (C-index) and the area under the receiver operating characteristic curve (AU-ROC). Calibration was evaluated using the Brier score and calibration plots. Decision curve analysis (DCA) was carried out to evaluate the clinical benefit. External validation was assessed using a validation cohort.

Missing data were imputed via multiple imputation by chained equation. All statistical analyses and data visualization were executed using R. Reported *P* values were two-sided, with significance set at $P < 0.05$.

RESULTS

Patients' characteristics

We enrolled 719 patients in our study—405 patients (56%) in the training and 314 patients (44%) in the validation

cohort. Demographic and clinical characteristics are shown in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2024.103661.

In the training cohort, the median age was 68 years (IQR 57-77 years), and most (61%) were male. More than half (67%) of the patients presented non-acral cutaneous melanoma. Twenty-eight percent exhibited *BRAF* mutation, whereas 26% had *NRAS* mutation. Upon therapy initiation, 21% and 38% had brain and liver metastasis, respectively. Thirty-six percent of patients received anti-PD-1 and CTLA-4 inhibitors, while 64% received anti-PD-1 monotherapy.

Within the validation cohort comprising 314 patients, the median age was 69 years (range 57-76 years), 64% of patients had non-acral cutaneous melanomas and 47% received combined anti-PD-1 and CTLA-4 inhibitors.

The median follow-up in the training cohort was 54 months (range 47-56 months). The median PFS after therapy initiation was 25 months [95% confidence interval (CI) 21-36 months] (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2024.103661).

The validation cohort had a median follow-up of 50 months (95% CI 45-58 months). The median PFS following therapy initiation was 5 months (95% CI 4-8 months) (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2024.103661).

Random forest model and Cox model

The RSF analysis showed that the most important factors influencing PFS after starting ICI were LDH, followed by the melanoma type and NLR. Factors including S100 level, BMI, liver metastasis, age at therapy start, ulceration in primary tumor, brain metastasis, and the specific type of ICI therapy also played a role with decreasing importance. On the other hand, *BRAF* and *NRAS* mutational status, tumor thickness, and sex did not improve the model (Figure 1 & Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2024.103661).

The 10 variables selected by RSF were included in the Cox model. Based on backward selection process, a model was constructed including the following variables: LDH, NLR, melanoma subtype, S100 levels, BMI $<$ 18.5, presence of brain metastasis, presence of liver metastasis, and ICI therapy type (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2024.103661 and Supplementary Figure S4, available at https://doi.org/10.1016/j.esmoop.2024.103661).

Nomogram

We constructed a nomogram based on the eight identified variables (Figure 2), also found online [Dynamic Nomogram Predicting Progression-Free Survival in Stage IV Melanoma Patients after First-Line Anti-PD-1-Based Immunotherapy (https://science1.shinyapps.io/Melanoma/)]. The relationship between the total points of the nomogram (score) of the patients in the training cohort and the PFS as continuous is depicted in Figure 2. Patients were split into three groups based on the quartiles of the score (Figure 3A and B). The first group had individuals with total points ranging from 0 to 74.88 (below the lower quartile), the second group spanned from 74.88 to 153.28 points (interquartile), and the final group exceeded 153.28 points (above upper quartile). We decided to divide into three groups as no significant differences were observed between patients having a score between the lower quartile and median and median and the upper quartile (Supplementary Figure S5, available at https://doi.org/10.1016/j.esmoop.2024.103661).

Among the three groups in the training cohort, differences in median PFS (mPFS) were observed, and the Kaplan—Meier curves showed that PFS between the three groups was statistically significantly different (log-rank test, $P < 0.001$) (Figure 3C). Pairwise comparisons, conducted using the log-rank test and adjusted with Benjamini Hochberg correction, revealed significant differences in survival outcomes among the three groups. The mPFS was not reached (95% CI 58 months-not applicable) for the first group. In the second group, the mPFS was 27.1 months (95% CI 19-36 months), with a hazard ratio (HR) of 2.50 (ref: first group: 95% CI 1.7-3.6, $P < 0.001$). The third group displayed an mPFS of 9 months (95% CI 6-14 months) and HR of 6.19 (ref: first group: 95% CI 4.1-9.3, $P < 0.001$) (Figure 3C).

In the training cohort, the same score stratified patients based on melanoma-specific survival (MSS) (Figure 3D).

Performance metrics of model

The C-index was 0.674 (se = 0.018) in the training cohort, indicating moderate discriminative capacity. We generated calibration plots, revealing good correlations across various timeframes encompassing 1-, 2-, 3-, and 5-year PFS (Figure 4A). The Brier score consistently remained below 0.25, thus showing the higher precision of our nomogram's predictions compared to null model (Figure 4B). The DCA yielded an area under net benefit (AU-NBC) of 0.077 (Figure 4C). The AU-ROC consistently approximated 0.7 across different time intervals (Figure 5). Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2024.103661, shows additional performance metrics. The C-index for different subgroups were as follows: 0.68 (se = 0.029) for females, 0.68 (se = 0.023) for males, 0.70 (se = 0.033) for *BRAF* mutated melanoma, 0.67 (se = 0.021) for *BRAF* wild-type, 0.71 (se = 0.026) for *NRAS* mutated melanoma, 0.69 (se = 0.022) for *NRAS* wild-type melanomas, 0.69 (se = 0.027) for patients $<$65 years of age, and 0.68 (se = 0.023) for patients aged $>$65 years.

Internal validation

The shrinkage coefficient was 1.0003, indicating a 0.03% lack of fit in the model. The optimism-corrected C-index was 0.68, indicating moderated correlation between the log hazard and the observed survival time (Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2024.103661).

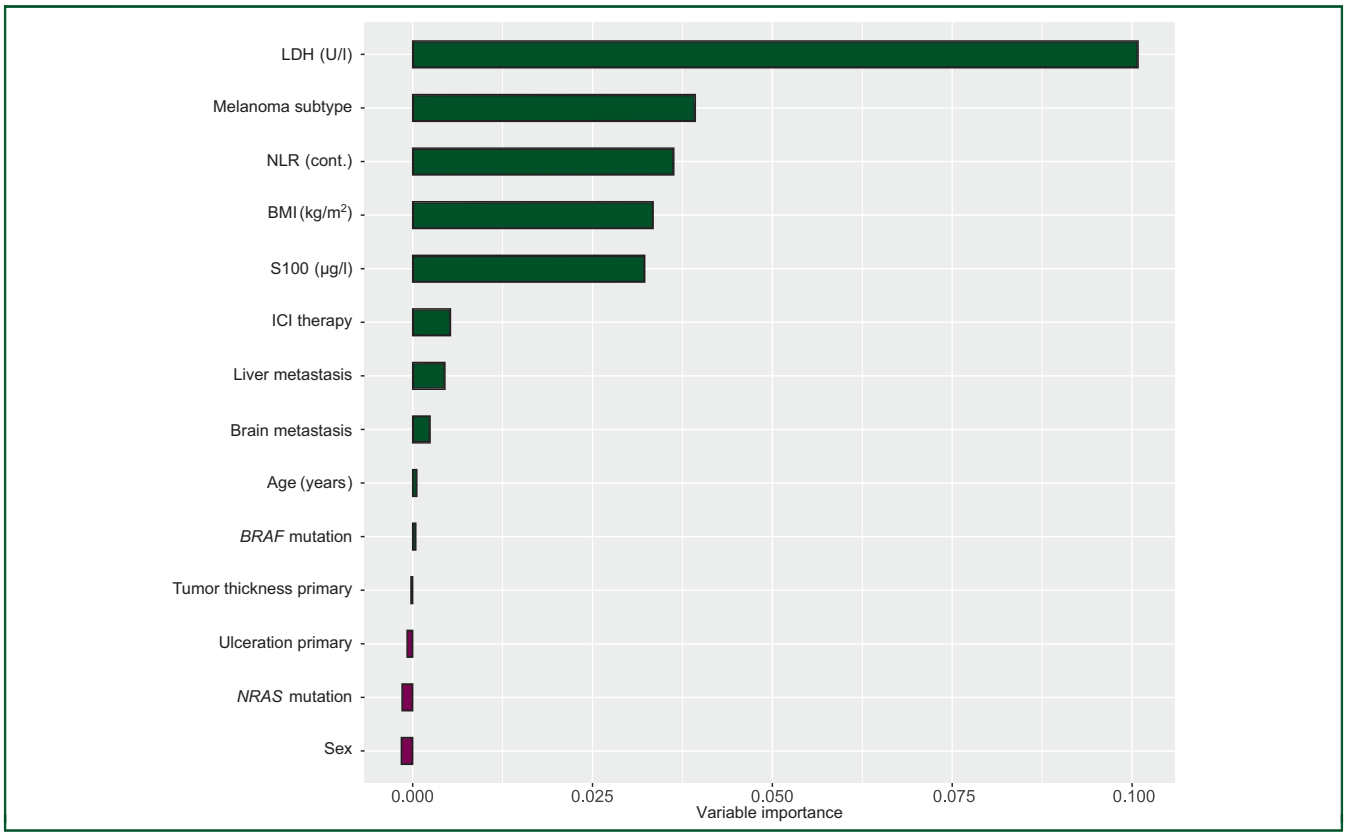


Figure 1. Plot showing the results of the random survival forest. The *x*-axis shows the variable importance of each variable. The variables with positive variable importance are depicted in green, while the ones with negative variable importance (vimp) are depicted in burgundy.
BMI, body mass index; ICI, immune checkpoint inhibitors; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio.

External validation

The performance of nomogram was evaluated using a cohort of 314 patients. Patients were divided into four groups according to their risk score based on the quartiles of the score in the training cohort.

Kaplan—Meier curves showed that the PFS between the four groups were statistically significantly different (log-rank test $P < 0.001$). In the validation cohort, the first group showed an mPFS of 35 months (95% CI 12 months-not applicable), the second group had an mPFS of 5 months (95% CI 4-8 months) and a HR of 2.07 (ref: first group: 95% CI 1.4-2.99), and the third had the shortest mPFS of 2 months (95% CI 1-3 months) and HR of 3.89 (ref: first group: 95% CI: 2.58-5.86) (Figure 3C).

The same score also stratified patients according to MSS in the validation cohort (Figure 3D).

The C-index of the validation cohort was 0.664 (se = 0.021) and the calibration plots showed good correlation (Figure 4A). The AU-NBC value was 0.079 (Figure 4C) and the AU-ROC approximated 0.7 across different time intervals (Figure 5).

DISCUSSION

We developed and validated a novel nomogram designed for predicting PFS in stage IV melanoma patients after first-line anti-PD-1-based therapy. This tool can tailor personalized therapeutic decisions and individualized patient follow-up strategies, and facilitate risk stratification.[22] This model is in the form of a dynamic, web-based nomogram to ensure accessibility and user-friendliness. The clinical utility has been assessed using DCA alongside other established performance metrics.[22]

Within AJCC v8 stage IV melanoma, no substages have been identified. The M category is subdivided based on the localization of metastases and LDH levels. Given the significant variability in survival outcomes among stage IV melanoma patients, there is a need to introduce a more nuanced stratification system.

We initiated our investigation by identifying predictors of PFS in patients undergoing ICI therapy. The variables finally included in our nomogram have exhibited predictive abilities in prior studies.[23,24]

Among the variables, LDH, markers of tumor burden, NLR, and melanoma subtype were significant PFS predictors. These findings align with earlier research, emphasizing the role of pretherapy LDH along with the melanoma subtype, underlining its distinct genomic profiles.[23,25-30] Claps et al. reviewed the role of LDH in cancer showing its role in immunosuppression, metabolism, and glycolysis.[31] Despite the counterintuitive role of neutrophils in tumors, elevated NLR has been correlated with unfavorable outcomes across various cancers. This may be attributed to the effect of lowered lymphocyte levels.[32-36] S100, although

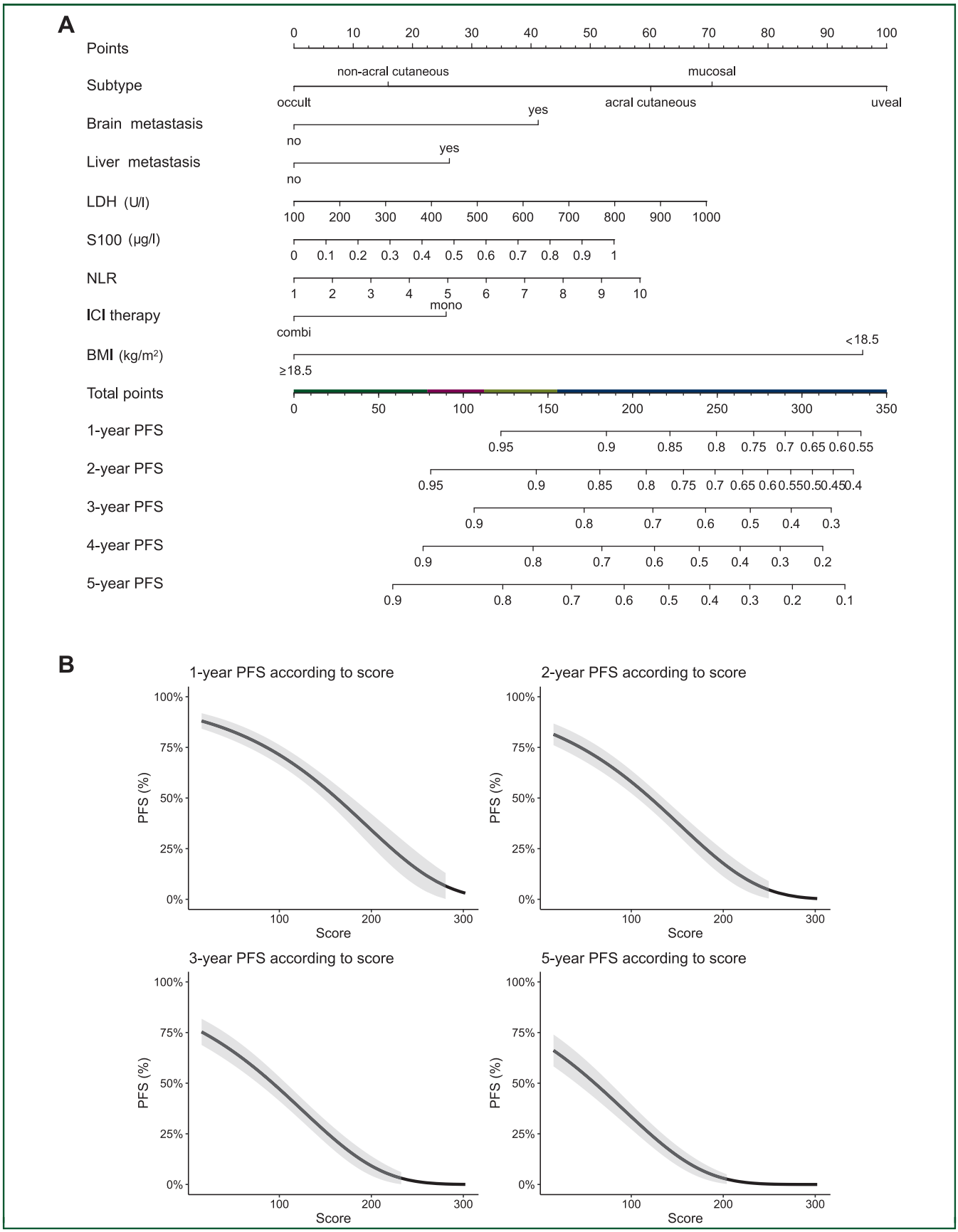


Figure 2. The nomogram and score creation. (A) Plot showing the nomogram that was built using the selected eight variables. The nomogram combines these eight clinicopathologic features to predict the PFS of stage IV melanoma patients after first-line anti-PD-1-based therapy. (B) Plots showing the relationship between the total points of the nomogram (score) and the PFS probability at 1, 2, 3, and 5 years after therapy initiation in the training cohort.
BMI, body mass index; ICI, immune checkpoint inhibitors; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; PD-1, programmed cell death protein 1; PFS, progression-free survival.

https://doi.org/10.1016/j.esmoop.2024.103661

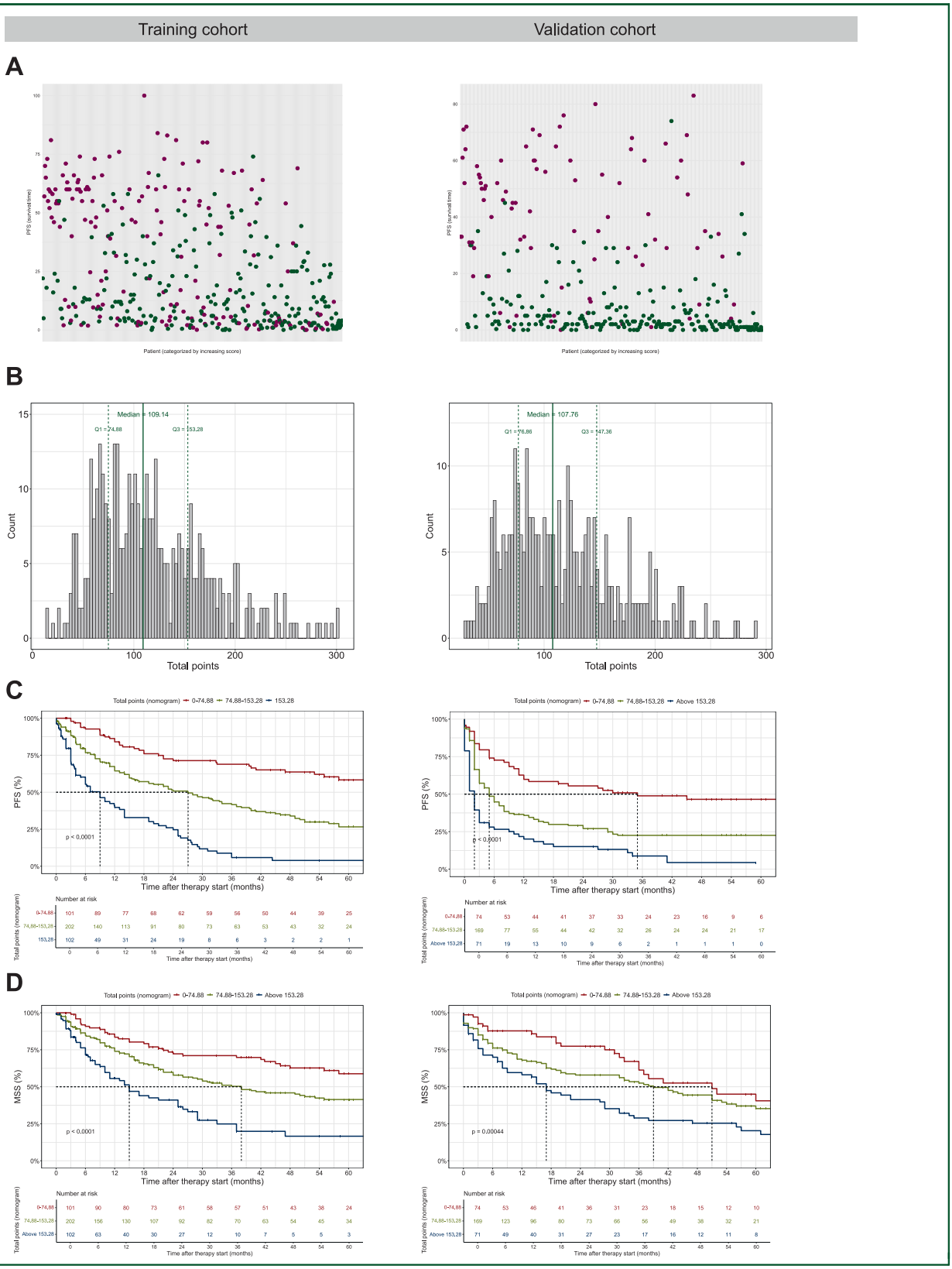


Figure 3. The score derived from the nomogram and the survival outcomes. (A) Plots that show the distribution of the score in the patients in the two cohorts. Green represents the patients who did develop progression after anti-PD-1-based therapy and burgundy dots represent patients who did not progress. (B) Plot showing the distribution, the median, the lower, and the upper quartile of the total points, calculated using the nomogram among patients in the two cohorts. Using the quartiles of the training cohort, three groups were created. (C) Kaplan—Meier curves showing the PFS of the three groups that were created using the quartiles of the total points in the training cohort in the training (left) and validation (right) cohorts. (D) Plot showing the MSS of the three groups defined by the total points of the nomogram in the training cohort for the training (left) and validation (right) cohorts.
MSS. melanoma-specific survival; PD-1, programmed cell death protein 1; PFS, progression-free survival.

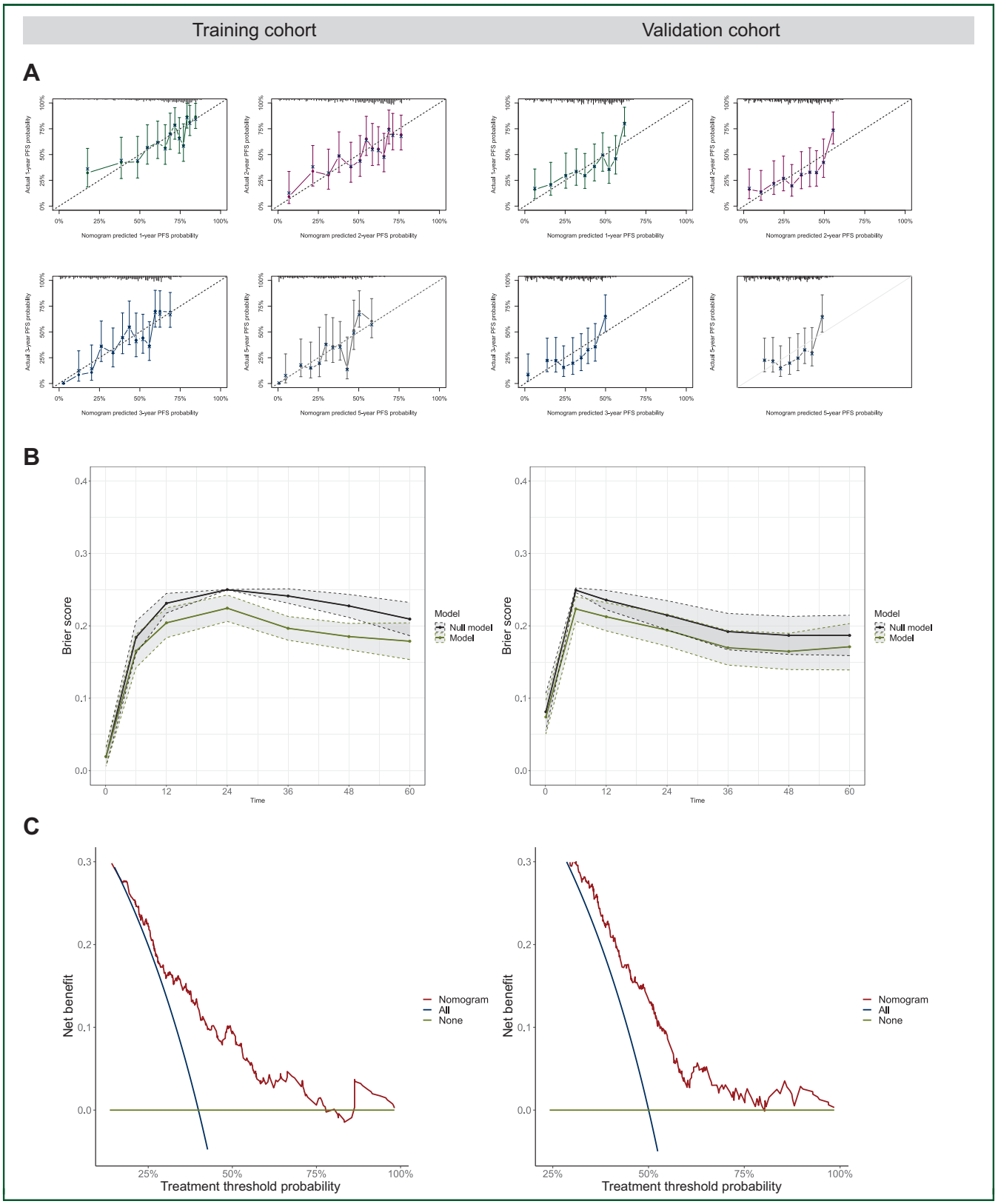


Figure 4. Performance metrics plots regarding progression-free survival and its clinical application. (A) Calibration curves of the model in the training (left) and validation (right) cohort. (B) Brier score in relation to time in months after therapy start for the training (left) and validation (right) cohort. (C) Decision curve analysis depicting the net clinical benefit of the model in the training (left) and validation (right) cohort.
PFS, progression-free survival.

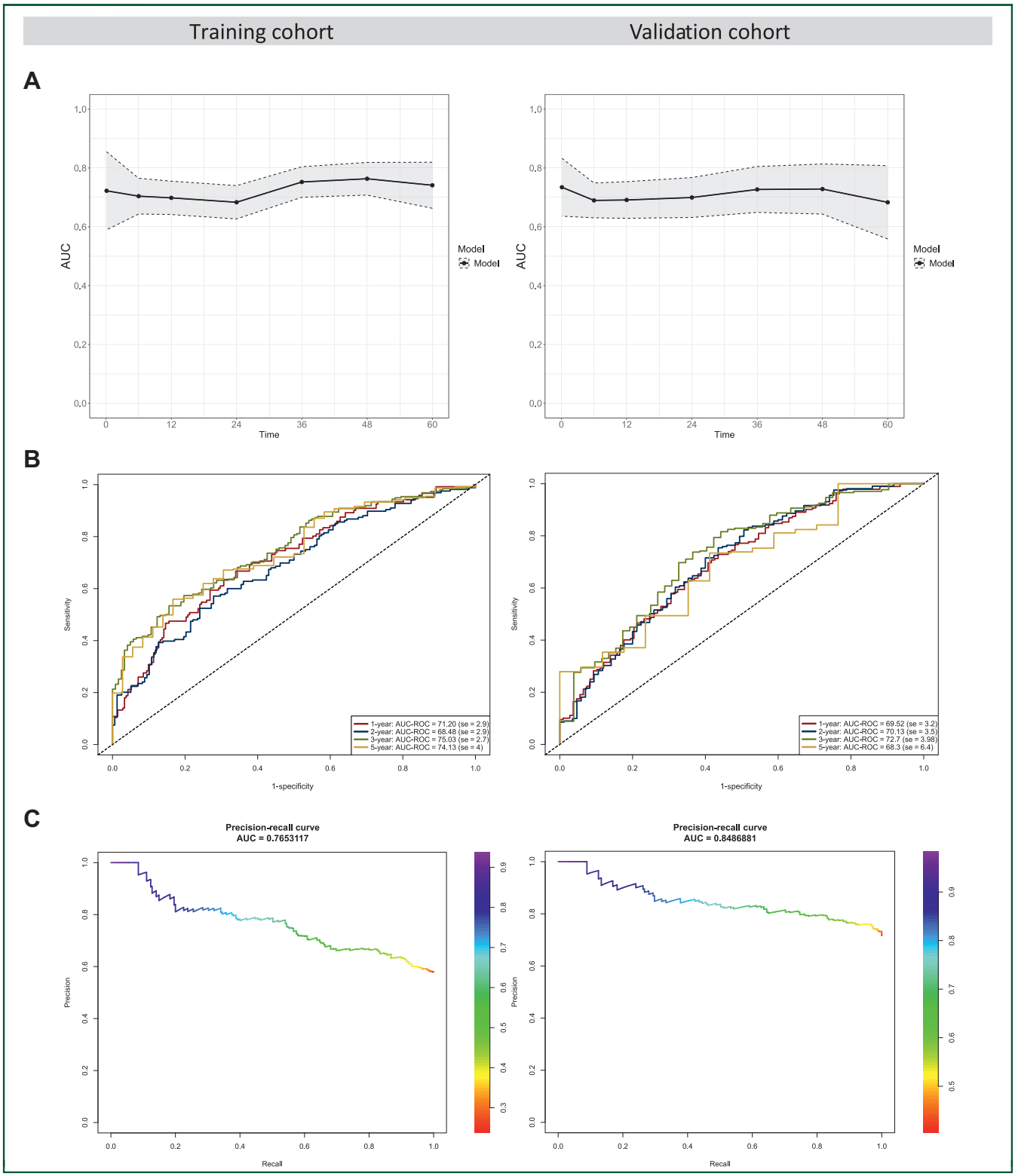


Figure 5. Performance metrics to predict risk of progression. (A) Area of the receiver operating characteristic curve (AU-ROC) in relation to time in months after therapy start for the training (left) and validation (right) cohorts. (B) ROC curves. (C) Precision and recall curves.

primarily used as a biomarker in daily clinical practice only in some countries, has demonstrated promise in risk prediction and should be integrated into nomograms.[23,27,37] Patients with brain metastasis exhibited worse outcomes compared to those without brain metastases, as reflected by the AJCC v8 classification, where presence of brain metastases is included in a separated substage M1d. The presence of hepatic metastases and the influence of hepatic microenvironment on immunotherapy resistance has also been demonstrated before.[24,26,38,39] Low BMI was

associated with worse outcomes, with underweight individuals showing poorer outcomes compared to those with normal or elevated BMI, similar to what other groups published before.[40,41]

Our work has significant strengths and one of the most important lies in its simplicity and user-friendly design.[42] The study underwent internal and external validation, yielding a nomogram tailored to the subgroup of stage IV patients undergoing first-line ICI. Handling of missing values through multiple imputations enhances its internal validity.

Given the ICI approval in 2015, our choice of PFS as the primary endpoint aligns with the clinical context. Our cohort of 719 melanoma patients from diverse European centers, and the long median follow-up of >50 months, contribute to the study's robustness. The decision to use upper and lower cut-offs for continuous variables (LDH, NLR, S100) aimed to reduce the impact of extreme values and enhance applicability.

This study does have limitations, including its retrospective nature. While the nomogram can be used as a predictive tool, it should not be misinterpreted that ICI lacks efficacy in patients with higher scores. The nomogram reflects only the features upon therapy initiation and does not consider the dynamics of biomarkers.[40,43,44] We included the LDH, S100, and NLR as continuous variables associated with survival; however, the exact relationship is more complex than linear. Higher pathological (above cut-off) values are correlated with worse outcomes. However, the absolute values of those with normal values (below cut-off) seem to be associated with different outcomes. Despite this, the overall score remains the critical determinant in prognosis. In the training cohort, the number of patients with *BRAF* mutation was lower than expected. Individuals with *BRAF* mutation and unfavorable baseline characteristics were most likely directed toward first-line targeted therapy at this time. Despite this, the nomogram did not identify a predictive role for *BRAF* mutation. Moreover, the training cohort had a significant number of acral melanoma patients. Partly, this relates to the high number of patients treated in Tuebingen. Acral melanomas, requiring specialized surgical treatment, are typically managed in experienced centers, where patients then remain in follow-up. Our model, shown from calibration plots in the validation cohort, predicted more favorable results than observed. The primary recruitment from reference centers across Europe may introduce bias.

Conclusions

We identified survival predictors in stage IV melanoma patients upon first-line anti-PD-1-based immunotherapy initiation and established a web-based nomogram requiring the input of eight features upon therapy initiation.

ACKNOWLEDGEMENTS

We thank all patients who consented to the analysis of their data and all colleagues involved in the patients' care.

FUNDING

None declared.

DISCLOSURE

TA reports personal honoraria from BMS, CeCaVa, Novartis, and Pierre-Fabre; institutional financial support from iFIT, NeraCare, Novartis, Sanofi, and SkylineDX, and institutional research grant from Novartis, outside the submitted work. AF served as consultant to Roche, Novartis, MSD, BMS, and Pierre-Fabre; received travel support from Roche, Novartis, BMS, and Pierre-Fabre; received speaker fees from Roche, Novartis, BMS, MSD, and CeGaT. She reports institutional research grants from BMS Stiftung Immunonkologie. LF reports grants or contracts from Hookipa Pharma, SAKK/Immunophotonics, DFG Grant (Deutsche Forschungsgemeinschaft), Philogen, and Mundipharma; consulting fees from Philogen, Sanofi, Novartis, and BMS; participation on Data Safety Board University of Basel and stocks or stock options from Hookipa Pharma, outside the submitted work. CG reports personal fees from Amgen; personal fees from MSD; grants and personal fees from Novartis; grants and personal fees from NeraCare; grants and personal fees from BMS; personal fees from Philogen; grants and personal fees from Roche; grants and personal fees from Sanofi, outside the submitted work. UL reports research support from MSD; consulting fees and honoraria from Sun Pharma, Sanofi (personal and institutional), MSD (personal and institutional), Novartis, Roche, Almirall Hermal; support for attending meeting from Sun Pharma and participation on a data safety monitoring board or advisory board from Sun Pharma, Sanofi, MSD, Novartis, Roche, Almirall Hermal, outside the submitted work. All other authors have declared no conflicts of interest.

DATA SHARING

Data from this research study will be made available to qualified researchers upon request.

REFERENCES

1. Jenkins RW, Fisher DE. Treatment of advanced melanoma in 2020 and beyond. *J Invest Dermatol*. 2021;141(1):23-31.
2. Tawbi HA, Schadendorf D, Lipson EJ, et al. Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. *N Engl J Med*. 2022;386(1):24-34.
3. Atkins MB, Julian C, Secrest MH, Lee J, Abajo-Guijarro AM, McKenna E. Real-world treatment patterns and overall survival in BRAF-mutant melanoma patients treated with immunotherapy or targeted therapy. *Future Oncol*. 2022;18(18):2233-2245.
4. Ascierto PA, Mandalà M, Ferrucci PF, et al. Sequencing of ipilimumab plus nivolumab and encorafenib plus binimetinib for untreated BRAF-mutated metastatic melanoma (SECOMBIT): a randomized, three-arm, open-label phase II trial. *J Clin Oncol*. 2023;41(2):212-221.
5. Atkins MB, Lee SJ, Chmielowski B, et al. Combination dabrafenib and trametinib versus combination nivolumab and ipilimumab for patients with advanced BRAF-mutant melanoma: the DREAMseq trial—ECOG-ACRIN EA6134. *J Clin Oncol*. 2023;41(2):186-197.
6. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med*. 2019;381(16):1535-1546.

7. Robert C, Ribas A, Schachter J, et al. Pembrolizumab versus ipilimumab in advanced melanoma (KEYNOTE-006): post-hoc 5-year results from an open-label, multicentre, randomised, controlled, phase 3 study. *Lancet Oncol*. 2019;20(9):1239-1251.
8. Johnson DB, Nebhan CA, Moslehi JJ, Balko JM. Immune-checkpoint inhibitors: long-term implications of toxicity. *Nat Rev Clin Oncol*. 2022;19(4):254-267.
9. Douglass J, Hsiue EH, Mog BJ, et al. Bispecific antibodies targeting mutant RAS neoantigens. *Sci Immunol*. 2021;6(57):eabd5515.
10. Schwartz LH, Litière S, de Vries E, et al. RECIST 1.1-Update and clarification: from the RECIST committee. *Eur J Cancer*. 2016;62:132-137.
11. Chatziioannou E, Leiter U, Thomas I, et al. Features and long-term outcomes of stage IV melanoma patients achieving complete response under anti-PD-1-based immunotherapy. *Am J Clin Dermatol*. 2023;24(3):453-467.
12. Robert C, Long GV, Larkin J, et al. 1082MO 5-year characterization of complete responses in patients with advanced melanoma who received nivolumab plus ipilimumab (NIVO+IPI) or NIVO alone. *Ann Oncol*. 2020;31:S734-S735.
13. Robert C, Ribas A, Hamid O, et al. Durable complete response after discontinuation of pembrolizumab in patients with metastatic melanoma. *J Clin Oncol*. 2018;36(17):1668-1674.
14. Brenner E, Schörg BF, Ahmetlić F, et al. Cancer immune control needs senescence induction by interferon-dependent cell cycle regulator pathways in tumours. *Nat Commun*. 2020;11(1):1335.
15. Keung EZ, Gershenwald JE. The eighth edition American Joint Committee on Cancer (AJCC) melanoma staging system: implications for melanoma treatment and care. *Expert Rev Anticancer Ther*. 2018;18(8):775-784.
16. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228-247.
17. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med*. 2015;373(1):23-34.
18. Ishwaran H, Kogalur U, Blackstone E, Lauer M. Random survival forests. *Ann Appl Stat*. 2008;2:841-860.
19. Pickett KL, Suresh K, Campbell KR, Davis S, Juarez-Colunga E. Random survival forests for dynamic predictions of a time-to-event outcome using a longitudinal biomarker. *BMC Med Res Methodol*. 2021;21(1):216.
20. Qiu X, Gao J, Yang J, et al. A comparison study of machine learning (random survival forest) and classic statistic (Cox proportional hazards) for predicting progression in high-grade glioma after proton and carbon ion radiotherapy. *Front Oncol*. 2020;10:551420.
21. Grant SW, Hickey GL, Head SJ. Statistical primer: multivariable regression considerations and pitfalls. *Eur J Cardiothorac Surg*. 2018;55(2):179-185.
22. Balachandran VP, Gonen M, Smith JJ, DeMatteo RP. Nomograms in oncology: more than meets the eye. *Lancet Oncol*. 2015;16(4):e173-e180.
23. Wagner NB, Forschner A, Leiter U, Garbe C, Eigentler TK. S100B and LDH as early prognostic markers for response and overall survival in melanoma patients treated with anti-PD-1 or combined anti-PD-1 plus anti-CTLA-4 antibodies. *Br J Cancer*. 2018;119(3):339-346.
24. Pires da Silva I, Lo S, Quek C, et al. Site-specific response patterns, pseudoprogression, and acquired resistance in patients with melanoma treated with ipilimumab combined with anti-PD-1 therapy. *Cancer*. 2020;126(1):86-97.
25. Conforti F, Pala L, Bagnardi V, et al. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. *Lancet Oncol*. 2018;19(6):737-746.
26. Dercle L, Ammari S, Roblin E, et al. High serum LDH and liver metastases are the dominant predictors of primary cancer resistance to anti-PD(L)1 immunotherapy. *Eur J Cancer*. 2022;177:80-93.
27. Weide B, Elsässer M, Büttner P, et al. Serum markers lactate dehydrogenase and S100B predict independently disease outcome in melanoma patients with distant metastasis. *Br J Cancer*. 2012;107(3):422-428.
28. Xu J, Zhao J, Wang J, Sun C, Zhu X. Prognostic value of lactate dehydrogenase for melanoma patients receiving anti-PD-1/PD-L1 therapy: a meta-analysis. *Medicine*. 2021;100(14):e25318.
29. Berner F, Bomze D, Diem S, et al. Association of checkpoint inhibitor-induced toxic effects with shared cancer and tissue antigens in non-small cell lung cancer. *JAMA Oncol*. 2019;5(7):1043-1047.
30. Diem S, Kasenda B, Spain L, et al. Serum lactate dehydrogenase as an early marker for outcome in patients treated with anti-PD-1 therapy in metastatic melanoma. *Br J Cancer*. 2016;114(3):256-261.
31. Claps G, Faouzi S, Quidville V, et al. The multiple roles of LDH in cancer. *Nat Rev Clin Oncol*. 2022;19(12):749-762.
32. Qi Y, Zhang Y, Fu X, et al. Platelet-to-lymphocyte ratio in peripheral blood: a novel independent prognostic factor in patients with melanoma. *Int Immunopharmacol*. 2018;56:143-147.
33. Zhou X, Du Y, Huang Z, et al. Prognostic value of PLR in various cancers: a meta-analysis. *PLoS ONE*. 2014;9(6):e101119.
34. Hedrick CC, Malanchi I. Neutrophils in cancer: heterogeneous and multifaceted. *Nat Rev Immunol*. 2022;22(3):173-187.
35. Cupp MA, Cariolou M, Tzoulaki I, Aune D, Evangelou E, Berlanga-Taylor AJ. Neutrophil to lymphocyte ratio and cancer prognosis: an umbrella review of systematic reviews and meta-analyses of observational studies. *BMC Med*. 2020;18(1):360.
36. Howard R, Kanetsky PA, Egan KM. Exploring the prognostic value of the neutrophil-to-lymphocyte ratio in cancer. *Sci Rep*. 2019;9(1):19673.
37. Sviatoha V, Tani E, Kleina R, Sperga M, Skoog L. Immunohistochemical analysis of the S100A1, S100B, CD44 and Bcl-2 antigens and the rate of cell proliferation assessed by Ki-67 antibody in benign and malignant melanocytic tumours. *Melanoma Res*. 2010;20(2):118-125.
38. Antoranz A, Van Herck Y, Bolognesi MM, et al. Mapping the immune landscape in metastatic melanoma reveals localized cell-cell interactions that predict immunotherapy response. *Cancer Res*. 2022;82(18):3275-3290.
39. Lee JC, Green MD, Huppert LA, Chow C, Pierce RH, Daud AI. The liver-immunity nexus and cancer immunotherapy. *Clin Cancer Res*. 2022;28(1):5-12.
40. McQuade JL, Daniel CR, Hess KR, et al. Association of body-mass index and outcomes in patients with metastatic melanoma treated with targeted therapy, immunotherapy, or chemotherapy: a retrospective, multicohort analysis. *Lancet Oncol*. 2018;19(3):310-322.
41. Yoo S-K, Chowell D, Valero C, Morris LGT, Chan TA. Outcomes among patients with or without obesity and with cancer following treatment with immune checkpoint blockade. *JAMA Network Open*. 2022;5(2):e220448.
42. Jalali A, Alvarez-Iglesias A, Roshan D, Newell J. Visualising statistical models using dynamic nomograms. *PLoS One*. 2019;14(11):e0225253.
43. Guida M, Bartolomeo N, Quaresmini D, et al. Basal and one-month differed neutrophil, lymphocyte and platelet values and their ratios strongly predict the efficacy of checkpoint inhibitors immunotherapy in patients with advanced BRAF wild-type melanoma. *J Transl Med*. 2022;20(1):159.
44. Kim J-Y, Jung EJ, Kim J-M, et al. Dynamic changes of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio predicts breast cancer prognosis. *BMC Cancer*. 2020;20(1):1206.

3. Discussion

The therapeutic landscape of unresectable stage IV melanoma has been transformed with the use of ICI, particularly those targeting the PD-1/PDL1 pathway. While these therapies have demonstrated durable and deep responses and significantly prolonged PFS and OS in a subset of patients, approximately 40-60%, prediction of response remains an unmet need, considering the fact that up to 50% do not respond to these therapies (Czarnecka et al. 2025; A. M. M. Eggermont et al. 2020; Hamid et al. 2021; Jason J. Luke et al. 2024; Robert et al. 2019). In these previous two works, therefore, we try to address this need by focusing on baseline clinical and laboratory parameters to predict survival after first-line ICI and on the long-term follow-up of complete responders, while also assessing biomarkers and characteristics related to them (Hamid et al. 2021; James Larkin et al. 2019).

Both of our studies emphasize the imperative for standardized, personalized, evidence-based decision algorithms in clinical practice that are not only clinically relevant but also biologically grounded. While baseline pre-therapy biomarkers offer the advantage of early prediction, preceding even radiological assessments, our work also investigates the dynamic longitudinal evolution of these markers during treatment, capturing shifts that further enhance prognostic assessment and therapeutic guidance, rendering them useful for monitoring of disease relapse and minimal residual disease.

Our investigation into PFS using a clinically integrated nomogram highlights the value of combining routine clinical and laboratory data into personalized prognostic tools. While the model’s predictive power is moderate, it marks an important step toward individualized care in advanced melanoma, as the M category of the AJCC does not accurately predict survival outcomes. Key variables like type of melanoma, high S100 protein, and a high NLR are not only indicators of tumor burden, but also reflections of an immune-resistant tumor microenvironment. These findings reinforce the need for risk stratification models in metastatic

melanoma to move beyond anatomical staging of AJCC, which includes the distant organ and the LDH, and incorporate broader systemic immune and metabolic biomarkers. For example, other molecular signatures like copy number alterations and IFN signatures should also be incorporated (Gershenwald and Scolyer 2018; Claps et al. 2022; Dercle et al. 2022; Costantini et al. 2025; Dai et al. 2024; Grasso et al. 2020).

While nomograms based only on baseline data offer valuable early prognostic insights, their static nature may limit adaptability as therapeutic options evolve. With the advent of new immunotherapeutic options, including also the anti-LAG-3 antibodies and evolving localized therapies or combination regimens, it is uncertain whether the original predictive markers will remain valid. Future prognostic models using machine learning approaches can model many different parameters (Gide et al. 2023; Reschke, Enk, and Hassel 2025; Lei et al. 2022).

Analyzing complete responders is important to understand not only the clinical aspects and their characteristics, but also the biology behind this. The remarkable durability of CR, even after stopping IT, suggests a stable immunologic equilibrium rather than simple tumor eradication as compared to previous therapies like conventional chemotherapy and TT, where resistance appears after CR. Long-term survival outcomes indicate that many of these patients maintain DC over the years, and when relapses occur, they tend to be delayed and often respond again to immune rechallenge. This pattern implies that persistent immune surveillance, mainly through IFN signalling, continues even in the absence of ongoing therapy, while defects in these pathways have not been acquired (Davies 2020; A. Betof Warner et al. 2020; Gauci et al. 2019; Boutros et al. 2023; Robert et al. 2013). Complete responders can also be associated with the fact that some cells, on the one hand, can be killed through CD8 cells, but on the other hand, some could become senescent, where these senescent cells are highly immunogenic and maintain immune surveillance. However, in some cases, chronic senescence can promote tumor relapse (Heidi Braumüller et al. 2013b; Brenner et al. 2020; Milanovic et al. 2018; Eyles et al. 2010; N. Müller-Hermelink et al. 2008a; Patton et al. 2021; Pollard 2016; Singvogel and Schittek 2024; Sosa, Bragado, and Aguirre-Ghiso 2014).

The observation that patients who achieve CR within the first three months of therapy rarely experience progression suggests that the timing of CR itself may carry prognostic significance. This is important so that we reconsider treatment duration, raising the possibility that an early CR could serve as a clinical signal for discontinuing therapy earlier than previously thought. Currently, clinical trials are assessing this by selective therapy discontinuation according to depth of response (Lodde et al. 2024; Ochenduszko et al. 2023). This is especially important for those developing high-grade toxicity.

Both studies raise an essential question, in patients with durable CR, is continuing therapy truly beneficial, or might it carry unnecessary risk of irAEs, which have been shown to be associated with increased psychological burden and healthcare burden. This optimal duration in patients, therefore, should be based on biomarkers and depth of response, with those having CR to be the first group to be investigated. In these individuals, extending IT may confer diminishing therapeutic benefit, while increasing exposure to late immune-related toxicities, especially cutaneous and endocrine, although also life-threatening toxicities, prolonged healthcare use, and psychological stress. Reports of late-onset adverse events, often occurring many months into treatment, stress the need to re-evaluate therapy duration in terms of both long-term efficacy and overall patient well-being (R.S. Goodman et al. 2023; Owen et al. 2021; Asher et al. 2021; Rubatto et al. 2023).

Recent data suggest that the goal in IT should not be prolonged exposure when no further benefit can be given. This is particularly important in the context of IT, where we observe the concepts of immune surveillance, equilibrium, and senescence as the minimal residual disease can remain dormant in a dynamic nature by the effects of the immune system. In this context, the dormant and senescent cells also favor antitumor inflammation, and further use of ICI does not make biological sense. Discontinuing therapy after a sustained CR is increasingly seen as a strategic, biomarker-guided decision, rather than one driven by toxicity. Trials such as DANTE and Safe Stop are now testing response-based discontinuation strategies using tools like early imaging and ctDNA. Studies show that many patients who stop anti-PD-1 IT after 6-12 months of CR maintain durable

remission, with low relapse rates and successful rechallenge when needed (Marron et al. 2021). Also, modalities that can assess whether the tumors are hot or cold, or also those monitoring residual disease, would be important to implement the strategy of earlier discontinuation of therapy (Amrane et al. 2019; Asher et al. 2021; Eyles et al. 2010).

These findings show how important the stratification of patients, early response evaluation, and informed decision-making are. Baseline clinical markers guide initial risk. Achieving a rapid CR and longitudinal measured biomarkers may give the opportunity for treatment de-escalation. Ongoing blood-based monitoring can further reveal relapse and minimal residual disease for therapy rechallenge, and progressions can be effectively treated with ICI rechallenge, exploiting the immune memory, or with TT exploiting MAPK dependence of *BRAF*-mutated melanoma (Coen et al. 2021; Lydia Warburton et al. 2023). In this context, for ICI rechallenge, it is important that no resistance has been acquired.

In light of all this, we need prospective validation of predictive tools in modern therapeutic landscape, given evolving treatments, molecular profiling of complete responders to uncover their immune, epigenomic and genomic features, health economic analyses to assess the cost-effectiveness of response-guided treatment de-escalation, and real-world guidelines that can help clinicians to make informed decisions regarding patient therapy as well as to understand the biology related to dormancy, senescence, immune equilibrium and minimal residual disease (Cartun et al. 2022; Felicity Newell, Pires da Silva, et al. 2022; Lim et al. 2023).

Publication No 1:

In this study, we retrospectively investigated a cohort of unresectable stage III/IV melanoma patients who achieved a CR as BOR following first-line anti-PD-1-based IT. The central aim was to investigate the clinical characteristics associated with CR, assess long-term outcomes in terms of survival after achieving CR and after therapy discontinuation, and explore the role of blood and clinical biomarkers in response durability. Longitudinal monitoring of certain biomarkers and comparison of the characteristics of CR with those not achieving CR was made. This analysis contributes to growing evidence suggesting that CR represents a biologically distinct and prognostically favorable subset within the broader population of metastatic melanoma patients that underwent therapy with ICI. CR is a surrogate marker for OS.

Our findings demonstrate that CR is associated with prolonged long-term survival outcomes, even after therapy cessation. The five-year PFS and OS rates of 79% and 83%, respectively, in CR patients underscore the potential for durable immune-mediated tumor control (Asher et al. 2021; Boutros et al. 2023). The fact that responses are durable after therapy cessation shows the immune equilibrium.
Importantly, neither the total duration of therapy nor the treatment duration after CR correlated significantly with improved survival, supporting the hypothesis that, for selected patients, prolonged treatment may not be necessary once a deep response has been achieved.

These observations raise important questions about the biological nature of CR. While CR, as defined by radiologic criteria, has traditionally been interpreted as an endpoint, our data suggests an immune equilibrium. As they also responded to rechallenge, these findings imply that the immune system retains some degree of capacity to re-engage tumor control (Eyles et al. 2010; N. Müller-Hermelink et al. 2008a; Patton et al. 2021; Pollard 2016; Singvogel and Schittek 2024; Sosa, Bragado, and Aguirre-Ghiso 2014).

The dynamics of blood-based biomarkers further support the utility of longitudinal monitoring, especially in those patients for whom therapy can be discontinued

(Burkey et al. 2025; Y. Guo, Xiang, et al. 2022). Normalization of S100 protein levels at the time of CR was a consistent finding, and re-elevation often preceded radiologic progression. Therefore, S100 can be used to monitor relapse and help in discontinuation of therapy. Disease monitoring is now with ctDNA in many tumors (Burkey et al. 2025; Schroeder et al. 2024; L. Warburton et al. 2020; Lydia Warburton et al. 2023).
Similarly, trends in LDH, NLR, and PLR appeared to reflect shifts in immune activity. These markers, particularly when evaluated dynamically, may serve as early indicators of relapse and help guide surveillance or the need for therapeutic reinitiation.

Among baseline characteristics, age, lower LDH and NLR levels, and the absence of liver metastases were significantly associated with achieving CR (Y. Guo, Xiang, et al. 2022; Li et al. 2022; Jialin Su et al. 2025; X. Zhou et al. 2014). The association with age is particularly intriguing, as older individuals have previously been observed to respond better to ICIs, possibly due to age-related changes in immune regulation, such as diminished T regulatory cell activity or increased TMB. However, among CR patients, those aged over 77 years had worse outcomes than those below, suggesting a complex relationship between immune senescence and response durability that needs further study (Wong, Nebhan, and Johnson 2021).

Of particular concern is the occurrence of late-onset irAEs, observed in 25% of CR patients, most commonly involving cutaneous, endocrine, or hepatic manifestations. These findings emphasize the importance of continued post-treatment surveillance not only for disease recurrence but also for delayed toxicity. As IT transitions toward more personalized and de-escalated paradigms, the ability to predict and prevent chronic irAEs will become increasingly important (Bastacky et al. 2021; Casagrande et al. 2024; Martins et al. 2019; Zhu et al. 2020; Fiamma Berner et al. 2019; F. Berner and Flatz 2023).
These irAEs might not only reflect toxicity but also signal robust immune activation and senescence, which could explain their association with improved treatment outcomes (Q. Sun et al. 2022; Karhapää et al. 2022). The management of these toxicities could be a challenge in order not to lose this equilibrium.

From the patient's perspective, psychosocial factors, including fear of stopping therapy or concerns about disease recurrence, can influence adherence to monitoring plans and must be addressed in future models. Research on the health economic burden is also needed to determine the value of early therapy discontinuation, balancing cost benefit with quality-adjusted survival outcomes, as further benefit with ICI may not be observed (Kasparian, McLoone, and Butow 2009; Zhang et al. 2023).

Recent data increasingly highlights the significance of T cell clonality and the persistence of the immune repertoire as key indicators of long-term tumor control. Sequencing of T cell receptors in blood or tumor tissue may help identify patients who maintain a durable response and immune surveillance, thereby supporting the rationale for discontinuing ICI safely after achieving a CR (Huuhtanen et al. 2022; Valpione et al. 2021; Porciello et al. 2022). TIL in metastases, therefore, hot tumors respond better to ICI (Eftychia Chatziioannou, Roßner, Aung, Rimm, Niessner, Keim, Serna-Higuita, Bonzheim, Cuellar, et al. 2023; Han et al. 2021).

Another area is the gut microbiome's influence on the immune system. Differences in microbial composition and the presence of specific bacterial species have been linked to both better response to ICI and increased susceptibility to immune-mediated colitis. Ongoing studies are expected to determine whether microbiome-based markers can serve as additional biomarkers (Hwang, Kim, and Kweon 2023; K.A. Lee et al. 2022; Björk et al. 2024).

Understanding the mechanisms behind immune escape, resistance, and tumor dormancy and senescence is essential to explain late relapses after CR. Chronic inflammation or senescence can, on the other hand, promote cancer. Alterations in genes associated with the IFN signalling pathway, the cell cycle, or tumor evasion via antigen loss and MHC downregulation may increase the risk for recurrence. Studying how immune memory controls dormant cells could make the surveillance timing and reinitiation of therapy better (Kawashima and Togashi 2023; Mengoni et al. 2025; Singvogel and Schittek 2024; Lim et al. 2023; Alsaafeen, Ali, and Elkord 2025; J.H. Lee, Shklovskaya, et al. 2020).

Sex and hormonal influences on outcomes of ICI remain uncertain. Estrogen, androgen levels, or menopausal status may impact immune responses, CR rates, and irAE patterns. Stratifying data by sex and hormonal context could improve biomarker discovery and treatment personalization (Petersen et al. 2024; Capone et al. 2018; Ma et al. 2022; Bhattacharya, Sadhukhan, and Saraswathy 2024).

Incorporating patient-reported outcomes and digital tools into care models can capture real-time quality-of-life data, enhancing toxicity management and guiding shared decisions, especially during treatment de-escalation (Basch, Mody, and Dueck 2020; Tolstrup et al. 2022).

Refining treatment sequencing is critical. Patients likely to respond to PD-1 monotherapy could avoid upfront combination regimens, while high-risk individuals may benefit from early intensification, combinatorial therapies, or closer follow-up. Predictive tools like our nomogram can help tailor these decisions, intensify monitoring (James Larkin et al. 2019; Z. Wang et al. 2024).
In addition, the application of artificial intelligence in radiomics offers a novel way to assess response durability. Advanced image analysis techniques can extract and quantify features such as tumor heterogeneity, shape, and density, traits that may correspond with biological behavior and immune status, minimal residual disease, etc. Detection of TILs or senescent cells could also be investigated. When integrated with molecular and clinical data, AI-driven radiomic models could provide a more comprehensive and individualized approach to estimating treatment needs and optimizing therapy duration beyond conventional metrics (Kothari 2022; Roisman et al. 2023; Ligero et al. 2024; McGale et al. 2023).

Moving forward, we propose that future research should focus on prospective trials assessing individualized treatment duration based on depth of response and biomarker dynamics. Additionally, efforts should be directed toward identifying molecular and immunologic correlates of CR, including TIL profiles, spatial immune architecture, and ctDNA kinetics. Understanding the biological basis of CR may ultimately enable us to convert responses into durable remission for a broader population (Burkey et al. 2025; E. Chatziioannou, Roßner, Aung, Rimm, Niessner, Keim, Serna-Higuita, Bonzheim, Kuhn Cuellar, et al. 2023; Eliason et al. 2025).

Publication No 2:

In the second work, following the previous work, we aimed to enhance the personalization of melanoma treatment by making a better biomarker. Therefore, we developed and externally validated a predictive nomogram for PFS in patients with unresectable stage IV melanoma being treated with first-line anti-PD-1-based IT. Recognizing that a substantial fraction of patients, up to 50%, fail to achieve long-term benefit from ICIs, we sought to identify baseline variables that could reliably predict therapeutic outcomes and aid in risk stratification at therapy initiation.

Our model was derived from a multicenter European cohort and incorporated eight clinically and laboratory accessible variables: LDH, NLR, melanoma subtype, serum S100 protein, BMI, presence of brain and liver metastases, and type of ICI therapy, whether monotherapy or combination. These variables were selected to be included in the nomogram following a selection process using first a random survival forest model and then Cox regression analysis.

LDH emerged as the most important prognostic factor, consistent with its role as a surrogate for tumor burden and hypoxic tumor microenvironments that favor immune evasion. It is also incorporated in the AJCC classification. NLR and S100, both systemic markers of inflammation and tumor activity, further enhanced the model's ability to discriminate between low- and high-risk patients. The inclusion of BMI is noteworthy, as low BMI has been associated with diminished treatment efficacy, potentially reflecting compromised nutritional or immunologic reserves (Petrelli et al. 2019; Wagner et al. 2018; Li et al. 2022; Richtig et al. 2018). The question here lies in whether BMI or muscle mass is the prognostic determinant.

The nomogram demonstrated moderate discriminatory capacity with a C-index of 0.67 and favorable calibration in the training and validation cohorts, although lower in the latter. Its capacity to stratify patients into discrete risk groups with significantly different PFS outcomes enables more informed clinical decision-making regarding treatment intensity, surveillance strategies, and early referral to clinical trials. Importantly, the model also facilitates shared decision-making with patients by providing individualized prognostic estimates.

Nonetheless, certain limitations must be acknowledged. First, it is a retrospective study. The model is based entirely on baseline characteristics and does not account for early treatment response or longitudinal biomarker changes. As such, its predictive utility may be enhanced by integration with dynamic variables such as early radiologic response, ctDNA kinetics, or on-treatment changes in systemic immune parameters. Furthermore, the generalizability of the model to patients treated with newer therapeutic combinations, such as anti-LAG-3 or TIL therapies, remains to be established (Burkey et al. 2025; Kreidieh and Tawbi 2023; Allison Betof Warner, Corrie, and Hamid 2023). We looked at clinical and blood biomarkers, and not at the tumor itself.

In addition to the clinical and biomarker findings from our studies, the study of the tumor itself, as well as tumor circulating cells, is important, as several future research directions could help advance the personalization of IT in advanced melanoma. Spatial transcriptomics and single-cell RNA sequencing can reveal the cellular composition and organization of the tumor microenvironment, immune cell interactions, and spatial immune signatures associated with long-lasting responses. Neoantigen profiling and genomic tracking of tumor evolution also hold promise for understanding immune escape and long-term DC. Patients with a fewer immune-evasive genomic alterations or not defects in IFN signalling may be good candidates for early treatment discontinuation without compromising outcome (Abbott et al. 2021; Alsaafeen, Ali, and Elkord 2025; Teresa Amaral et al. 2020; Barriga et al. 2022; Eliason et al. 2025; Falahat et al. 2019; Grasso et al. 2020; Han et al. 2021; Kawashima and Togashi 2023; Kreidieh and Tawbi 2023; Larson et al. 2022; J.H. Lee, Shklovskaya, et al. 2020; D. Liu et al. 2019; S. Liu et al. 2023; Felicity Newell, Pires da Silva, et al. 2022; Sade-Feldman et al. 2017; Shin et al. 2017; Zaretsky et al. 2016).

The main concept from our melanoma model, baseline risk stratification, early response assessment, and informed treatment adjustment, could inform similar strategies in other IT-treated cancers, including renal cell cancer and lung cancer.

Looking ahead, we believe that a prospective validation of this tool in cohorts and for its integration into clinical trials aimed at treatment personalization is important. Its advantage lies in its easy application. Its application may be broadened to include decision-support algorithms for early treatment modification, such as escalation or de-escalation based on predicted response. Finally, the model provides a foundation for incorporating emerging molecular and digital pathology data into more predictive models.

4. Summary

This research explores two critical aspects of systemic IT in unresectable stage IV melanoma patients who underwent treatment with first-line anti-PD-1-based agents. The central goals were, first, to assess the clinical outcomes of response and survival as well as the characteristics of patients achieving a CR, and second, to develop a baseline risk model capable of predicting PFS prior to therapy initiation.

To address these aims, two retrospective analyses were conducted. One focused on a monocentric cohort of patients who achieved CR under ICI, with particular attention to treatment duration, biomarker trends, relapse patterns, and long-term survival after therapy cessation. The other involved the construction and validation of a nomogram using multicenter data from a large patient population, incorporating common clinical and laboratory parameters to estimate individualized PFS probabilities.

The findings revealed that CR is strongly associated with excellent long-term survival outcomes, with five-year PFS and OS rates exceeding 75% in this subgroup. Importantly, treatment discontinuation after CR did not negatively impact outcomes, suggesting that indefinite therapy may not be necessary for all patients and giving insight into the biology behind CR in favor of immune equilibrium, dormancy, and senescence. Predictive features of CR included age, low LDH and PLR, and absence of liver metastases. Dynamic blood markers such as S100 and LDH proved useful in monitoring remission status and early signs of relapse, and therefore for minimal residual disease (Khoury et al. 2021; Lodde et al. 2024; Perez, Samlowski, and Lopez-Flores 2022; Robert et al. 2018; Warner et al. 2020; Eyles et al. 2010; N. Müller-Hermelink et al. 2008a; Pollard 2016; Singvogel and Schittek 2024; Sosa, Bragado, and Aguirre-Ghiso 2014).

In parallel, the PFS nomogram demonstrated that pretreatment factors such as blood inflammatory markers, BMI, metastasis sites, and melanoma subtype can stratify patients by risk with good accuracy. This tool provides a foundation for early prognostic guidance and could assist in personalizing treatment intensity, therapy duration, sequential and combinatorial regimen, and follow-up strategies.

The broader implications of these studies highlight a shift toward more individualized and biologically informed management of metastatic melanoma. Rather than defaulting to prolonged or indefinite IT, evidence from this work supports the potential for response-guided de-escalation in selected patients. The concept of CR as immune equilibrium, rather than absolute eradication, calls for further research into biomarkers that can define durable remission and guide retreatment when necessary (Reschke, Enk, and Hassel 2025).

In conclusion, this integrated research underscores the value of both predictive modeling and deep response characterization in optimizing IT for advanced melanoma. Future studies should validate these approaches prospectively and explore novel biomarkers that can refine risk stratification, treatment discontinuation protocols, and long-term disease monitoring in real-world clinical settings.

5. German summary -Zusammenfassung

Diese Arbeit befasst sich mit zwei komplementären Aspekten der Immuntherapie bei Patienten mit einem nicht resezierbaren Melanom im Stadium IV, die mit einer anti-PD-1-basierten Behandlung therapiert wurden. Ziel war es zum einen, die langfristigen klinischen Verläufe und Merkmale von Patienten zu untersuchen, die ein komplettes Ansprechen (Complete Response) unter Immuncheckpoint-Inhibition erreichen, und zum anderen ein prädiktives Modell zu entwickeln, das anhand von Basisdaten vor Therapiebeginn das progressionsfreie Überleben prognostizieren kann.

Im ersten Teil der Untersuchung wurde eine retrospektive Analyse einer Kohorte von Patienten durchgeführt, die unter anti-PD-1-Therapie ein CR erreichten. Dabei wurden klinische Parameter, Blutbiomarker, wie LDH und S100, Dauer der Behandlung, Auftreten von Rückfällen und Langzeitüberleben analysiert. Die Ergebnisse zeigen, dass ein CR mit exzellentem Langzeitüberleben assoziiert ist: Das progressionsfreie und das Gesamtüberleben lagen nach fünf Jahren bei über 75 %. Bemerkenswerterweise hatte die Beendigung der Therapie nach CR keinen negativen Einfluss auf den Behandlungserfolg, was darauf hinweist, dass eine zeitlich begrenzte Therapie bei ausgewählten Patienten ausreichend sein kann.

Niedrige LDH- und PLR-Werte, das Fehlen von Lebermetastasen sowie ein höheres Alter zum Zeitpunkt der Therapie waren signifikant mit einem CR assoziiert (Asher et al. 2021; Bastacky et al. 2021; A. Betof Warner et al. 2020; Bhatia, Tykodi, and Thompson 2009; Boutros et al. 2023; Fahey, Gracie, and Johnson 2023; Kataoka and Hirano 2018; Khoury et al. 2021; Lodde et al. 2024; Marron et al. 2021; Mayer et al. 2025; Patnaik et al. 2021; Perez, Samlowski, and Lopez-Flores 2022; Robert et al. 2018; Robert et al. 2013; Sacco et al. 2024; Swami et al. 2019; Trojaniello et al. 2023; S.J. Wang, Dougan, and Dougan 2023; Warner et al. 2020; Eyles et al. 2010; Nele Müller-Hermelink et al. 2008b; Pollard 2016).

Im zweiten Teil wurde ein klinisch anwendbares Nomogramm entwickelt und in einer multizentrischen Kohorte validiert, um das individuelle Risiko eines Fortschreitens der Erkrankung unter ICI-Therapie vorherzusagen. Das Modell basiert auf leicht verfügbaren Basisparametern wie LDH, NLR, S100, Melanomsubtyp, BMI sowie der Metastasierung in Leber oder Gehirn. Trotz einer moderaten prädiktiven Genauigkeit stellt das Nomogramm ein praktikables Instrument zur Risikostratifizierung dar, das potenziell zur personalisierten Therapieplanung beitragen kann.

Die Diskussion der Ergebnisse legt nahe, dass ein CR weniger auf eine vollständige Tumoreradikation, sondern eher auf eine stabile Immunkontrolle hinweist. Die Reaktion auf erneute Immuntherapie bei Rückfällen stützt diese Hypothese zusätzlich. Zugleich wird die Notwendigkeit in Frage gestellt, Immuntherapien über Jahre hinweg fortzusetzen, wenn ein tiefes und frühes Ansprechen bereits erreicht ist. Eine Umorientierung hin zu einer biologisch adaptiven Therapiestrategie, bei der Therapieintensität und -dauer individuell angepasst werden, scheint nicht nur möglich, sondern auch medizinisch sinnvoll zu sein (Abbott et al. 2021; A. Betof Warner et al. 2020; Boutros et al. 2023; Fahey, Gracie, and Johnson 2023; Kataoka and Hirano 2018; Khoury et al. 2021; Lodde et al. 2024; Marron et al. 2021; Mayer et al. 2025; Patnaik et al. 2021; Perez, Samlowski, and Lopez-Flores 2022; Pillai et al. 2022; Robert et al. 2018; Robert et al. 2013).

Zusammenfassend unterstützt diese Arbeit einen Paradigmenwechsel in der Behandlung des metastasierten Melanoms: weg von standardisierten Langzeittherapien, hin zu einer intelligenten, datenbasierten und

patientenzentrierten Immuntherapie. Zukünftige Studien sollten sich auf prospektive Validierung, die Integration molekularer Biomarker und die Entwicklung dynamischer Entscheidungsalgorithmen konzentrieren, um eine nachhaltige und individualisierte Behandlung zu gewährleisten.

6. Bibliography / List of References

Abbott, C. W., S. M. Boyle, R. M. Pyke, et al. 2021. "Prediction of Immunotherapy Response in Melanoma through Combined Modeling of Neoantigen Burden and Immune-Related Resistance Mechanisms." *Clin Cancer Res* 27 (15): 4265-4276. https://doi.org/10.1158/1078-0432.Ccr-20-4314.

Agarwala, S. S. 2015. "Intralesional therapy for advanced melanoma: promise and limitation." *Curr Opin Oncol* 27 (2): 151-6. https://doi.org/10.1097/cco.0000000000000158.

Akbani, Rehan, Kadir C Akdemir, B. Arman Aksoy, et al. 2015. "Genomic Classification of Cutaneous Melanoma." *Cell* 161 (7): 1681-1696. https://doi.org/https://doi.org/10.1016/j.cell.2015.05.044. https://www.sciencedirect.com/science/article/pii/S0092867415006340.

Alsaafeen, Besan H., Bassam R. Ali, and Eyad Elkord. 2025. "Resistance mechanisms to immune checkpoint inhibitors: updated insights." *Molecular Cancer* 24 (1): 20. https://doi.org/10.1186/s12943-024-02212-7. https://doi.org/10.1186/s12943-024-02212-7.

Alves Costa Silva, Carolina, Francesco Facchinetti, Bertrand Routy, et al. 2020. "New pathways in immune stimulation: targeting OX40." *ESMO Open* 5 (1): e000573. https://doi.org/https://doi.org/10.1136/esmoopen-2019-000573. https://www.sciencedirect.com/science/article/pii/S2059702920300041.

Amaral, T., E. Chatziioannou, A. Nuebling, et al. 2024. "Identification of patients at high risk for relapse by Merlin Assay (CP-GEP) in an independent cohort of patients with melanoma who did not undergo sentinel lymph node biopsy: head and neck subgroup analysis." *EJC Skin Cancer* 2.

Amaral, Teresa, Eftychia Chatziioannou, Alica Nuebling, et al. 2025. "Risk stratification using the Merlin Assay (CP-GEP) in an independent cohort of 930 patients with clinical stage I/II melanoma who did not undergo sentinel lymph node biopsy." *European Journal of Cancer*: 115372. https://doi.org/https://doi.org/10.1016/j.ejca.2025.115372. https://www.sciencedirect.com/science/article/pii/S0959804925001534.

Amaral, Teresa, Lena Nanz, Rudolf Stadler, et al. 2024. "Isolated melanoma cells in sentinel lymph node in stage IIIA melanoma correlate with a favorable prognosis similar to stage IB." *European Journal of Cancer* 201: 113912.

Amaral, Teresa, Martin Schulze, Tobias Sinnberg, et al. 2020. "Are Pathogenic Germline Variants in Metastatic Melanoma Associated with Resistance to Combined Immunotherapy?" *Cancers* 12 (5): 1101-1101. https://doi.org/10.3390/CANCERS12051101. https://pmc.ncbi.nlm.nih.gov/articles/PMC7281129/.

Amrane, Karim, Delphine Le Goupil, Gilles Quere, et al. 2019. "Prediction of response to immune checkpoint inhibitor therapy using 18F-FDG PET/CT in patients with melanoma." *Medicine* 98 (29): e16417. https://doi.org/10.1097/md.0000000000016417. https://journals.lww.com/md-journal/fulltext/2019/07190/prediction_of_response_to_immune_checkpoint.26.aspx.

Andrews, M. C., G. Li, R. P. Graf, et al. 2024. "Predictive Impact of Tumor Mutational Burden on Real-World Outcomes of First-Line Immune Checkpoint Inhibition in Metastatic Melanoma." *JCO Precis Oncol* 8: e2300640. https://doi.org/10.1200/po.23.00640.

Antony, F. C., G. Sanclemente, H. Shaikh, et al. 2006. "Pigment synthesizing melanoma (so-called animal type melanoma): a clinicopathological study of 14 cases of a poorly known distinctive variant of melanoma." *Histopathology* 48 (6): 754-62. https://doi.org/10.1111/j.1365-2559.2006.02411.x.

Aoude, Lauren G., Antonia L. Pritchard, Carla Daniela Robles-Espinoza, et al. 2014. "Nonsense mutations in the shelterin complex genes ACD and TERF2IP in familial

melanoma." *Journal of the National Cancer Institute* 107 (2). https://doi.org/10.1093/JNCI/DJU408. https://pubmed.ncbi.nlm.nih.gov/25505254/.

Arnold, Melina, Deependra Singh, Mathieu Laversanne, et al. 2022. "Global Burden of Cutaneous Melanoma in 2020 and Projections to 2040." *JAMA Dermatology* 158 (5): 495-503. https://doi.org/10.1001/jamadermatol.2022.0160. https://doi.org/10.1001/jamadermatol.2022.0160.

Ascierto, P. A., G. V. Long, C. Robert, et al. 2019. "Survival Outcomes in Patients With Previously Untreated BRAF Wild-Type Advanced Melanoma Treated With Nivolumab Therapy: Three-Year Follow-up of a Randomized Phase 3 Trial." *JAMA Oncol* 5 (2): 187-194. https://doi.org/10.1001/jamaoncol.2018.4514.

Asher, N., N. Israeli-Weller, R. Shapira-Frommer, et al. 2021. "Immunotherapy Discontinuation in Metastatic Melanoma: Lessons from Real-Life Clinical Experience." *Cancers (Basel)* 13 (12). https://doi.org/10.3390/cancers13123074.

Atkins, M. B., J. Hsu, S. Lee, et al. 2008. "Phase III trial comparing concurrent biochemotherapy with cisplatin, vinblastine, dacarbazine, interleukin-2, and interferon alfa-2b with cisplatin, vinblastine, and dacarbazine alone in patients with metastatic malignant melanoma (E3695): a trial coordinated by the Eastern Cooperative Oncology Group." *J Clin Oncol* 26 (35): 5748-54. https://doi.org/10.1200/jco.2008.17.5448.

Atkins, M. B., A. Tarhini, M. Rael, et al. 2019. "Comparative efficacy of combination immunotherapy and targeted therapy in the treatment of BRAF-mutant advanced melanoma: a matching-adjusted indirect comparison." *Immunotherapy* 11 (7): 617-629. https://doi.org/10.2217/imt-2018-0208.

Awada, Gil, and Bart Neyns. 2022. "Melanoma with genetic alterations beyond the BRAFV600 mutation: management and new insights." *Current Opinion in Oncology* 34 (2): 115-122. https://doi.org/10.1097/cco.0000000000000817. https://journals.lww.com/co-oncology/fulltext/2022/03000/melanoma_with_genetic_alterations_beyond_the.2.aspx.

Bailey, Teresa A., John F. Thompson, and Richard A. Scolyer. 2020. "Desmoplastic Melanoma." In *Melanoma*, edited by David E. Fisher and Boris C. Bastian, 1-13. New York, NY: Springer New York.

Ball, Somedeb, Raktim K. Ghosh, Sariya Wongsaengsak, et al. 2019. "Cardiovascular Toxicities of Immune Checkpoint Inhibitors." *JACC* 74 (13): 1714-1727. https://doi.org/doi:10.1016/j.jacc.2019.07.079. https://www.jacc.org/doi/abs/10.1016/j.jacc.2019.07.079.

Baptista Freitas, Marta, Laurence Desmyter, Cindy Badoer, et al. 2024. "POT1 tumour predisposition: a broader spectrum of associated malignancies and proposal for additional screening program." *European Journal of Human Genetics 2024 32:8* 32 (8): 980-986. https://doi.org/10.1038/s41431-024-01611-0. https://www.nature.com/articles/s41431-024-01611-0.

Barreiro-Capurro, Alicia, Juan J. Andrés-Lencina, Sebastian Podlipnik, et al. 2021. "Differences in cutaneous melanoma survival between the 7th and 8th edition of the American Joint Committee on Cancer (AJCC). A multicentric population-based study." *European Journal of Cancer* 145: 29-37. https://doi.org/https://doi.org/10.1016/j.ejca.2020.11.036. https://www.sciencedirect.com/science/article/pii/S0959804920313733.

Barriga, Francisco M., Kaloyan M. Tsanov, Yu-Jui Ho, et al. 2022. "MACHETE identifies interferon-encompassing chromosome 9p21.3 deletions as mediators of immune evasion and metastasis." *Nature Cancer* 3 (11): 1367-1385. https://doi.org/10.1038/s43018-022-00443-5. https://doi.org/10.1038/s43018-022-00443-5.

Barroso-Sousa, Romualdo, William T. Barry, Ana C. Garrido-Castro, et al. 2018. "Incidence of Endocrine Dysfunction Following the Use of Different Immune Checkpoint Inhibitor Regimens: A Systematic Review and Meta-analysis." *JAMA Oncology* 4 (2): 173-182.

https://doi.org/10.1001/jamaoncol.2017.3064.
https://doi.org/10.1001/jamaoncol.2017.3064.
Bartlett, E. K., M. Gupta, J. Datta, et al. 2014. "Prognosis of patients with melanoma and microsatellitosis undergoing sentinel lymph node biopsy." *Ann Surg Oncol* 21 (3): 1016-23. https://doi.org/10.1245/s10434-013-3388-5.
Basch, Ethan, Gita N. Mody, and Amylou C. Dueck. 2020. "Electronic Patient-Reported Outcomes as Digital Therapeutics to Improve Cancer Outcomes." *JCO Oncology Practice* 16 (9): 541-542. https://doi.org/10.1200/op.20.00264. https://ascopubs.org/doi/abs/10.1200/OP.20.00264.
Bastacky, Melissa L., Hong Wang, Dylan Fortman, et al. 2021. "Immune-Related Adverse Events in PD-1 Treated Melanoma and Impact Upon Anti-Tumor Efficacy: A Real World Analysis." *Frontiers in Oncology* Volume 11 - 2021. https://doi.org/10.3389/fonc.2021.749064. https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2021.749064.
Beadling, Carol, Erick Jacobson-Dunlop, F. Stephen Hodi, et al. 2008. "KIT Gene Mutations and Copy Number in Melanoma Subtypes." *Clinical Cancer Research* 14 (21): 6821-6828. https://doi.org/10.1158/1078-0432.Ccr-08-0575. https://doi.org/10.1158/1078-0432.CCR-08-0575.
Berner, F., and L. Flatz. 2023. "Autoimmunity in immune checkpoint inhibitor-induced immune-related adverse events: A focus on autoimmune skin toxicity and pneumonitis." *Immunol Rev* 318 (1): 37-50. https://doi.org/10.1111/imr.13258.
Berner, Fiamma, David Bomze, Stefan Diem, et al. 2019. "Association of Checkpoint Inhibitor–Induced Toxic Effects With Shared Cancer and Tissue Antigens in Non–Small Cell Lung Cancer." *JAMA Oncology* 5 (7): 1043-1047. https://doi.org/10.1001/jamaoncol.2019.0402. https://doi.org/10.1001/jamaoncol.2019.0402.
Besser, M. J., R. Shapira-Frommer, O. Itzhaki, et al. 2013. "Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: intent-to-treat analysis and efficacy after failure to prior immunotherapies." *Clin Cancer Res* 19 (17): 4792-800. https://doi.org/10.1158/1078-0432.Ccr-13-0380.
Betof Warner, A., J. S. Palmer, A. N. Shoushtari, et al. 2020. "Long-Term Outcomes and Responses to Retreatment in Patients With Melanoma Treated With PD-1 Blockade." *J Clin Oncol* 38 (15): 1655-1663. https://doi.org/10.1200/jco.19.01464.
Betof Warner, Allison, Pippa G. Corrie, and Omid Hamid. 2023. "Tumor-Infiltrating Lymphocyte Therapy in Melanoma: Facts to the Future." *Clinical Cancer Research* 29 (10): 1835-1854. https://doi.org/10.1158/1078-0432.Ccr-22-1922. https://doi.org/10.1158/1078-0432.CCR-22-1922.
Bhatia, S., S. S. Tykodi, and J. A. Thompson. 2009. "Treatment of metastatic melanoma: an overview." *Oncology (Williston Park)* 23 (6): 488-96.
Bhattacharya, Sombodhi, Debasmita Sadhukhan, and Radha Saraswathy. 2024. "Role of sex in immune response and epigenetic mechanisms." *Epigenetics & Chromatin* 17 (1): 1. https://doi.org/10.1186/s13072-024-00525-x. https://doi.org/10.1186/s13072-024-00525-x.
Biermann, Jana, Johannes C. Melms, Amit Dipak Amin, et al. 2022. "Dissecting the treatment-naive ecosystem of human melanoma brain metastasis." *Cell* 185 (14): 2591-2608.e30. https://doi.org/https://doi.org/10.1016/j.cell.2022.06.007. https://www.sciencedirect.com/science/article/pii/S0092867422007127.
Björk, Johannes R., Laura A. Bolte, Andrew Maltez Thomas, et al. 2024. "Longitudinal gut microbiome changes in immune checkpoint blockade-treated advanced melanoma." *Nature Medicine* 30 (3): 785-796. https://doi.org/10.1038/s41591-024-02803-3. https://doi.org/10.1038/s41591-024-02803-3.
Blank, Christian U., Minke W. Lucas, Richard A. Scolyer, et al. 2024. "Neoadjuvant Nivolumab and Ipilimumab in Resectable Stage III Melanoma." *New England Journal of Medicine* 391 (18): 1696-1708. https://doi.org/doi:10.1056/NEJMoa2402604. https://www.nejm.org/doi/full/10.1056/NEJMoa2402604.

Boriani, F., F. O'Leary, M. Tohill, et al. 2014. "Acral Lentiginous Melanoma - misdiagnosis, referral delay and 5 years specific survival according to site." *Eur Rev Med Pharmacol Sci* 18 (14): 1990-6.

Bosetti, C., C. La Vecchia, L. Naldi, et al. 2004. "Mortality from cutaneous malignant melanoma in Europe. Has the epidemic levelled off?" *Melanoma Res* 14 (4): 301-9. https://doi.org/10.1097/01.cmr.0000136710.75287.1c.

Boutros, C., D. Belkadi-Sadou, A. Marchand, et al. 2023. "Cured or Not? Long-term Outcomes of Immunotherapy Responders. Focus on Melanoma." *Curr Oncol Rep* 25 (9): 989-996. https://doi.org/10.1007/s11912-023-01429-x.

Bradford, P. T., A. M. Goldstein, M. L. McMaster, et al. 2009. "Acral lentiginous melanoma: incidence and survival patterns in the United States, 1986-2005." *Arch Dermatol* 145 (4): 427-34. https://doi.org/10.1001/archdermatol.2008.609.

Braumüller, H., T. Wieder, E. Brenner, et al. 2013a. "T-helper-1-cell cytokines drive cancer into senescence." *Nature* 494 (7437): 361-5. https://doi.org/10.1038/nature11824.

Braumüller, Heidi, Thomas Wieder, Ellen Brenner, et al. 2013b. "T-helper-1-cell cytokines drive cancer into senescence." *Nature* 494 (7437): 361-365. https://doi.org/10.1038/nature11824. https://doi.org/10.1038/nature11824.

Bregman, B., S. Teitsson, I. Orsini, et al. 2020. "Cost-Utility Analysis of Nivolumab in Adjuvant Treatment of Melanoma in France." *Dermatol Ther (Heidelb)* 10 (6): 1331-1343. https://doi.org/10.1007/s13555-020-00446-z.

Brenner, E., B. F. Schörg, F. Ahmetlić, et al. 2020. "Cancer immune control needs senescence induction by interferon-dependent cell cycle regulator pathways in tumours." *Nat Commun* 11 (1): 1335. https://doi.org/10.1038/s41467-020-14987-6.

Brombin, A., and E. E. Patton. 2024. "Melanocyte lineage dynamics in development, growth and disease." *Development* 151 (15). https://doi.org/10.1242/dev.201266.

Broseghini, E., F. Venturi, G. Veronesi, et al. 2025. "Exploring the Common Mutational Landscape in Cutaneous Melanoma and Pancreatic Cancer." *Pigment Cell Melanoma Res* 38 (1): e13210. https://doi.org/10.1111/pcmr.13210.

Buchbinder, E. I., and A. Desai. 2016. "CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition." *Am J Clin Oncol* 39 (1): 98-106. https://doi.org/10.1097/coc.0000000000000239.

Burd, Christin E., Wenjin Liu, Minh V. Huynh, et al. 2014. "Mutation-Specific RAS Oncogenicity Explains NRAS Codon 61 Selection in Melanoma." *Cancer Discovery* 4 (12): 1418-1429. https://doi.org/10.1158/2159-8290.Cd-14-0729. https://doi.org/10.1158/2159-8290.CD-14-0729.

Burkey, Caroline, Fauzia Hollnagel, Janmesh D. Patel, et al. 2025. "Circulating tumor DNA (ctDNA) dynamics during anti-PD-1 based therapy to predict clinical outcomes in advanced stage melanoma: A multicenter retrospective study." *Journal of Clinical Oncology* 43 (16_suppl): 9523-9523. https://doi.org/10.1200/JCO.2025.43.16_suppl.9523. https://doi.org/10.1200/JCO.2025.43.16_suppl.9523.

Bushara, O., J. Tidwell, J. R. Wester, et al. 2023. "The Current State of Neoadjuvant Therapy in Resectable Advanced Stage Melanoma." *Cancers (Basel)* 15 (13). https://doi.org/10.3390/cancers15133344.

Cai, Letong, Yuchen Li, Jiaxiong Tan, et al. 2023. "Targeting LAG-3, TIM-3, and TIGIT for cancer immunotherapy." *Journal of Hematology & Oncology* 16 (1): 101. https://doi.org/10.1186/s13045-023-01499-1. https://doi.org/10.1186/s13045-023-01499-1.

Cai, Yuan-jun, Long-feng Ke, Wen-wen Zhang, et al. 2021. "Recurrent KRAS, KIT and SF3B1 mutations in melanoma of the female genital tract." *BMC Cancer* 21 (1): 677. https://doi.org/10.1186/s12885-021-08427-x. https://doi.org/10.1186/s12885-021-08427-x.

Cancer Genome Atlas, Network. 2015. "Genomic Classification of Cutaneous Melanoma." *Cell* 161 (7): 1681-1696. https://doi.org/10.1016/j.cell.2015.05.044. http://europepmc.org/abstract/MED/26091043

https://doi.org/10.1016/j.cell.2015.05.044

https://europepmc.org/articles/PMC4580370

https://europepmc.org/articles/PMC4580370?pdf=render.

Capone, Imerio, Paolo Marchetti, Paolo Antonio Ascierto, et al. 2018. "Sexual Dimorphism of Immune Responses: A New Perspective in Cancer Immunotherapy." *Frontiers in Immunology* Volume 9 - 2018. https://doi.org/10.3389/fimmu.2018.00552. https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2018.00552.

Cartun, Z., W. G. Kunz, L. Heinzerling, et al. 2022. "Cost-effectiveness of Response-Adapted De-escalation of Immunotherapy in Advanced Melanoma." *JAMA Dermatol* 158 (12): 1387-1393. https://doi.org/10.1001/jamadermatol.2022.4556.

Casagrande, Silvia, Giulia Boscato Sopetto, Giovanni Bertalot, et al. 2024. "Immune-Related Adverse Events Due to Cancer Immunotherapy: Immune Mechanisms and Clinical Manifestations." *Cancers* 16 (7): 1440. https://www.mdpi.com/2072-6694/16/7/1440.

Cascinelli, N., A. Morabito, M. Santinami, et al. 1998. "Immediate or delayed dissection of regional nodes in patients with melanoma of the trunk: a randomised trial. WHO Melanoma Programme." *Lancet* 351 (9105): 793-6. https://doi.org/10.1016/s0140-6736(97)08260-3.

Chang, Michael S, Rebecca I Hartman, Nicole Trepanowski, et al. 2022. "Cumulative Erythemal Ultraviolet Radiation and Risk of Cancer in 3 Large US Prospective Cohorts." *American Journal of Epidemiology* 191 (10): 1742-1752. https://doi.org/10.1093/aje/kwac101. https://doi.org/10.1093/aje/kwac101.

Chapman, Paul B., Lawrence H. Einhorn, Michael L. Meyers, et al. 1999. "Phase III Multicenter Randomized Trial of the Dartmouth Regimen Versus Dacarbazine in Patients With Metastatic Melanoma." *Journal of Clinical Oncology* 17 (9): 2745-2745. https://doi.org/10.1200/jco.1999.17.9.2745. https://ascopubs.org/doi/abs/10.1200/JCO.1999.17.9.2745.

Chatziioannou, E., J. Roßner, T. N. Aung, et al. 2023. "Deep learning-based scoring of tumour-infiltrating lymphocytes is prognostic in primary melanoma and predictive to PD-1 checkpoint inhibition in melanoma metastases." *EBioMedicine* 93: 104644. https://doi.org/10.1016/j.ebiom.2023.104644.

Chatziioannou, Eftychia, Jana Roßner, Thazin New Aung, et al. 2023. "Deep learning-based scoring of tumour-infiltrating lymphocytes is prognostic in primary melanoma and predictive to PD-1 checkpoint inhibition in melanoma metastases." *EBioMedicine* 93.

Chen, G., Y. Yu, Y. Qi, et al. 2024. "Comparative analysis of PD-L1 expression and molecular alterations in primary versus metastatic lung adenocarcinoma: a real-world study in China." *Front Oncol* 14: 1393686. https://doi.org/10.3389/fonc.2024.1393686.

Chen, J. Y., G. Hruby, R. A. Scolyer, et al. 2008. "Desmoplastic neurotropic melanoma: a clinicopathologic analysis of 128 cases." *Cancer* 113 (10): 2770-8. https://doi.org/10.1002/cncr.23895.

Chen, L. L., N. Jaimes, C. A. Barker, et al. 2013. "Desmoplastic melanoma: a review." *J Am Acad Dermatol* 68 (5): 825-33. https://doi.org/10.1016/j.jaad.2012.10.041.

Cheng, Tiffany W., Madeline C. Ahern, and Alessio Giubellino. 2022. "The Spectrum of Spitz Melanocytic Lesions: From Morphologic Diagnosis to Molecular Classification." *Frontiers in Oncology* Volume 12 - 2022. https://doi.org/10.3389/fonc.2022.889223. https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2022.889223.

Chesney, J., K. D. Lewis, H. Kluger, et al. 2022. "Efficacy and safety of lifileucel, a one-time autologous tumor-infiltrating lymphocyte (TIL) cell therapy, in patients with advanced melanoma after progression on immune checkpoint inhibitors and targeted therapies: pooled analysis of consecutive cohorts of the C-144-01 study." *J Immunother Cancer* 10 (12). https://doi.org/10.1136/jitc-2022-005755.

Claps, Giuseppina, Sara Faouzi, Virginie Quidville, et al. 2022. "The multiple roles of LDH in cancer." *Nature Reviews Clinical Oncology* 19 (12): 749-762. https://doi.org/10.1038/s41571-022-00686-2. https://doi.org/10.1038/s41571-022-00686-2.

Coen, O., P. Corrie, H. Marshall, et al. 2021. "The DANTE trial protocol: a randomised phase III trial to evaluate the Duration of ANti-PD-1 monoclonal antibody Treatment in patients with metastatic mElanoma." *BMC Cancer* 21 (1): 761. https://doi.org/10.1186/s12885-021-08509-w.

Conforti, C., and I. Zalaudek. 2021. "Epidemiology and Risk Factors of Melanoma: A Review." *Dermatol Pract Concept* 11 (Suppl 1): e2021161S. https://doi.org/10.5826/dpc.11S1a161S.

Conway, Jake R., Eric Kofman, Shirley S. Mo, et al. 2018. "Genomics of response to immune checkpoint therapies for cancer: implications for precision medicine." *Genome Medicine* 10 (1): 93. https://doi.org/10.1186/s13073-018-0605-7. https://doi.org/10.1186/s13073-018-0605-7.

Costantini, S., G. Madonna, M. Capone, et al. 2025. "Metabolomic signatures in liquid biopsy are associated with overall survival in metastatic melanoma patients treated with immune checkpoint inhibitor therapy." *J Exp Clin Cancer Res* 44 (1): 119. https://doi.org/10.1186/s13046-025-03378-8.

Crowson, A. N., C. M. Magro, and M. C. Mihm, Jr. 1999. "Malignant melanoma with prominent pigment synthesis: "animal type" melanoma--a clinical and histological study of six cases with a consideration of other melanocytic neoplasms with prominent pigment synthesis." *Hum Pathol* 30 (5): 543-50. https://doi.org/10.1016/s0046-8177(99)90199-5.

Czarnecka, Anna M., Paweł Teterycz, Krzysztof Ostaszewski, et al. 2025. "Discontinuation of Immune Checkpoint Inhibition in Patients with Advanced Unresectable Melanoma Achieving CR, PR, or SD." *Targeted Oncology*. https://doi.org/10.1007/s11523-025-01156-2. https://doi.org/10.1007/s11523-025-01156-2.

Dai, J., J. Jia, F. Zhang, et al. 2024. "Comparative Epigenetic Profiling Reveals Distinct Features of Mucosal Melanomas Associated with Immune Cell Infiltration and Their Clinical Implications." *Cancer Res Commun* 4 (5): 1351-1362. https://doi.org/10.1158/2767-9764.Crc-23-0406.

Danielli, R., R. Patuzzo, A. M. Di Giacomo, et al. 2015. "Intralesional administration of L19-IL2/L19-TNF in stage III or stage IVM1a melanoma patients: results of a phase II study." *Cancer Immunol Immunother* 64 (8): 999-1009. https://doi.org/10.1007/s00262-015-1704-6.

Das, Satya, and Douglas B. Johnson. 2019. "Immune-related adverse events and anti-tumor efficacy of immune checkpoint inhibitors." *Journal for ImmunoTherapy of Cancer* 7 (1): 306. https://doi.org/10.1186/s40425-019-0805-8. https://doi.org/10.1186/s40425-019-0805-8.

Davies, Michael A. 2020. "Is It Safe to Stop Anti–PD-1 Immunotherapy in Patients With Metastatic Melanoma Who Achieve a Complete Response?" *Journal of Clinical Oncology* 38 (15): 1645-1647. https://doi.org/10.1200/jco.20.00136. https://ascopubs.org/doi/abs/10.1200/JCO.20.00136.

Dercle, Laurent, Samy Ammari, Elvire Roblin, et al. 2022. "High serum LDH and liver metastases are the dominant predictors of primary cancer resistance to anti-PD(L)1 immunotherapy." *European Journal of Cancer* 177: 80-93. https://doi.org/https://doi.org/10.1016/j.ejca.2022.08.034. https://www.sciencedirect.com/science/article/pii/S0959804922007572.

DeWane, M. E., A. Kelsey, M. Oliviero, et al. 2019. "Melanoma on chronically sun-damaged skin: Lentigo maligna and desmoplastic melanoma." *J Am Acad Dermatol* 81 (3): 823-833. https://doi.org/10.1016/j.jaad.2019.03.066.

Dian, Yating, Yihuang Liu, Furong Zeng, et al. 2024. "Efficacy and safety of tebentafusp in patients with metastatic uveal melanoma: A systematic review and meta-analysis." *Human Vaccines & Immunotherapeutics* 20 (1): 2374647. https://doi.org/10.1080/21645515.2024.2374647. https://doi.org/10.1080/21645515.2024.2374647.

Diem, S., B. Kasenda, L. Spain, et al. 2016. "Serum lactate dehydrogenase as an early marker for outcome in patients treated with anti-PD-1 therapy in metastatic

melanoma." *British Journal of Cancer* 114 (3): 256-261. https://doi.org/10.1038/bjc.2015.467. https://doi.org/10.1038/bjc.2015.467.
Dreier, M. R., and I. L. de la Serna. 2022. "SWI/SNF Chromatin Remodeling Enzymes in Melanoma." *Epigenomes* 6 (1). https://doi.org/10.3390/epigenomes6010010.
Druskovich, Christina, Jesse Kelley, Jason Aubrey, et al. 2025. "A Review of Melanoma Subtypes: Genetic and Treatment Considerations." *Journal of Surgical Oncology* 131 (3): 356-364. https://doi.org/https://doi.org/10.1002/jso.27953. https://onlinelibrary.wiley.com/doi/abs/10.1002/jso.27953.
Dupin, Elisabeth, and Nicole M. Le Douarin. 2003. "Development of melanocyte precursors from the vertebrate neural crest." *Oncogene* 22 (20): 3016-3023. https://doi.org/10.1038/sj.onc.1206460. https://doi.org/10.1038/sj.onc.1206460.
Eggermont, A. M. M., C. U. Blank, M. Mandala, et al. 2020. "Longer Follow-Up Confirms Recurrence-Free Survival Benefit of Adjuvant Pembrolizumab in High-Risk Stage III Melanoma: Updated Results From the EORTC 1325-MG/KEYNOTE-054 Trial." *J Clin Oncol* 38 (33): 3925-3936. https://doi.org/10.1200/jco.20.02110.
Eggermont, A. M. M., O. Hamid, G. V. Long, et al. 2022. "Optimal systemic therapy for high-risk resectable melanoma." *Nat Rev Clin Oncol* 19 (7): 431-439. https://doi.org/10.1038/s41571-022-00630-4.
Eggermont, Alexander M.M., Christian U. Blank, Mario Mandala, et al. 2018. "Adjuvant Pembrolizumab versus Placebo in Resected Stage III Melanoma." *New England Journal of Medicine* 378 (19): 1789-1801. https://doi.org/doi:10.1056/NEJMoa1802357. https://www.nejm.org/doi/full/10.1056/NEJMoa1802357.
Eisenhauer, E. A., P. Therasse, J. Bogaerts, et al. 2009. "New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1)." *Eur J Cancer* 45 (2): 228-47. https://doi.org/10.1016/j.ejca.2008.10.026.
Eliason, J., S. Krishnan, Y. Fukuda, et al. 2025. "Characterizing spatial immune architecture in metastatic melanoma using high-dimensional multiplex imaging." *Front Immunol* 16: 1560778. https://doi.org/10.3389/fimmu.2025.1560778.
Eljilany, I., E. Castellano, and A. A. Tarhini. 2023. "Adjuvant Therapy for High-Risk Melanoma: An In-Depth Examination of the State of the Field." *Cancers (Basel)* 15 (16). https://doi.org/10.3390/cancers15164125.
Ellebaek, Eva, Shawez Khan, Lars Bastholt, et al. 2024. "PD-L1 is a biomarker of real-world clinical outcomes for anti-CTLA-4 plus anti-PD-1 or anti-PD-1 monotherapy in metastatic melanoma." *European Journal of Cancer* 198. https://doi.org/10.1016/j.ejca.2023.113476. https://doi.org/10.1016/j.ejca.2023.113476.
Elwood, J. M., R. P. Gallagher, G. B. Hill, et al. 1985. "Cutaneous melanoma in relation to intermittent and constant sun exposure--the Western Canada Melanoma Study." *Int J Cancer* 35 (4): 427-33. https://doi.org/10.1002/ijc.2910350403.
Espinosa, E., I. Márquez-Rodas, A. Soria, et al. 2017. "Predictive factors of response to immunotherapy-a review from the Spanish Melanoma Group (GEM)." *Ann Transl Med* 5 (19): 389. https://doi.org/10.21037/atm.2017.08.10.
Eyles, J., A. L. Puaux, X. Wang, et al. 2010. "Tumor cells disseminate early, but immunosurveillance limits metastatic outgrowth, in a mouse model of melanoma." *J Clin Invest* 120 (6): 2030-9. https://doi.org/10.1172/jci42002.
Fager, Anna, Matilda Samuelsson, Roger Olofsson Bagge, et al. 2025. "Immune checkpoint inhibitor therapy is associated with a decreased risk of developing melanoma brain metastases." *BJC Reports* 3 (1): 22. https://doi.org/10.1038/s44276-025-00137-2. https://doi.org/10.1038/s44276-025-00137-2.
Fahey, C. C., T. J. Gracie, and D. B. Johnson. 2023. "Immune checkpoint inhibitors: maximizing benefit whilst minimizing toxicity." *Expert Rev Anticancer Ther* 23 (7): 673-683. https://doi.org/10.1080/14737140.2023.2215435.
Falahat, R., P. Perez-Villarroel, A. W. Mailloux, et al. 2019. "STING Signaling in Melanoma Cells Shapes Antigenicity and Can Promote Antitumor T-cell Activity." *Cancer Immunol Res* 7 (11): 1837-1848. https://doi.org/10.1158/2326-6066.Cir-19-0229.

Faries, Mark B., John F. Thompson, Alistair J. Cochran, et al. 2017. "Completion Dissection or Observation for Sentinel-Node Metastasis in Melanoma." *New England Journal of Medicine* 376 (23): 2211-2222. https://doi.org/doi:10.1056/NEJMoa1613210. https://www.nejm.org/doi/full/10.1056/NEJMoa1613210.

Farrow, N. E., V. Raman, T. P. Williams, et al. 2020. "Adjuvant Therapy is Effective for Melanoma Patients with a Positive Sentinel Lymph Node Biopsy Who Forego Completion Lymphadenectomy." *Ann Surg Oncol* 27 (13): 5121-5125. https://doi.org/10.1245/s10434-020-08478-7.

Ferguson, Peter M., Jeffrey E. Gershenwald, and Richard A. Scolyer. 2018. "Staging of Cutaneous Melanoma: Is There Room for Further Improvement?" *JAMA Network Open* 1 (1): e180086-e180086. https://doi.org/10.1001/jamanetworkopen.2018.0086. https://doi.org/10.1001/jamanetworkopen.2018.0086.

Forchhammer, Stephan, Oltin Tiberiu Pop, Matthias Hahn, et al. 2024. "Expression of the tumor antigens NY-ESO-1, tyrosinase, MAGE-A3, and TPTE in pediatric and adult melanoma: a retrospective case control study." *Virchows Archiv*. https://doi.org/10.1007/s00428-024-03846-0. https://doi.org/10.1007/s00428-024-03846-0.

Fortuna, Ana, and Teresa Amaral. 2024. "Multidisciplinary approach and treatment of acral and mucosal melanoma." *Frontiers in Oncology* Volume 14 - 2024. https://doi.org/10.3389/fonc.2024.1340408. https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2024.1340408.

Fujisawa, Y., K. Yoshino, A. Otsuka, et al. 2018. "Retrospective study of advanced melanoma patients treated with ipilimumab after nivolumab: Analysis of 60 Japanese patients." *J Dermatol Sci* 89 (1): 60-66. https://doi.org/10.1016/j.jdermsci.2017.10.009.

Fukushima, T., S. Kobayashi, and M. Ueno. 2024. "The correlation between immune-related adverse events and efficacy of immune checkpoint inhibitors." *Jpn J Clin Oncol* 54 (9): 949-958. https://doi.org/10.1093/jjco/hyae067.

Gamboa, A. C., M. Lowe, M. L. Yushak, et al. 2020. "Surgical Considerations and Systemic Therapy of Melanoma." *Surg Clin North Am* 100 (1): 141-159. https://doi.org/10.1016/j.suc.2019.09.012.

Gandini, S., F. Sera, M. S. Cattaruzza, et al. 2005. "Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure." *Eur J Cancer* 41 (1): 45-60. https://doi.org/10.1016/j.ejca.2004.10.016.

Garbe, Claus, Ana-Maria Forsea, Teresa Amaral, et al. 2024. "Skin cancers are the most frequent cancers in fair-skinned populations, but we can prevent them." *European Journal of Cancer* 204. https://doi.org/10.1016/j.ejca.2024.114074. https://doi.org/10.1016/j.ejca.2024.114074.

García-Lozano, J. A., G. Salerni, A. Cuellar-Barboza, et al. 2020. "Rapid Dermoscopic Changes in Nodular Melanoma." *Dermatol Pract Concept* 10 (1): e2020016. https://doi.org/10.5826/dpc.1001a16.

Garg, Manik, Dominique-Laurent Couturier, Jérémie Nsengimana, et al. 2021. "Tumour gene expression signature in primary melanoma predicts long-term outcomes." *Nature Communications* 12 (1): 1137. https://doi.org/10.1038/s41467-021-21207-2. https://doi.org/10.1038/s41467-021-21207-2.

Gauci, Marie-Léa, Emilie Lanoy, Stéphane Champiat, et al. 2019. "Long-Term Survival in Patients Responding to Anti–PD-1/PD-L1 Therapy and Disease Outcome upon Treatment Discontinuation." *Clinical Cancer Research* 25 (3): 946-956. https://doi.org/10.1158/1078-0432.Ccr-18-0793. https://doi.org/10.1158/1078-0432.CCR-18-0793.

Gaughan, Elizabeth M., and Bethany J. Horton. 2022. "Outcomes From Cytotoxic Chemotherapy Following Progression on Immunotherapy in Metastatic Melanoma: An Institutional Case-Series." *Frontiers in Oncology* Volume 12 - 2022. https://doi.org/10.3389/fonc.2022.855782. https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2022.855782.

Gershenwald, Jeffrey E., and Richard A. Scolyer. 2018. "Melanoma Staging: American Joint Committee on Cancer (AJCC) 8th Edition and Beyond." *Annals of Surgical Oncology*

25 (8): 2105-2110. https://doi.org/10.1245/s10434-018-6513-7. https://doi.org/10.1245/s10434-018-6513-7.

Gide, T. N., E. C. Paver, Z. Yaseen, et al. 2023. "Lag-3 expression and clinical outcomes in metastatic melanoma patients treated with combination anti-lag-3 + anti-PD-1-based immunotherapies." *Oncoimmunology* 12 (1): 2261248. https://doi.org/10.1080/2162402x.2023.2261248.

Goldinger, S. M., K. Buder-Bakhaya, S. N. Lo, et al. 2022. "Chemotherapy after immune checkpoint inhibitor failure in metastatic melanoma: a retrospective multicentre analysis." *Eur J Cancer* 162: 22-33. https://doi.org/10.1016/j.ejca.2021.11.022.

Goodman, Aaron M., Shumei Kato, Lyudmila Bazhenova, et al. 2017. "Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers." *Molecular Cancer Therapeutics* 16 (11): 2598-2608. https://doi.org/10.1158/1535-7163.Mct-17-0386. https://doi.org/10.1158/1535-7163.MCT-17-0386.

Goodman, Rachel S., Aleigha Lawless, Rachel Woodford, et al. 2023. "Extended Follow-Up of Chronic Immune-Related Adverse Events Following Adjuvant Anti–PD-1 Therapy for High-Risk Resected Melanoma." *JAMA Network Open* 6 (8): e2327145-e2327145. https://doi.org/10.1001/jamanetworkopen.2023.27145. https://doi.org/10.1001/jamanetworkopen.2023.27145.

Grasso, C. S., J. Tsoi, M. Onyshchenko, et al. 2020. "Conserved Interferon-γ Signaling Drives Clinical Response to Immune Checkpoint Blockade Therapy in Melanoma." *Cancer Cell* 38 (4): 500-515.e3. https://doi.org/10.1016/j.ccell.2020.08.005.

Greaves, W. O., S. Verma, K. P. Patel, et al. 2013. "Frequency and spectrum of BRAF mutations in a retrospective, single-institution study of 1112 cases of melanoma." *J Mol Diagn* 15 (2): 220-6. https://doi.org/10.1016/j.jmoldx.2012.10.002.

Griewank, K. G., R. Murali, J. A. Puig-Butille, et al. 2014. "TERT promoter mutation status as an independent prognostic factor in cutaneous melanoma." *J Natl Cancer Inst* 106 (9). https://doi.org/10.1093/jnci/dju246.

Guo, P., X. Wei, Z. Guo, et al. 2024. "Clinicopathological features, current status, and progress of primary central nervous system melanoma diagnosis and treatment." *Pigment Cell Melanoma Res* 37 (2): 265-275. https://doi.org/10.1111/pcmr.13140.

Guo, Y., Y. Chen, L. Zhang, et al. 2022. "TERT Promoter Mutations and Telomerase in Melanoma." *J Oncol* 2022: 6300329. https://doi.org/10.1155/2022/6300329.

Guo, Y., D. Xiang, J. Wan, et al. 2022. "Focus on the Dynamics of Neutrophil-to-Lymphocyte Ratio in Cancer Patients Treated with Immune Checkpoint Inhibitors: A Meta-Analysis and Systematic Review." *Cancers (Basel)* 14 (21). https://doi.org/10.3390/cancers14215297.

Hahn, H. M., K. G. Lee, W. Choi, et al. 2019. "An updated review of mucosal melanoma: Survival meta-analysis." *Mol Clin Oncol* 11 (2): 116-126. https://doi.org/10.3892/mco.2019.1870.

Hamid, O., C. Robert, A. Daud, et al. 2021. "Long-term outcomes in patients with advanced melanoma who had initial stable disease with pembrolizumab in KEYNOTE-001 and KEYNOTE-006." *Eur J Cancer* 157: 391-402. https://doi.org/10.1016/j.ejca.2021.08.013.

Han, Guangchun, Guoliang Yang, Dapeng Hao, et al. 2021. "9p21 loss confers a cold tumor immune microenvironment and primary resistance to immune checkpoint therapy." *Nature Communications* 12 (1): 5606. https://doi.org/10.1038/s41467-021-25894-9. https://doi.org/10.1038/s41467-021-25894-9.

Harms, P. W., T. L. Hocker, L. Zhao, et al. 2016. "Loss of p16 expression and copy number changes of CDKN2A in a spectrum of spitzoid melanocytic lesions." *Hum Pathol* 58: 152-160. https://doi.org/10.1016/j.humpath.2016.07.029.

Hassel, Jessica C. 2022. "Checkpoint blocker induced autoimmunity as an indicator for tumour efficacy in melanoma." *British Journal of Cancer* 126 (2): 163-164. https://doi.org/10.1038/s41416-021-01390-1. https://doi.org/10.1038/s41416-021-01390-1.

Hassel, Jessica C., Sophie Piperno-Neumann, Piotr Rutkowski, et al. 2023. "Three-Year Overall Survival with Tebentafusp in Metastatic Uveal Melanoma." *New England Journal of Medicine* 389 (24): 2256-2266. https://doi.org/doi:10.1056/NEJMoa2304753. https://www.nejm.org/doi/full/10.1056/NEJMoa2304753.

Heppt, M. V., T. Siepmann, J. Engel, et al. 2017. "Prognostic significance of BRAF and NRAS mutations in melanoma: a German study from routine care." *BMC Cancer* 17 (1): 536. https://doi.org/10.1186/s12885-017-3529-5.

Hieken, Tina J., Firas Kreidieh, Veronica Aedo-Lopez, et al. 2023. "Neoadjuvant Immunotherapy in Melanoma: The Paradigm Shift." *American Society of Clinical Oncology Educational Book* (43): e390614. https://doi.org/10.1200/edbk_390614. https://ascopubs.org/doi/abs/10.1200/EDBK_390614.

Holman, D. M., J. B. King, A. White, et al. 2023. "Acral lentiginous melanoma incidence by sex, race, ethnicity, and stage in the United States, 2010-2019." *Prev Med* 175: 107692. https://doi.org/10.1016/j.ypmed.2023.107692.

Holmberg, C. J., R. Mikiver, K. Isaksson, et al. 2023. "Prognostic Significance of Sentinel Lymph Node Status in Thick Primary Melanomas (> 4 mm)." *Ann Surg Oncol* 30 (13): 8026-8033. https://doi.org/10.1245/s10434-023-14050-w.

Hu, T., W. Sun, Y. Xu, et al. 2021. "Combination of pembrolizumab plus temozolomide therapy in unresectable and advanced melanoma: a multicenter retrospective analysis in China." *Ann Transl Med* 9 (21): 1625. https://doi.org/10.21037/atm-21-5738.

Hu, Y., A. Briggs, R. L. Gennarelli, et al. 2020. "Sentinel Lymph Node Biopsy for T1b Melanoma: Balancing Prognostic Value and Cost." *Ann Surg Oncol* 27 (13): 5248-5256. https://doi.org/10.1245/s10434-020-08558-8.

Huang, Alexander C., and Roberta Zappasodi. 2022. "A decade of checkpoint blockade immunotherapy in melanoma: understanding the molecular basis for immune sensitivity and resistance." *Nature Immunology* 23 (5): 660-670. https://doi.org/10.1038/s41590-022-01141-1. https://doi.org/10.1038/s41590-022-01141-1.

Huang, F. W., E. Hodis, M. J. Xu, et al. 2013. "Highly recurrent TERT promoter mutations in human melanoma." *Science* 339 (6122): 957-9. https://doi.org/10.1126/science.1229259.

Hussain, R. N., A. Chiu, B. Pittam, et al. 2023. "Proton beam radiotherapy for choroidal and ciliary body melanoma in the UK—national audit of referral patterns of 1084 cases." *Eye* 37 (5): 1033-1036. https://doi.org/10.1038/s41433-022-02178-0. https://doi.org/10.1038/s41433-022-02178-0.

Hussain, Zahra, Martin J. Heaton, Andrew P. Snelling, et al. 2023. "Risk Stratification of Sentinel Node Metastasis Disease Burden and Phenotype in Stage III Melanoma Patients." *Annals of Surgical Oncology* 30 (3): 1808-1819. https://doi.org/10.1245/s10434-022-12804-6. https://doi.org/10.1245/s10434-022-12804-6.

Huuhtanen, Jani, Liang Chen, Emmi Jokinen, et al. 2022. "Evolution and modulation of antigen-specific T cell responses in melanoma patients." *Nature Communications* 13 (1): 5988. https://doi.org/10.1038/s41467-022-33720-z. https://doi.org/10.1038/s41467-022-33720-z.

Hwang, S. W., M. K. Kim, and M. N. Kweon. 2023. "Gut microbiome on immune checkpoint inhibitor therapy and consequent immune-related colitis: a review." *Intest Res* 21 (4): 433-442. https://doi.org/10.5217/ir.2023.00019.

Hynes, M. C., P. Nguyen, P. A. Groome, et al. 2022. "A population-based validation study of the 8th edition UICC/AJCC TNM staging system for cutaneous melanoma." *BMC Cancer* 22 (1): 720. https://doi.org/10.1186/s12885-022-09781-0.

Idriss, M. H., L. Rizwan, A. Sferuzza, et al. 2015. "Nevoid melanoma: A study of 43 cases with emphasis on growth pattern." *J Am Acad Dermatol* 73 (5): 836-42. https://doi.org/10.1016/j.jaad.2015.07.003.

Indini, Alice, Fausto Roila, Francesco Grossi, et al. 2022. "Molecular Profiling and Novel Therapeutic Strategies for Mucosal Melanoma: A Comprehensive Review." *International Journal of Molecular Sciences* 23 (1): 147. https://www.mdpi.com/1422-0067/23/1/147.

Janka, Eszter Anna, Tünde Várvölgyi, Zoltán Sipos, et al. 2021. "Predictive Performance of Serum S100B Versus LDH in Melanoma Patients: A Systematic Review and Meta-Analysis." *Frontiers in Oncology* Volume 11 - 2021. https://doi.org/10.3389/fonc.2021.772165. https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2021.772165.

Jenkins, R. W., and R. J. Sullivan. 2016. "NRAS mutant melanoma: an overview for the clinician for melanoma management." *Melanoma Manag* 3 (1): 47-59. https://doi.org/10.2217/mmt.15.40.

Jiminez, Victoria, Kelsey B. Montgomery, Britany Hollenquest, et al. 2025. "Long-term outcomes of nodal surveillance practices in sentinel node positive melanoma: An early post MSLT-II cohort." *The American Journal of Surgery* 239. https://doi.org/10.1016/j.amjsurg.2024.116049. https://doi.org/10.1016/j.amjsurg.2024.116049.

Johnson, Douglas B., Justin M. Balko, Margaret L. Compton, et al. 2016. "Fulminant Myocarditis with Combination Immune Checkpoint Blockade." *New England Journal of Medicine* 375 (18): 1749-1755. https://doi.org/doi:10.1056/NEJMoa1609214. https://www.nejm.org/doi/full/10.1056/NEJMoa1609214.

Jurlander, Rebecca Schou, Louise Mahncke Guldbrandt, Rikke B. Holmstroem, et al. 2024. "Immune-related adverse events in a nationwide cohort of real-world melanoma patients treated with adjuvant anti-PD1 – Seasonal variation and association with outcome." *European Journal of Cancer* 212. https://doi.org/10.1016/j.ejca.2024.115053. https://doi.org/10.1016/j.ejca.2024.115053.

Kalbasi, A., M. Tariveranmoshabad, K. Hakimi, et al. 2020. "Uncoupling interferon signaling and antigen presentation to overcome immunotherapy resistance due to JAK1 loss in melanoma." *Sci Transl Med* 12 (565). https://doi.org/10.1126/scitranslmed.abb0152.

Karhapää, H., S. Mäkelä, H. Laurén, et al. 2022. "Immune checkpoint inhibitors, endocrine adverse events, and outcomes of melanoma." *Endocr Connect* 11 (2). https://doi.org/10.1530/ec-21-0562.

Kasparian, Nadine A., Jordana K. McLoone, and Phyllis N. Butow. 2009. "Psychological Responses and Coping Strategies Among Patients With Malignant Melanoma: A Systematic Review of the Literature." *Archives of Dermatology* 145 (12): 1415-1427. https://doi.org/10.1001/archdermatol.2009.308. https://doi.org/10.1001/archdermatol.2009.308.

Kasprzak, J. M., and Y. G. Xu. 2015. "Diagnosis and management of lentigo maligna: a review." *Drugs Context* 4: 212281. https://doi.org/10.7573/dic.212281.

Kataoka, Yuki, and Katsuya Hirano. 2018. "Which criteria should we use to evaluate the efficacy of immunecheckpoint inhibitors?" *Annals of Translational Medicine* 6 (11): 222. https://atm.amegroups.org/article/view/19463.

Kawashima, Shusuke, and Yosuke Togashi. 2023. "Resistance to immune checkpoint inhibitors and the tumor microenvironment." *Experimental Dermatology* 32 (3): 240-249. https://doi.org/https://doi.org/10.1111/exd.14716. https://onlinelibrary.wiley.com/doi/abs/10.1111/exd.14716.

Kendra, Kari, Shay Bellasea, Zeynep Eroglu, et al. 2023. "Abstract CT009: S1512: High response rate with single agent anti-PD-1 in patients with metastatic desmoplastic melanoma." *Cancer Research* 83 (8_Supplement): CT009-CT009. https://doi.org/10.1158/1538-7445.Am2023-ct009. https://doi.org/10.1158/1538-7445.AM2023-CT009.

Keung, E. Z., and J. E. Gershenwald. 2018. "The eighth edition American Joint Committee on Cancer (AJCC) melanoma staging system: implications for melanoma treatment and care." *Expert Rev Anticancer Ther* 18 (8): 775-784. https://doi.org/10.1080/14737140.2018.1489246.

Khan, Muhammad, Sumbal Arooj, and Hua Wang. 2020. "NK Cell-Based Immune Checkpoint Inhibition." *Frontiers in Immunology* 11. https://doi.org/10.3389/fimmu.2020.00167. https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2020.00167.

Khayat, David, Olivier Rixe, Ginette Martin, et al. 2003. "Surgical margins in cutaneous melanoma (2 cm versus 5 cm for lesions measuring less than 2.1-mm thick)." *Cancer* 97 (8): 1941-1946. https://doi.org/https://doi.org/10.1002/cncr.11272. https://acsjournals.onlinelibrary.wiley.com/doi/abs/10.1002/cncr.11272.

Khoja, Leila, Minnie Kibiro, Ur Metser, et al. 2016. "Patterns of response to anti-PD-1 treatment: an exploratory comparison of four radiological response criteria and associations with overall survival in metastatic melanoma patients." *British Journal of Cancer* 115 (10): 1186-1192. https://doi.org/10.1038/bjc.2016.308. https://doi.org/10.1038/bjc.2016.308.

Khoury, S., G. C. Knapp, A. Fyfe, et al. 2021. "Durability of Complete Response to Intralesional Interleukin-2 for In-Transit Melanoma." *J Cutan Med Surg* 25 (4): 364-370. https://doi.org/10.1177/1203475420988862.

Khunger, Monica, Adrian V. Hernandez, Vinay Pasupuleti, et al. 2017. "Programmed Cell Death 1 (PD-1) Ligand (PD-L1) Expression in Solid Tumors As a Predictive Biomarker of Benefit From PD-1/PD-L1 Axis Inhibitors: A Systematic Review and Meta-Analysis." *JCO Precision Oncology* (1): 1-15. https://doi.org/10.1200/po.16.00030. https://ascopubs.org/doi/abs/10.1200/PO.16.00030.

Kim, J. Y., A. Kronbichler, M. Eisenhut, et al. 2019. "Tumor Mutational Burden and Efficacy of Immune Checkpoint Inhibitors: A Systematic Review and Meta-Analysis." *Cancers (Basel)* 11 (11). https://doi.org/10.3390/cancers11111798.

Kirkwood, John M., Michele Del Vecchio, Jeffrey Weber, et al. 2023. "Adjuvant nivolumab in resected stage IIB/C melanoma: primary results from the randomized, phase 3 CheckMate 76K trial." *Nature Medicine* 29 (11): 2835-2843. https://doi.org/10.1038/s41591-023-02583-2. https://doi.org/10.1038/s41591-023-02583-2.

Knispel, Sarah, Maximilian Gassenmaier, Alexander M. Menzies, et al. 2021. "Outcome of melanoma patients with elevated LDH treated with first-line targeted therapy or PD-1-based immune checkpoint inhibition." *European Journal of Cancer* 148: 61-75. https://doi.org/10.1016/j.ejca.2021.01.034. https://doi.org/10.1016/j.ejca.2021.01.034.

Kothari, G. 2022. "Role of radiomics in predicting immunotherapy response." *J Med Imaging Radiat Oncol* 66 (4): 575-591. https://doi.org/10.1111/1754-9485.13426.

Krauthammer, M., Y. Kong, A. Bacchiocchi, et al. 2015. "Exome sequencing identifies recurrent mutations in NF1 and RASopathy genes in sun-exposed melanomas." *Nat Genet* 47 (9): 996-1002. https://doi.org/10.1038/ng.3361.

Kreidieh, Firas Y., and Hussein A. Tawbi. 2023. "The introduction of LAG-3 checkpoint blockade in melanoma: immunotherapy landscape beyond PD-1 and CTLA-4 inhibition." *Therapeutic Advances in Medical Oncology* 15: 17588359231186027. https://doi.org/10.1177/17588359231186027. https://doi.org/10.1177/17588359231186027.

Kretschmer, Lutz, Christina Mitteldorf, Simin Hellriegel, et al. 2021. "The sentinel node invasion level (SNIL) as a prognostic parameter in melanoma." *Modern Pathology* 34 (10): 1839-1849. https://doi.org/10.1038/s41379-021-00835-5. https://doi.org/10.1038/s41379-021-00835-5.

Kriza, Chase, Brian Martin, Christine N. Bailey, et al. 2024. "Integrating the melanoma 31-gene expression profile test with clinical and pathologic features can provide personalized precision estimates for sentinel lymph node positivity: an independent performance cohort." *World Journal of Surgical Oncology* 22 (1): 228. https://doi.org/10.1186/s12957-024-03512-4. https://doi.org/10.1186/s12957-024-03512-4.

Kuzmanovszki, D., N. Kiss, B. Tóth, et al. 2022. "Anti-PD-1 Monotherapy in Advanced Melanoma-Real-World Data from a 77-Month-Long Retrospective Observational Study." *Biomedicines* 10 (7). https://doi.org/10.3390/biomedicines10071737.

Larkin, J., M. Del Vecchio, M. Mandalá, et al. 2023. "Adjuvant Nivolumab versus Ipilimumab in Resected Stage III/IV Melanoma: 5-Year Efficacy and Biomarker Results from CheckMate 238." *Clin Cancer Res* 29 (17): 3352-3361. https://doi.org/10.1158/1078-0432.Ccr-22-3145.

Larkin, James, Vanna Chiarion-Sileni, Rene Gonzalez, et al. 2019. "Five-Year Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma." *New England Journal of Medicine* 381 (16): 1535-1546. https://doi.org/10.1056/NEJMoa1910836. https://www.nejm.org/doi/full/10.1056/NEJMoa1910836.

Larson, R. C., M. C. Kann, S. R. Bailey, et al. 2022. "CAR T cell killing requires the IFNγR pathway in solid but not liquid tumours." *Nature* 604 (7906): 563-570. https://doi.org/10.1038/s41586-022-04585-5.

Lazarev, S., V. Gupta, K. Hu, et al. 2014. "Mucosal melanoma of the head and neck: a systematic review of the literature." *Int J Radiat Oncol Biol Phys* 90 (5): 1108-18. https://doi.org/10.1016/j.ijrobp.2014.03.042.

Ledderose, Stephan, Carola Ledderose, and Georg J. Ledderose. 2024. "Expression of immune checkpoint molecules TIGIT and TIM-3 by tumor-infiltrating lymphocytes predicts poor outcome in sinonasal mucosal melanoma." *Pathology - Research and Practice* 260: 155468. https://doi.org/https://doi.org/10.1016/j.prp.2024.155468. https://www.sciencedirect.com/science/article/pii/S0344033824003790.

Lee, J. H., E. Shklovskaya, S. Y. Lim, et al. 2020. "Transcriptional downregulation of MHC class I and melanoma de- differentiation in resistance to PD-1 inhibition." *Nat Commun* 11 (1): 1897. https://doi.org/10.1038/s41467-020-15726-7.

Lee, Karla A., Andrew Maltez Thomas, Laura A. Bolte, et al. 2022. "Cross-cohort gut microbiome associations with immune checkpoint inhibitor response in advanced melanoma." *Nature Medicine* 28 (3): 535-544. https://doi.org/10.1038/s41591-022-01695-5. https://doi.org/10.1038/s41591-022-01695-5.

Lee, M., R. M. Samstein, C. Valero, et al. 2020. "Tumor mutational burden as a predictive biomarker for checkpoint inhibitor immunotherapy." *Hum Vaccin Immunother* 16 (1): 112-115. https://doi.org/10.1080/21645515.2019.1631136.

Lei, H., X. Li, W. Ma, et al. 2022. "Comparison of nomogram and machine-learning methods for predicting the survival of non-small cell lung cancer patients." *Cancer Innov* 1 (2): 135-145. https://doi.org/10.1002/cai2.24.

Leiter, U., R. Stadler, C. Mauch, et al. 2019. "Final Analysis of DeCOG-SLT Trial: No Survival Benefit for Complete Lymph Node Dissection in Patients With Melanoma With Positive Sentinel Node." *J Clin Oncol* 37 (32): 3000-3008. https://doi.org/10.1200/jco.18.02306.

Li, Y., Y. Meng, H. Sun, et al. 2022. "The Prognostic Significance of Baseline Neutrophil-to-Lymphocyte Ratio in Melanoma Patients Receiving Immunotherapy." *J Immunother* 45 (1): 43-50. https://doi.org/10.1097/cji.0000000000000392.

Ligero, Marta, Bente Gielen, Victor Navarro, et al. 2024. "A whirl of radiomics-based biomarkers in cancer immunotherapy, why is large scale validation still lacking?" *npj Precision Oncology* 8 (1): 42. https://doi.org/10.1038/s41698-024-00534-9. https://doi.org/10.1038/s41698-024-00534-9.

Lim, Su Yin, Elena Shklovskaya, Jenny H. Lee, et al. 2023. "The molecular and functional landscape of resistance to immune checkpoint blockade in melanoma." *Nature Communications* 14 (1): 1516. https://doi.org/10.1038/s41467-023-36979-y. https://doi.org/10.1038/s41467-023-36979-y.

Linos, E., W. Q. Li, J. Han, et al. 2017. "Lifetime ultraviolet radiation exposure and lentigo maligna melanoma." *Br J Dermatol* 176 (6): 1666-1668. https://doi.org/10.1111/bjd.15218.

Lipson, Evan J., F. Stephen Hodi, Hussein Tawbi, et al. 2025. "Nivolumab plus relatlimab in advanced melanoma: RELATIVITY-047 4-year update." *European Journal of Cancer* 225: 115547. https://doi.org/https://doi.org/10.1016/j.ejca.2025.115547. https://www.sciencedirect.com/science/article/pii/S0959804925003296.

Liu, D., B. Schilling, D. Liu, et al. 2019. "Integrative molecular and clinical modeling of clinical outcomes to PD1 blockade in patients with metastatic melanoma." *Nat Med* 25 (12): 1916-1927. https://doi.org/10.1038/s41591-019-0654-5.

Liu, L., S. Hou, A. Zhu, et al. 2024. "The prognostic value of circulating tumor DNA in malignant melanoma patients treated with immune checkpoint inhibitors: a systematic review and meta-analysis." *Front Immunol* 15: 1520441. https://doi.org/10.3389/fimmu.2024.1520441.

Liu, Sixue, Prashanthi Dharanipragada, Shirley H. Lomeli, et al. 2023. "Multi-organ landscape of therapy-resistant melanoma." *Nature Medicine* 29 (5): 1123-1134. https://doi.org/10.1038/s41591-023-02304-9. https://doi.org/10.1038/s41591-023-02304-9.

Liubinas, S. V., N. Maartens, and K. J. Drummond. 2010. "Primary melanocytic neoplasms of the central nervous system." *J Clin Neurosci* 17 (10): 1227-32. https://doi.org/10.1016/j.jocn.2010.01.017.

Lodde, G. C., F. Zhao, R. Herbst, et al. 2024. "Early versus late response to PD-1-based immunotherapy in metastatic melanoma." *Eur J Cancer* 210: 114295. https://doi.org/10.1016/j.ejca.2024.114295.

Long, G. V., K. T. Flaherty, D. Stroyakovskiy, et al. 2017. "Dabrafenib plus trametinib versus dabrafenib monotherapy in patients with metastatic BRAF V600E/K-mutant melanoma: long-term survival and safety analysis of a phase 3 study." *Annals of Oncology* 28 (7): 1631-1639. https://doi.org/https://doi.org/10.1093/annonc/mdx176. https://www.sciencedirect.com/science/article/pii/S0923753419322707.

Long, G. V., A. Hauschild, M. Santinami, et al. 2024. "Final Results for Adjuvant Dabrafenib plus Trametinib in Stage III Melanoma." *N Engl J Med* 391 (18): 1709-1720. https://doi.org/10.1056/NEJMoa2404139.

Luke, J. J., R. Bao, R. F. Sweis, et al. 2019. "WNT/β-catenin Pathway Activation Correlates with Immune Exclusion across Human Cancers." *Clin Cancer Res* 25 (10): 3074-3083. https://doi.org/10.1158/1078-0432.Ccr-18-1942.

Luke, Jason J., Paolo A. Ascierto, Muhammad A. Khattak, et al. 2024. "Pembrolizumab Versus Placebo as Adjuvant Therapy in Resected Stage IIB or IIC Melanoma: Final Analysis of Distant Metastasis-Free Survival in the Phase III KEYNOTE-716 Study." *Journal of Clinical Oncology* 42 (14): 1619-1624. https://doi.org/10.1200/jco.23.02355. https://ascopubs.org/doi/abs/10.1200/JCO.23.02355.

Ma, Junfu, Yanxin Yao, Ye Tian, et al. 2022. "Advances in sex disparities for cancer immunotherapy: unveiling the dilemma of Yin and Yang." *Biology of Sex Differences* 13 (1): 58. https://doi.org/10.1186/s13293-022-00469-5. https://doi.org/10.1186/s13293-022-00469-5.

Man, W., and G. Wang. 2019. "Incidence, Outcomes and Predictors of Primary Central Nervous System Melanoma: A SEER-Based Study." *World Neurosurg* 129: e782-e790. https://doi.org/10.1016/j.wneu.2019.06.030.

Mandalà, M., B. Merelli, and D. Massi. 2016. "PD-L1 in melanoma: facts and myths." *Melanoma Manag* 3 (3): 187-194. https://doi.org/10.2217/mmt-2016-0013.

Mao, Lili, Zhonghui Qi, Li Zhang, et al. 2021. Immunotherapy in Acral and Mucosal Melanoma: Current Status and Future Directions. *Frontiers in immunology* 12: 680407. Accessed 2021. https://doi.org/10.3389/fimmu.2021.680407.

Marron, T. U., A. E. Ryan, S. M. Reddy, et al. 2021. "Considerations for treatment duration in responders to immune checkpoint inhibitors." *J Immunother Cancer* 9 (3). https://doi.org/10.1136/jitc-2020-001901.

Martins, Filipe, Latifyan Sofiya, Gerasimos P. Sykiotis, et al. 2019. "Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance." *Nature Reviews Clinical Oncology* 16 (9): 563-580. https://doi.org/10.1038/s41571-019-0218-0. https://doi.org/10.1038/s41571-019-0218-0.

Mayer, K. E., L. Warburton, A. Zaremba, et al. 2025. "Survival after cessation of immunotherapies in melanoma: A systematic review and meta-analysis." *J Eur Acad Dermatol Venereol*. https://doi.org/10.1111/jdv.20672.

McGale, J., J. Hama, R. Yeh, et al. 2023. "Artificial Intelligence and Radiomics: Clinical Applications for Patients with Advanced Melanoma Treated with Immunotherapy." *Diagnostics (Basel)* 13 (19). https://doi.org/10.3390/diagnostics13193065.

McKean, Meredith A., and Rodabe N. Amaria. 2018. "Multidisciplinary treatment strategies in high-risk resectable melanoma: Role of adjuvant and neoadjuvant therapy." *Cancer Treatment Reviews* 70: 144-153. https://doi.org/https://doi.org/10.1016/j.ctrv.2018.08.011. https://www.sciencedirect.com/science/article/pii/S0305737218301476.

Mengoni, M., F. O. Mahlo, E. Gaffal, et al. 2025. "Downregulation of MHC-I on Melanoma Cells and Decreased CD8+ T-Cell Infiltration Are Associated With Metastatic Spread and Resistance to Immunotherapy." *Lab Invest* 105 (3): 102209. https://doi.org/10.1016/j.labinv.2024.102209.

Milanovic, M., D. N. Y. Fan, D. Belenki, et al. 2018. "Senescence-associated reprogramming promotes cancer stemness." *Nature* 553 (7686): 96-100. https://doi.org/10.1038/nature25167.

Möhn, Nora, Gernot Beutel, Ralf Gutzmer, et al. 2019. "Neurological Immune Related Adverse Events Associated with Nivolumab, Ipilimumab, and Pembrolizumab Therapy—Review of the Literature and Future Outlook." *Journal of Clinical Medicine* 8 (11): 1777. https://www.mdpi.com/2077-0383/8/11/1777.

Mor, Adam, and Marianne Strazza. 2022. "Bridging the Gap: Connecting the Mechanisms of Immune-Related Adverse Events and Autoimmunity Through PD-1." *Frontiers in Cell and Developmental Biology* Volume 9 - 2021. https://doi.org/10.3389/fcell.2021.790386. https://www.frontiersin.org/journals/cell-and-developmental-biology/articles/10.3389/fcell.2021.790386.

Morrison, Carl, Sarabjot Pabla, Jeffrey M. Conroy, et al. 2018. "Predicting response to checkpoint inhibitors in melanoma beyond PD-L1 and mutational burden." *Journal for ImmunoTherapy of Cancer* 6 (1): 32. https://doi.org/10.1186/s40425-018-0344-8. https://doi.org/10.1186/s40425-018-0344-8.

Mort, R. L., I. J. Jackson, and E. E. Patton. 2015. "The melanocyte lineage in development and disease." *Development* 142 (4): 620-32. https://doi.org/10.1242/dev.106567.

Morton, Donald L., John F. Thompson, Alistair J. Cochran, et al. 2006. "Sentinel-Node Biopsy or Nodal Observation in Melanoma." *New England Journal of Medicine* 355 (13): 1307-1317. https://doi.org/doi:10.1056/NEJMoa060992. https://www.nejm.org/doi/full/10.1056/NEJMoa060992.

Müller-Hermelink, N., H. Braumüller, B. Pichler, et al. 2008a. "TNFR1 signaling and IFN-gamma signaling determine whether T cells induce tumor dormancy or promote multistage carcinogenesis." *Cancer Cell* 13 (6): 507-18. https://doi.org/10.1016/j.ccr.2008.04.001.

Müller-Hermelink, Nele, Heidi Braumüller, Bernd Pichler, et al. 2008b. "TNFR1 Signaling and IFN-γ Signaling Determine whether T Cells Induce Tumor Dormancy or Promote Multistage Carcinogenesis." *Cancer Cell* 13 (6): 507-518. https://doi.org/https://doi.org/10.1016/j.ccr.2008.04.001. https://www.sciencedirect.com/science/article/pii/S1535610808001219.

Myers, D. J., and E. A. Hyde. 2021. "Aggressive Nodular Malignant Melanoma." *Cureus* 13 (8): e16819. https://doi.org/10.7759/cureus.16819.

Nacchiero, Eleonora, Massimo Giotta, Fabio Robusto, et al. 2024. "The role of wide local excision of a primary lesion in cutaneous malignant melanoma: a retrospective analysis of its usefulness in local and general control of disease." *Melanoma Research* 34 (6). https://journals.lww.com/melanomaresearch/fulltext/2024/12000/the_role_of_wide_local_excision_of_a_primary.6.aspx.

Nakamura, Y., and Y. Fujisawa. 2018. "Diagnosis and Management of Acral Lentiginous Melanoma." *Curr Treat Options Oncol* 19 (8): 42. https://doi.org/10.1007/s11864-018-0560-y.

Nandini, Acharya, Sabatos-Peyton Catherine, and Anderson Ana Carrizosa. 2020. "Tim-3 finds its place in the cancer immunotherapy landscape." *Journal for ImmunoTherapy*

of Cancer 8 (1): e000911. https://doi.org/10.1136/jitc-2020-000911. https://jitcsite-bmj.vercel.app/content/8/1/e000911.

Nassar, K. W., and A. C. Tan. 2020. "The mutational landscape of mucosal melanoma." *Semin Cancer Biol* 61: 139-148. https://doi.org/10.1016/j.semcancer.2019.09.013.

Newell, F., P. A. Johansson, J. S. Wilmott, et al. 2022. "Comparative Genomics Provides Etiologic and Biological Insight into Melanoma Subtypes." *Cancer Discov* 12 (12): 2856-2879. https://doi.org/10.1158/2159-8290.Cd-22-0603.

Newell, Felicity, Ines Pires da Silva, Peter A. Johansson, et al. 2022. "Multiomic profiling of checkpoint inhibitor-treated melanoma: Identifying predictors of response and resistance, and markers of biological discordance." *Cancer Cell* 40 (1): 88-102.e7. https://doi.org/https://doi.org/10.1016/j.ccell.2021.11.012. https://www.sciencedirect.com/science/article/pii/S1535610821006127.

Newell, Felicity, James S. Wilmott, Peter A. Johansson, et al. 2020. "Whole-genome sequencing of acral melanoma reveals genomic complexity and diversity." *Nature Communications* 11 (1): 5259. https://doi.org/10.1038/s41467-020-18988-3. https://doi.org/10.1038/s41467-020-18988-3.

Nissan, Moriah H., Christine A. Pratilas, Alexis M. Jones, et al. 2014. "Loss of NF1 in Cutaneous Melanoma Is Associated with RAS Activation and MEK Dependence." *Cancer Research* 74 (8): 2340-2350. https://doi.org/10.1158/0008-5472.Can-13-2625. https://doi.org/10.1158/0008-5472.CAN-13-2625.

Ochenduszko, S., J. García Sanchez, M. J. J. Fita, et al. 2023. "Characteristics and outcomes of advanced melanoma patients with complete response and elective discontinuation of first-line anti-programmed death-1 monotherapy: A real-world multicentre observational cohort study." *Pigment Cell Melanoma Res* 36 (5): 388-398. https://doi.org/10.1111/pcmr.13093.

Oh, A., D. M. Tran, L. C. McDowell, et al. 2017. "Cost-Effectiveness of Nivolumab-Ipilimumab Combination Therapy Compared with Monotherapy for First-Line Treatment of Metastatic Melanoma in the United States." *J Manag Care Spec Pharm* 23 (6): 653-664. https://doi.org/10.18553/jmcp.2017.23.6.653.

Owen, C. N., X. Bai, T. Quah, et al. 2021. "Delayed immune-related adverse events with anti-PD-1-based immunotherapy in melanoma." *Ann Oncol* 32 (7): 917-925. https://doi.org/10.1016/j.annonc.2021.03.204.

Park, Hyo Jung, Gun Ha Kim, Kyung Won Kim, et al. 2021. "Comparison of RECIST 1.1 and iRECIST in Patients Treated with Immune Checkpoint Inhibitors: A Systematic Review and Meta-Analysis." *Cancers* 13 (1): 120. https://www.mdpi.com/2072-6694/13/1/120.

Patnaik, A., T. A. Yap, H. C. Chung, et al. 2021. "Safety and Clinical Activity of a New Anti-PD-L1 Antibody as Monotherapy or Combined with Targeted Therapy in Advanced Solid Tumors: The PACT Phase Ia/Ib Trial." *Clin Cancer Res* 27 (5): 1267-1277. https://doi.org/10.1158/1078-0432.Ccr-20-2821.

Patton, E. E., K. L. Mueller, D. J. Adams, et al. 2021. "Melanoma models for the next generation of therapies." *Cancer Cell* 39 (5): 610-631. https://doi.org/10.1016/j.ccell.2021.01.011.

Pedersen, Sidsel, Emma Lund Johansen, Karen Louise Højholt, et al. 2025. "Survival improvements in patients with melanoma brain metastases and leptomeningeal disease in the modern era: Insights from a nationwide study (2015–2022)." *European Journal of Cancer* 217. https://doi.org/10.1016/j.ejca.2025.115253. https://doi.org/10.1016/j.ejca.2025.115253.

Pellerino, A., R. M. Verdijk, L. Nichelli, et al. 2024. "Primary Meningeal Melanocytic Tumors of the Central Nervous System: A Review from the Ultra-Rare Brain Tumors Task Force of the European Network for Rare Cancers (EURACAN)." *Cancers (Basel)* 16 (14). https://doi.org/10.3390/cancers16142508.

Perez, L., W. Samlowski, and R. Lopez-Flores. 2022. "Outcome of Elective Checkpoint Inhibitor Discontinuation in Patients with Metastatic Melanoma Who Achieved a Complete Remission: Real-World Data." *Biomedicines* 10 (5). https://doi.org/10.3390/biomedicines10051144.

Petersen, Soeren Kjaer, Christian Rønn Hansen, Eva Ellebaek, et al. 2024. "Does patient sex affect the treatment outcome of immune checkpoint inhibitors? A Danish, observational melanoma study." *European Journal of Cancer* 205. https://doi.org/10.1016/j.ejca.2024.114099. https://doi.org/10.1016/j.ejca.2024.114099.

Petrelli, F., R. Ardito, B. Merelli, et al. 2019. "Prognostic and predictive role of elevated lactate dehydrogenase in patients with melanoma treated with immunotherapy and BRAF inhibitors: a systematic review and meta-analysis." *Melanoma Res* 29 (1): 1-12. https://doi.org/10.1097/cmr.0000000000000520.

Pham, J. P., A. M. Joshua, I. P. da Silva, et al. 2023. "Chemotherapy in Cutaneous Melanoma: Is There Still a Role?" *Curr Oncol Rep* 25 (6): 609-621. https://doi.org/10.1007/s11912-023-01385-6.

Pillai, M., Y. Jiang, P. C. Lorigan, et al. 2022. "Clinical feasibility and treatment outcomes with nonselected autologous tumor-infiltrating lymphocyte therapy in patients with advanced cutaneous melanoma." *Am J Cancer Res* 12 (8): 3967-3984.

Piulats, J. M., C. Watkins, M. Costa-García, et al. 2024. "Overall survival from tebentafusp versus nivolumab plus ipilimumab in first-line metastatic uveal melanoma: a propensity score-weighted analysis." *Ann Oncol* 35 (3): 317-326. https://doi.org/10.1016/j.annonc.2023.11.013.

Placke, Jan-Malte, Luisa Sophie Rajcsanyi, Rudolf Herbst, et al. 2025. "Presence of brain metastasis differentially impacts long-term survival after first-line therapy in melanoma depending on BRAF mutation status." *Frontiers in Immunology* Volume 16 - 2025. https://doi.org/10.3389/fimmu.2025.1536642. https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2025.1536642.

Pollard, Jeffrey W. 2016. "Defining Metastatic Cell Latency." *The New England journal of medicine* 375 (3): 280-282. https://doi.org/10.1056/NEJMCIBR1606716. https://pubmed.ncbi.nlm.nih.gov/27468066/.

Porciello, Nicla, Ornella Franzese, Lorenzo D'Ambrosio, et al. 2022. "T-cell repertoire diversity: friend or foe for protective antitumor response?" *Journal of Experimental & Clinical Cancer Research* 41 (1): 356. https://doi.org/10.1186/s13046-022-02566-0. https://doi.org/10.1186/s13046-022-02566-0.

Postow, Michael A., Jason Chesney, Anna C. Pavlick, et al. 2015. "Nivolumab and Ipilimumab versus Ipilimumab in Untreated Melanoma." *New England Journal of Medicine* 372 (21): 2006-2017. https://doi.org/doi:10.1056/NEJMoa1414428. https://www.nejm.org/doi/full/10.1056/NEJMoa1414428.

Potrony, M., C. Badenas, P. Aguilera, et al. 2015. "Update in genetic susceptibility in melanoma." *Ann Transl Med* 3 (15): 210. https://doi.org/10.3978/j.issn.2305-5839.2015.08.11.

Puzanov, I., A. Diab, K. Abdallah, et al. 2017. "Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group." *Journal for ImmunoTherapy of Cancer* 5 (1): 95. https://doi.org/10.1186/s40425-017-0300-z. https://doi.org/10.1186/s40425-017-0300-z.

Quon, P. L., Y. Xiao, S. Sorensen, et al. 2019. "Economic Evaluation of Nivolumab Plus Ipilimumab Combination as First-Line Treatment for Patients with Advanced Melanoma in Canada." *Pharmacoecon Open* 3 (3): 321-331. https://doi.org/10.1007/s41669-018-0112-1.

Rabbie, R., P. Ferguson, K. Wong, et al. 2021. "The mutational landscape of melanoma brain metastases presenting as the first visceral site of recurrence." *Br J Cancer* 124 (1): 156-160. https://doi.org/10.1038/s41416-020-01090-2.

Radny, P., U. M. Caroli, J. Bauer, et al. 2003. "Phase II trial of intralesional therapy with interleukin-2 in soft-tissue melanoma metastases." *Br J Cancer* 89 (9): 1620-6. https://doi.org/10.1038/sj.bjc.6601320.

Raimondi, S., M. Suppa, and S. Gandini. 2020. "Melanoma Epidemiology and Sun Exposure." *Acta Derm Venereol* 100 (11): adv00136. https://doi.org/10.2340/00015555-3491.

Ramani, N. S., A. C. Morani, and S. Zhang. 2022. "MET Gene High Copy Number (Amplification/Polysomy) Identified in Melanoma for Potential Targeted Therapy." *Am J Clin Pathol* 157 (4): 502-505. https://doi.org/10.1093/ajcp/aqab171.

Ray, A., M. A. Williams, S. M. Meek, et al. 2016. "A phase I study of intratumoral ipilimumab and interleukin-2 in patients with advanced melanoma." *Oncotarget* 7 (39): 64390-64399. https://doi.org/10.18632/oncotarget.10453.

Redmer, Torben, Elisa Schumann, Kristin Peters, et al. 2024. "MET receptor serves as a promising target in melanoma brain metastases." *Acta Neuropathologica* 147 (1): 44. https://doi.org/10.1007/s00401-024-02694-1. https://doi.org/10.1007/s00401-024-02694-1.

Reschke, Robin, Alexander H. Enk, and Jessica C. Hassel. 2025. "Prognostic Biomarkers in Evolving Melanoma Immunotherapy." *American Journal of Clinical Dermatology* 26 (2): 213-223. https://doi.org/10.1007/s40257-024-00910-y. https://doi.org/10.1007/s40257-024-00910-y.

Rhodin, Kristen E., Denisse Porras Fimbres, Danielle N. Burner, et al. 2022. "Melanoma lymph node metastases – moving beyond quantity in clinical trial design and contemporary practice." *Frontiers in Oncology* Volume 12 - 2022. https://doi.org/10.3389/fonc.2022.1021057. https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2022.1021057.

Richtig, G., C. Hoeller, M. Wolf, et al. 2018. "Body mass index may predict the response to ipilimumab in metastatic melanoma: An observational multi-centre study." *PLoS One* 13 (10): e0204729. https://doi.org/10.1371/journal.pone.0204729.

Robert, C., A. Ribas, O. Hamid, et al. 2018. "Durable Complete Response After Discontinuation of Pembrolizumab in Patients With Metastatic Melanoma." *J Clin Oncol* 36 (17): 1668-1674. https://doi.org/10.1200/jco.2017.75.6270.

Robert, C., J. Schachter, G. V. Long, et al. 2015. "Pembrolizumab versus Ipilimumab in Advanced Melanoma." *N Engl J Med* 372 (26): 2521-32. https://doi.org/10.1056/NEJMoa1503093.

Robert, Caroline, Antoni Ribas, Jacob Schachter, et al. 2019. "Pembrolizumab versus ipilimumab in advanced melanoma (KEYNOTE-006): post-hoc 5-year results from an open-label, multicentre, randomised, controlled, phase 3 study." *The Lancet Oncology* 20 (9): 1239-1251. https://doi.org/10.1016/S1470-2045(19)30388-2. https://doi.org/10.1016/S1470-2045(19)30388-2.

Robert, Caroline, Dirk Schadendorf, Marianne Messina, et al. 2013. "Efficacy and Safety of Retreatment with Ipilimumab in Patients with Pretreated Advanced Melanoma Who Progressed after Initially Achieving Disease Control." *Clinical Cancer Research* 19 (8): 2232-2239. https://doi.org/10.1158/1078-0432.Ccr-12-3080. https://doi.org/10.1158/1078-0432.CCR-12-3080.

Rohaan, M. W., T. H. Borch, J. H. van den Berg, et al. 2022. "Tumor-Infiltrating Lymphocyte Therapy or Ipilimumab in Advanced Melanoma." *N Engl J Med* 387 (23): 2113-2125. https://doi.org/10.1056/NEJMoa2210233.

Roisman, Laila C., Waleed Kian, Alaa Anoze, et al. 2023. "Radiological artificial intelligence - predicting personalized immunotherapy outcomes in lung cancer." *npj Precision Oncology* 7 (1): 125. https://doi.org/10.1038/s41698-023-00473-x. https://doi.org/10.1038/s41698-023-00473-x.

Rubatto, M., P. Fava, I. Stanganelli, et al. 2023. "Discontinuation of anti-PD1 in advanced melanoma: an observational retrospective study from the Italian Melanoma Intergroup." *Eur J Cancer* 187: 25-35. https://doi.org/10.1016/j.ejca.2023.03.020.

Rubió-Casadevall, J., A. Carbó-Bagué, M. Puigdemont, et al. 2021. "Population-based analysis of the prevalence of BRAF mutation in patients diagnosed with cutaneous melanoma and its significance as a prognostic factor." *Eur J Dermatol* 31 (5): 616-622. https://doi.org/10.1684/ejd.2021.4136.

Rutkowski, Piotr, and Paulina Jagodzińska-Mucha. 2023. "What role can LAG-3-blocking antibodies play in melanoma therapy?" *Expert Opinion on Biological Therapy* 23 (12): 1155-1158. https://doi.org/10.1080/14712598.2023.2281495. https://doi.org/10.1080/14712598.2023.2281495.

Rydén, Viktoria, Ali Inan El-Naggar, Anthoula Koliadi, et al. 2024. "The role of dacarbazine and temozolomide therapy after treatment with immune checkpoint inhibitors in malignant melanoma patients: A case series and meta-analysis." *Pigment Cell & Melanoma Research* 37 (3): 352-362. https://doi.org/https://doi.org/10.1111/pcmr.13156. https://onlinelibrary.wiley.com/doi/abs/10.1111/pcmr.13156.

Sacco, J. J., R. D. Carvajal, M. O. Butler, et al. 2024. "Long-term survival follow-up for tebentafusp in previously treated metastatic uveal melanoma." *J Immunother Cancer* 12 (6). https://doi.org/10.1136/jitc-2024-009028.

Sachin, R. Jhawar, Wang Shang-Jui, Thandoni Aditya, et al. 2023. "Combination oncolytic virus, radiation therapy, and immune checkpoint inhibitor treatment in anti-PD-1-refractory cancer." *Journal for ImmunoTherapy of Cancer* 11 (7): e006780. https://doi.org/10.1136/jitc-2023-006780. https://jitcsite-bmj.vercel.app/content/11/7/e006780.

Sade-Feldman, M., Y. J. Jiao, J. H. Chen, et al. 2017. "Resistance to checkpoint blockade therapy through inactivation of antigen presentation." *Nat Commun* 8 (1): 1136. https://doi.org/10.1038/s41467-017-01062-w.

Sadeq, Mohammed Ahmed, Mohamed Hady Ashry, Reem Mohammed Farouk Ghorab, et al. 2023. "Causes of death among patients with cutaneous melanoma: a US population-based study." *Scientific Reports* 13 (1): 10257. https://doi.org/10.1038/s41598-023-37333-4. https://doi.org/10.1038/s41598-023-37333-4.

Schadendorf, D., J. J. Luke, P. A. Ascierto, et al. 2024. "Pembrolizumab versus placebo as adjuvant therapy in resected stage IIB or IIC melanoma: Outcomes in histopathologic subgroups from the randomized, double-blind, phase 3 KEYNOTE-716 trial." *J Immunother Cancer* 12 (3). https://doi.org/10.1136/jitc-2023-007501.

Schroeder, Christopher, Sergios Gatidis, Olga Kelemen, et al. 2024. "Tumour-informed liquid biopsies to monitor advanced melanoma patients under immune checkpoint inhibition." *Nature Communications* 15 (1): 8750. https://doi.org/10.1038/s41467-024-52923-0. https://doi.org/10.1038/s41467-024-52923-0.

Schumann, K., K. C. Klespe, C. Mauch, et al. 2025. "Switching PD-1 to BRAF + MEK inhibition improves recurrence-free survival in patients receiving a second course of adjuvant melanoma therapy." *J Eur Acad Dermatol Venereol*. https://doi.org/10.1111/jdv.20708.

Scolyer, R. A., G. V. Long, and J. F. Thompson. 2011. "Evolving concepts in melanoma classification and their relevance to multidisciplinary melanoma patient care." *Mol Oncol* 5 (2): 124-36. https://doi.org/10.1016/j.molonc.2011.03.002.

Scolyer, Richard A., Robert V. Rawson, Jeffrey E. Gershenwald, et al. 2020. "Melanoma pathology reporting and staging." *Modern Pathology* 33: 15-24. https://doi.org/https://doi.org/10.1038/s41379-019-0402-x. https://www.sciencedirect.com/science/article/pii/S0893395222007165.

Seidel, Judith A., Atsushi Otsuka, and Kenji Kabashima. 2018. "Anti-PD-1 and Anti-CTLA-4 Therapies in Cancer: Mechanisms of Action, Efficacy, and Limitations." *Frontiers in Oncology* Volume 8 - 2018. https://doi.org/10.3389/fonc.2018.00086. https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2018.00086.

Sergi, Maria Chiara, Elisabetta Filoni, Giacomo Triggiano, et al. 2023. "Mucosal Melanoma: Epidemiology, Clinical Features, and Treatment." *Current Oncology Reports* 25 (11): 1247-1258. https://doi.org/10.1007/s11912-023-01453-x. https://doi.org/10.1007/s11912-023-01453-x.

Seth, Rahul, Sanjiv S. Agarwala, Hans Messersmith, et al. 2023. "Systemic Therapy for Melanoma: ASCO Guideline Update." *Journal of Clinical Oncology* 41 (30): 4794-4820. https://doi.org/10.1200/jco.23.01136. https://ascopubs.org/doi/abs/10.1200/JCO.23.01136.

Shaikh, Ladan, Richard W. Sagebiel, Carlos M. M. Ferreira, et al. 2005. "The Role of Microsatellites as a Prognostic Factor in Primary Malignant Melanoma." *Archives of Dermatology* 141 (6): 739-742. https://doi.org/10.1001/archderm.141.6.739. https://doi.org/10.1001/archderm.141.6.739.

Shannon, A. B., J. S. Zager, and M. C. Perez. 2024. "Clinical Characteristics and Special Considerations in the Management of Rare Melanoma Subtypes." *Cancers (Basel)* 16 (13). https://doi.org/10.3390/cancers16132395.

Sharma, A. N., D. P. Foulad, L. Doan, et al. 2021. "Mohs surgery for the treatment of lentigo maligna and lentigo maligna melanoma - a systematic review." *J Dermatolog Treat* 32 (2): 157-163. https://doi.org/10.1080/09546634.2019.1690624.

Shin, D. S., J. M. Zaretsky, H. Escuin-Ordinas, et al. 2017. "Primary Resistance to PD-1 Blockade Mediated by JAK1/2 Mutations." *Cancer Discov* 7 (2): 188-201. https://doi.org/10.1158/2159-8290.Cd-16-1223.

Shivaji, U. N., L. Jeffery, X. Gui, et al. 2019. "Immune checkpoint inhibitor-associated gastrointestinal and hepatic adverse events and their management." *Therap Adv Gastroenterol* 12: 1756284819884196. https://doi.org/10.1177/1756284819884196.

Singh, P., H. J. Kim, and R. A. Schwartz. 2016. "Superficial spreading melanoma: an analysis of 97 702 cases using the SEER database." *Melanoma Res* 26 (4): 395-400. https://doi.org/10.1097/cmr.0000000000000245.

Singvogel, Kathrin, and Birgit Schittek. 2024. "Dormancy of cutaneous melanoma." *Cancer Cell International* 24 (1): 88. https://doi.org/10.1186/s12935-024-03278-5. https://doi.org/10.1186/s12935-024-03278-5.

Sneyd, Mary Jane, and Brian Cox. 2013. "A comparison of trends in melanoma mortality in New Zealand and Australia: the two countries with the highest melanoma incidence and mortality in the world." *BMC Cancer* 13 (1): 372. https://doi.org/10.1186/1471-2407-13-372. https://doi.org/10.1186/1471-2407-13-372.

Snyman, Marelize, Rachel Elizabeth Walsdorf, Sophia Nicole Wix, et al. 2024. "The metabolism of melanin synthesis—From melanocytes to melanoma." *Pigment Cell & Melanoma Research* 37 (4): 438-452. https://doi.org/https://doi.org/10.1111/pcmr.13165. https://onlinelibrary.wiley.com/doi/abs/10.1111/pcmr.13165.

Song, Y., R. J. Straker, 3rd, X. Xu, et al. 2020. "Neoadjuvant Versus Adjuvant Immune Checkpoint Blockade in the Treatment of Clinical Stage III Melanoma." *Ann Surg Oncol* 27 (8): 2915-2926. https://doi.org/10.1245/s10434-019-08174-1.

Sosa, M. S., P. Bragado, and J. A. Aguirre-Ghiso. 2014. "Mechanisms of disseminated cancer cell dormancy: an awakening field." *Nat Rev Cancer* 14 (9): 611-22. https://doi.org/10.1038/nrc3793.

Soumoy, L., V. Quidville, C. Sarre-Pradon, et al. 2024. "3MO LDH isoforms shape the immune microenvironment and impact treatment response and survival in metastatic melanoma." *Immuno-Oncology and Technology* 24. https://doi.org/10.1016/j.iotech.2024.100887. https://doi.org/10.1016/j.iotech.2024.100887.

Spathis, A., A. C. Katoulis, V. Damaskou, et al. 2019. "BRAF Mutation Status in Primary, Recurrent, and Metastatic Malignant Melanoma and Its Relation to Histopathological Parameters." *Dermatol Pract Concept* 9 (1): 54-62. https://doi.org/10.5826/dpc.0901a13.

Sperduto, P. W., A. K. S. Salama, and C. Anders. 2023. "Progress for patients with melanoma brain metastases." *Neuro Oncol* 25 (7): 1321-1322. https://doi.org/10.1093/neuonc/noad050.

Su, J., Y. Fu, Z. Cui, et al. 2023. "Relatlimab: a novel drug targeting immune checkpoint LAG-3 in melanoma therapy." *Front Pharmacol* 14: 1349081. https://doi.org/10.3389/fphar.2023.1349081.

Su, Jialin, Yuning Li, Shuhua Tan, et al. 2025. "Pretreatment neutrophil-to-lymphocyte ratio is associated with immunotherapy efficacy in patients with advanced cancer: a systematic review and meta-analysis." *Scientific Reports* 15 (1): 446.

https://doi.org/10.1038/s41598-024-84890-3. https://doi.org/10.1038/s41598-024-84890-3.
Sun, C., S. España, N. Richarz, et al. 2024. "Targeted therapy or immunotherapy in BRAF-mutated metastatic melanoma: a Spanish center's decade of experience." *Front Oncol* 14: 1322116. https://doi.org/10.3389/fonc.2024.1322116.
Sun, J., K. M. Karasaki, and J. M. Farma. 2024. "The Use of Gene Expression Profiling and Biomarkers in Melanoma Diagnosis and Predicting Recurrence: Implications for Surveillance and Treatment." *Cancers (Basel)* 16 (3). https://doi.org/10.3390/cancers16030583.
Sun, Q., H. Sun, N. Wu, et al. 2022. "Patients with melanoma treated with immune checkpoint inhibitors who had non-thyroid endocrine and skin immune-related adverse events have better prognosis: A systematic review and meta-analysis." *Front Oncol* 12: 976224. https://doi.org/10.3389/fonc.2022.976224.
Susok, L., M. Stücker, F. G. Bechara, et al. 2021. "Multivariate analysis of prognostic factors in patients with nodular melanoma." *J Cancer Res Clin Oncol* 147 (9): 2759-2764. https://doi.org/10.1007/s00432-021-03562-1.
Sussman, T. A., and P. A. Ott. 2024. "Adjuvant immunotherapy for melanoma patients: progress and opportunities." *ESMO Open* 9 (5): 102962. https://doi.org/10.1016/j.esmoop.2024.102962.
Swami, U., V. Monga, A. D. Bossler, et al. 2019. "Durable Clinical Benefit in Patients with Advanced Cutaneous Melanoma after Discontinuation of Anti-PD-1 Therapies Due to Immune-Related Adverse Events." *J Oncol* 2019: 1856594. https://doi.org/10.1155/2019/1856594.
Switzer, B., I. Puzanov, J. J. Skitzki, et al. 2022. "Managing Metastatic Melanoma in 2022: A Clinical Review." *JCO Oncol Pract* 18 (5): 335-351. https://doi.org/10.1200/op.21.00686.
Tang, Wei, Jun Chen, Tianlong Ji, et al. 2023. "TIGIT, a novel immune checkpoint therapy for melanoma." *Cell Death & Disease* 14 (7): 466. https://doi.org/10.1038/s41419-023-05961-3. https://doi.org/10.1038/s41419-023-05961-3.
Tawbi, Hussein A., F. Stephen Hodi, Evan J. Lipson, et al. 2025. "Three-Year Overall Survival With Nivolumab Plus Relatlimab in Advanced Melanoma From RELATIVITY-047." *Journal of Clinical Oncology* 43 (13): 1546-1552. https://doi.org/10.1200/jco.24.01124. https://ascopubs.org/doi/abs/10.1200/JCO.24.01124.
Tawbi, Hussein A., Dirk Schadendorf, Evan J. Lipson, et al. 2022. "Relatlimab and Nivolumab versus Nivolumab in Untreated Advanced Melanoma." *New England Journal of Medicine* 386 (1): 24-34. https://doi.org/10.1056/NEJMoa2109970. https://www.nejm.org/doi/full/10.1056/NEJMoa2109970.
Taylor, John, Aesha Gandhi, Elin Gray, et al. 2023. "Checkpoint inhibitor immune-related adverse events: A focused review on autoantibodies and B cells as biomarkers, advancements and future possibilities." *Frontiers in Immunology* Volume 13 - 2022. https://doi.org/10.3389/fimmu.2022.991433. https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.991433.
Teixidó, Cristina, Maria González-Cao, Niki Karachaliou, et al. 2015. "Predictive factors for immunotherapy in melanoma." *Annals of Translational Medicine* 3 (15): 208. https://atm.amegroups.org/article/view/6579.
Teng, Y. S., and S. Yu. 2023. "Molecular Mechanisms of Cutaneous Immune-Related Adverse Events (irAEs) Induced by Immune Checkpoint Inhibitors." *Curr Oncol* 30 (7): 6805-6819. https://doi.org/10.3390/curroncol30070498.
Thomas, J. Meirion, Julia Newton-Bishop, Roger A'Hern, et al. 2004. "Excision Margins in High-Risk Malignant Melanoma." *New England Journal of Medicine* 350 (8): 757-766. https://doi.org/doi:10.1056/NEJMoa030681. https://www.nejm.org/doi/full/10.1056/NEJMoa030681.
Tjokrowidjaja, A., L. Browne, and H. Soudy. 2022. "External validation of the American Joint Committee on Cancer melanoma staging system eighth edition using the surveillance, epidemiology, and end results program." *Asia Pac J Clin Oncol* 18 (5): e280-e288. https://doi.org/10.1111/ajco.13689.

Tolstrup, L. K., H. Pappot, L. Bastholt, et al. 2022. "Impact of patient-reported outcomes on symptom monitoring during treatment with checkpoint inhibitors: health-related quality of life among melanoma patients in a randomized controlled trial." *J Patient Rep Outcomes* 6 (1): 8. https://doi.org/10.1186/s41687-022-00414-5.

Trindade, F. M., M. L. P. de Freitas, and F. V. Bittencourt. 2021. "Dermoscopic evaluation of superficial spreading melanoma." *An Bras Dermatol* 96 (2): 139-147. https://doi.org/10.1016/j.abd.2020.06.012.

Trojaniello, C., F. Sparano, E. Cioli, et al. 2023. "Sequencing Targeted and Immune Therapy in BRAF-Mutant Melanoma: Lessons Learned." *Curr Oncol Rep* 25 (6): 623-634. https://doi.org/10.1007/s11912-023-01402-8.

Tsatsou, F., M. Trakatelli, A. Patsatsi, et al. 2012. "Extrinsic aging: UV-mediated skin carcinogenesis." *Dermatoendocrinol* 4 (3): 285-97. https://doi.org/10.4161/derm.22519.

Ulmer, A., K. Dietz, M. Werner-Klein, et al. 2018. "The sentinel lymph node spread determines quantitatively melanoma seeding to non-sentinel lymph nodes and survival." *Eur J Cancer* 91: 1-10. https://doi.org/10.1016/j.ejca.2017.12.002.

Utjés, D., J. Malmstedt, J. Teras, et al. 2019. "2-cm versus 4-cm surgical excision margins for primary cutaneous melanoma thicker than 2 mm: long-term follow-up of a multicentre, randomised trial." *Lancet* 394 (10197): 471-477. https://doi.org/10.1016/s0140-6736(19)31132-8.

Valentini, R., J. Quinn, and M. J. Murphy. 2025. "Nevoid melanoma." *Clin Dermatol* 43 (1): 29-35. https://doi.org/10.1016/j.clindermatol.2025.01.012.

Valpione, Sara, Piyushkumar A. Mundra, Elena Galvani, et al. 2021. "The T cell receptor repertoire of tumor infiltrating T cells is predictive and prognostic for cancer survival." *Nature Communications* 12 (1): 4098. https://doi.org/10.1038/s41467-021-24343-x. https://doi.org/10.1038/s41467-021-24343-x.

van Akkooi, A. C., Z. I. Nowecki, C. Voit, et al. 2008. "Sentinel node tumor burden according to the Rotterdam criteria is the most important prognostic factor for survival in melanoma patients: a multicenter study in 388 patients with positive sentinel nodes." *Ann Surg* 248 (6): 949-55. https://doi.org/10.1097/SLA.0b013e31818fefe0.

van Breeschoten, Jesper, Michel W. J. M. Wouters, Doranne L. Hilarius, et al. 2021. "First-line BRAF/MEK inhibitors versus anti-PD-1 monotherapy in BRAFV600-mutant advanced melanoma patients: a propensity-matched survival analysis." *British Journal of Cancer* 124 (7): 1222-1230. https://doi.org/10.1038/s41416-020-01229-1. https://doi.org/10.1038/s41416-020-01229-1.

van der Ploeg, A. P., A. C. van Akkooi, P. Rutkowski, et al. 2011. "Prognosis in patients with sentinel node-positive melanoma is accurately defined by the combined Rotterdam tumor load and Dewar topography criteria." *J Clin Oncol* 29 (16): 2206-14. https://doi.org/10.1200/jco.2010.31.6760.

van Not, Olivier J., Rik J. Verheijden, Alfonsus J. M. van den Eertwegh, et al. 2022. "Association of Immune-Related Adverse Event Management With Survival in Patients With Advanced Melanoma." *JAMA Oncology* 8 (12): 1794-1801. https://doi.org/10.1001/jamaoncol.2022.5041. https://doi.org/10.1001/jamaoncol.2022.5041.

Vanni, I., E. T. Tanda, F. Spagnolo, et al. 2020. "The Current State of Molecular Testing in the BRAF-Mutated Melanoma Landscape." *Front Mol Biosci* 7: 113. https://doi.org/10.3389/fmolb.2020.00113.

Vanni, Irene, Enrica Teresa Tanda, Bruna Dalmasso, et al. 2020. "Non-BRAF Mutant Melanoma: Molecular Features and Therapeutical Implications." *Frontiers in Molecular Biosciences* Volume 7 - 2020. https://doi.org/10.3389/fmolb.2020.00172. https://www.frontiersin.org/journals/molecular-biosciences/articles/10.3389/fmolb.2020.00172.

Velho, T. R. 2012. "Metastatic melanoma - a review of current and future drugs." *Drugs Context* 2012: 212242. https://doi.org/10.7573/dic.212242.

Venna, Suraj S., Suresh Thummala, Mehdi Nosrati, et al. 2013. "Analysis of sentinel lymph node positivity in patients with thin primary melanoma." *Journal of the American*

Academy of Dermatology 68 (4): 560-567. https://doi.org/https://doi.org/10.1016/j.jaad.2012.08.045. https://www.sciencedirect.com/science/article/pii/S0190962212010511.

Verma, V., T. Sprave, W. Haque, et al. 2018. "A systematic review of the cost and cost-effectiveness studies of immune checkpoint inhibitors." *J Immunother Cancer* 6 (1): 128. https://doi.org/10.1186/s40425-018-0442-7.

Versluis, J. M., A. M. Menzies, K. Sikorska, et al. 2023. "Survival update of neoadjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma in the OpACIN and OpACIN-neo trials." *Ann Oncol* 34 (4): 420-430. https://doi.org/10.1016/j.annonc.2023.01.004.

Vredeveld, L. C., P. A. Possik, M. A. Smit, et al. 2012. "Abrogation of BRAFV600E-induced senescence by PI3K pathway activation contributes to melanomagenesis." *Genes Dev* 26 (10): 1055-69. https://doi.org/10.1101/gad.187252.112.

Vyas, R., J. J. Keller, K. Honda, et al. 2015. "A systematic review and meta-analysis of animal-type melanoma." *J Am Acad Dermatol* 73 (6): 1031-9. https://doi.org/10.1016/j.jaad.2015.08.016.

Wadasadawala, T., S. Trivedi, T. Gupta, et al. 2010. "The diagnostic dilemma of primary central nervous system melanoma." *J Clin Neurosci* 17 (8): 1014-1017. https://doi.org/10.1016/j.jocn.2009.12.020.

Wagner, N. B., A. Forschner, U. Leiter, et al. 2018. "S100B and LDH as early prognostic markers for response and overall survival in melanoma patients treated with anti-PD-1 or combined anti-PD-1 plus anti-CTLA-4 antibodies." *Br J Cancer* 119 (3): 339-346. https://doi.org/10.1038/s41416-018-0167-x.

Wang, D. Y., and D. B. Johnson. 2016. "Advances in the development of intralesional therapies for melanoma." *Melanoma Manag* 3 (4): 259-266. https://doi.org/10.2217/mmt-2016-0020.

Wang, Meng, Ishani Banik, A. Hunter Shain, et al. 2022. "Integrated genomic analyses of acral and mucosal melanomas nominate novel driver genes." *Genome Medicine* 14 (1): 65. https://doi.org/10.1186/s13073-022-01068-0. https://doi.org/10.1186/s13073-022-01068-0.

Wang, S. J., S. K. Dougan, and M. Dougan. 2023. "Immune mechanisms of toxicity from checkpoint inhibitors." *Trends Cancer* 9 (7): 543-553. https://doi.org/10.1016/j.trecan.2023.04.002.

Wang, Z., X. Zou, H. Wang, et al. 2024. "Companion diagnostics and predictive biomarkers for PD-1/PD-L1 immune checkpoint inhibitors therapy in malignant melanoma." *Front Immunol* 15: 1454720. https://doi.org/10.3389/fimmu.2024.1454720.

Warburton, L., L. Calapre, M. R. Pereira, et al. 2020. "Circulating Tumour DNA in Advanced Melanoma Patients Ceasing PD1 Inhibition in the Absence of Disease Progression." *Cancers (Basel)* 12 (11). https://doi.org/10.3390/cancers12113486.

Warburton, Lydia, Anna Reid, Benhur Amanuel, et al. 2023. "Detectable ctDNA at the time of treatment cessation of ipilimumab and nivolumab for toxicity predicts disease progression in advanced melanoma patients." *Frontiers in Oncology* Volume 13 - 2023. https://doi.org/10.3389/fonc.2023.1280730. https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2023.1280730.

Warner, Allison Betof, Jessica S. Palmer, Alexander N. Shoushtari, et al. 2020. "Long-Term Outcomes and Responses to Retreatment in Patients With Melanoma Treated With PD-1 Blockade." *Journal of Clinical Oncology* 38 (15): 1655-1663. https://doi.org/10.1200/jco.19.01464. https://ascopubs.org/doi/abs/10.1200/JCO.19.01464.

Watson, Alexander S., Siddhartha Goutam, Igor Stukalin, et al. 2022. "Association of Immune-Related Adverse Events, Hospitalization, and Therapy Resumption With Survival Among Patients With Metastatic Melanoma Receiving Single-Agent or Combination Immunotherapy." *JAMA Network Open* 5 (12): e2245596-e2245596. https://doi.org/10.1001/jamanetworkopen.2022.45596. https://doi.org/10.1001/jamanetworkopen.2022.45596.

Weber, J. S., T. Poretta, B. D. Stwalley, et al. 2023. "Nivolumab versus placebo as adjuvant therapy for resected stage III melanoma: a propensity weighted indirect treatment comparison and number needed to treat analysis for recurrence-free survival and overall survival." *Cancer Immunol Immunother* 72 (4): 945-954. https://doi.org/10.1007/s00262-022-03302-5.

Whiteman, David C., William J. Pavan, and Boris C. Bastian. 2011. "The melanomas: a synthesis of epidemiological, clinical, histopathological, genetic, and biological aspects, supporting distinct subtypes, causal pathways, and cells of origin." *Pigment Cell & Melanoma Research* 24 (5): 879-897. https://doi.org/https://doi.org/10.1111/j.1755-148X.2011.00880.x. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1755-148X.2011.00880.x.

Whitman, E. D., V. P. Koshenkov, B. R. Gastman, et al. 2021. "Integrating 31-Gene Expression Profiling With Clinicopathologic Features to Optimize Cutaneous Melanoma Sentinel Lymph Node Metastasis Prediction." *JCO Precis Oncol* 5. https://doi.org/10.1200/po.21.00162.

Wiesner, T., M. Kiuru, S. N. Scott, et al. 2015. "NF1 Mutations Are Common in Desmoplastic Melanoma." *Am J Surg Pathol* 39 (10): 1357-62. https://doi.org/10.1097/pas.0000000000000451.

Wolchok, J. D., V. Chiarion-Sileni, R. Gonzalez, et al. 2017. "Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma." *N Engl J Med* 377 (14): 1345-1356. https://doi.org/10.1056/NEJMoa1709684.

Wong, Selina K., Caroline A. Nebhan, and Douglas B. Johnson. 2021. "Impact of Patient Age on Clinical Efficacy and Toxicity of Checkpoint Inhibitor Therapy." *Frontiers in Immunology* Volume 12 - 2021. https://doi.org/10.3389/fimmu.2021.786046. https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2021.786046.

Wu, Chiao-En, Chan-Keng Yang, Meng-Ting Peng, et al. 2020. "The association between immune-related adverse events and survival outcomes in Asian patients with advanced melanoma receiving anti-PD-1 antibodies." *BMC Cancer* 20 (1): 1018. https://doi.org/10.1186/s12885-020-07508-7. https://doi.org/10.1186/s12885-020-07508-7.

Xu, C., Y. P. Chen, X. J. Du, et al. 2018. "Comparative safety of immune checkpoint inhibitors in cancer: systematic review and network meta-analysis." *Bmj* 363: k4226. https://doi.org/10.1136/bmj.k4226.

Xu, Y., K. Ma, F. Zhang, et al. 2023. "Association between baseline C-reactive protein level and survival outcomes for cancer patients treated with immunotherapy: A meta-analysis." *Exp Ther Med* 26 (2): 361. https://doi.org/10.3892/etm.2023.12060.

Ye, Ting, Jie-Ying Zhang, Xin-Yi Liu, et al. 2022. "The Predictive Value of MAP2K1/2 Mutations on Efficiency of Immunotherapy in Melanoma." *Frontiers in Immunology* Volume 12 - 2021. https://doi.org/10.3389/fimmu.2021.785526. https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2021.785526.

Yirgin, Inci Kizildag, Sukru Mehmet Erturk, Izzet Dogan, et al. 2021. "Are radiologists ready to evaluate true response to immunotherapy?" *Insights into Imaging* 12 (1): 29. https://doi.org/10.1186/s13244-021-00968-w. https://doi.org/10.1186/s13244-021-00968-w.

Yousaf, A., F. J. Tjien-Fooh, B. Rentroia-Pacheco, et al. 2021. "Validation of CP-GEP (Merlin Assay) for predicting sentinel lymph node metastasis in primary cutaneous melanoma patients: A U.S. cohort study." *Int J Dermatol* 60 (7): 851-856. https://doi.org/10.1111/ijd.15594.

Yun, J., J. Lee, J. Jang, et al. 2011. "KIT amplification and gene mutations in acral/mucosal melanoma in Korea." *Apmis* 119 (6): 330-5. https://doi.org/10.1111/j.1600-0463.2011.02737.x.

Zaretsky, Jesse M., Angel Garcia-Diaz, Daniel S. Shin, et al. 2016. "Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma." *New England Journal of Medicine* 375 (9): 819-829. https://doi.org/10.1056/NEJMoa1604958. https://www.nejm.org/doi/full/10.1056/NEJMoa1604958.

Zedek, D. C., and T. H. McCalmont. 2011. "Spitz nevi, atypical spitzoid neoplasms, and spitzoid melanoma." *Clin Lab Med* 31 (2): 311-20. https://doi.org/10.1016/j.cll.2011.03.008.

Zembowicz, A., M. McCusker, C. Chiarelli, et al. 2001. "Morphological analysis of nevoid melanoma: a study of 20 cases with a review of the literature." *Am J Dermatopathol* 23 (3): 167-75. https://doi.org/10.1097/00000372-200106000-00001.

Zhang, S., A. G. Bensimon, R. Xu, et al. 2023. "Cost-Effectiveness Analysis of Pembrolizumab as an Adjuvant Treatment of Resected Stage IIB or IIC Melanoma in the United States." *Adv Ther* 40 (7): 3038-3055. https://doi.org/10.1007/s12325-023-02525-x.

Zhao, J., C. Galvez, K. E. Beckermann, et al. 2021. "Novel insights into the pathogenesis and treatment of NRAS mutant melanoma." *Expert Rev Precis Med Drug Dev* 6 (4): 281-294. https://doi.org/10.1080/23808993.2021.1938545.

Zhou, Lei, Yun Zhong, Le Han, et al. 2025. "Global, regional, and national trends in the burden of melanoma and non-melanoma skin cancer: insights from the global burden of disease study 1990–2021." *Scientific Reports* 15 (1): 5996. https://doi.org/10.1038/s41598-025-90485-3. https://doi.org/10.1038/s41598-025-90485-3.

Zhou, Xin, Yiping Du, Zebo Huang, et al. 2014. "Prognostic Value of PLR in Various Cancers: A Meta-Analysis." *PLOS ONE* 9 (6): e101119. https://doi.org/10.1371/journal.pone.0101119. https://doi.org/10.1371/journal.pone.0101119.

Zhu, Shicong, Yang Fu, Bo Zhu, et al. 2020. "Pneumonitis Induced by Immune Checkpoint Inhibitors: From Clinical Data to Translational Investigation." *Frontiers in Oncology* Volume 10 - 2020. https://doi.org/10.3389/fonc.2020.01785. https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2020.01785.

7. Declaration and contributions

7.1. Declaration

This work was carried out in the University Hospital in Tübingen, Dermatology Department, under the supervision of Prof. Dr. med. Lukas Flatz und Frau Dr. Teresa Amaral. Herewith I, Chatziioannou Eftychia, declare that I have written this thesis by myself, and that I have not used any sources and aids other than those indicated. The references are presented according to standard rules for publication and standard citation guidelines. Further, I declare I have contributed to the major part of the following publications that are included in this work. All co-authors agreed to the contributions to the publications.

Tübingen, 25.7.2025

Date

Signature Eftychia Chatziioannou

7.2. Contributions of other authors

This work was carried out in the University Hospital in Tübingen, Dermatology Department, under the supervision of Prof. Dr. med. Lukas Flatz und Frau Dr. Teresa Amaral.

Publication 1:

Title: Features and long-term outcomes of stage IV melanoma patients achieving complete response under anti-PD-1-based immunotherapy

Authors: Chatziioannou Eftychia, Leiter Ulrike, Thomas Ioannis, Keim Ulrike, Seeber Olivia, Meiwes Andreas, Boessenecker Isabell, Gonzalez Stephanie Sanchez, Torres Francisco Merraz and Niessner, Heike, Sinnberg Tobias, Forschner Andrea, Flatz Lukas, Amaral Teresa

Eftychia Chatziioannou: research concept, selection of methods, recruitment of patients, data acquisition, data analysis, interpretation of results, manuscript writing

Ulrike Leiter: selection of methods, recruitment of patients, data acquisition, manuscript writing.

Ioannis Thomas: recruitment of patients, manuscript writing.

Ulrike Keim: recruitment of patients, manuscript writing.

Olivia Seeber: recruitment of patients, manuscript writing.

Andreas Meiwes: recruitment of patients, manuscript writing

Isabell Boessenecker: recruitment of patients, manuscript writing

Stephanie Sanchez Gonzalez: recruitment of patients, manuscript writing

Francisco Merraz Torres: recruitment of patients, manuscript writing

Heike Niessner: recruitment of patients, manuscript writing

Tobias Sinnberg: recruitment of patients, data analysis, manuscript writing

Andrea Forschner: recruitment of patients, manuscript writing.

Lukas Flatz: research concept, recruitment of patients, data analysis, manuscript writing

Teresa Amaral: research concept, recruitment of patients, data acquisition, data analysis, interpretation of results, manuscript writing

Publication 2: Nomogram for predicting survival after first-line anti-PD-1-based immunotherapy in unresectable stage IV melanoma: a multicenter international study

Authors: Eftychia Chatziioannou, Serna-Higuita Lina Maria, Kreft Sophia, Kandolf Lidija, Dujovic Branko, Reinhardt Lydia, Tamara Etterlin, Márquez-Rodas Ivan, Fortuna Ana Raquel Ferraz Pereira, Nübling Alica, Niessner Heike, Forschner Andrea, Garbe Claus, Popovic Aleksandar, Mirjana Balić, Meier Friedegund, Eigentler Thomas, Leiter Ulrike, Flatz Lukas, Sinnberg Tobias, Amaral Teresa.

Eftychia Chatziioannou: research concept, selection of methods, recruitment of patients, data acquisition, data analysis, interpretation of results, manuscript writing.

Serna-Higuita Lina Maria: data analysis, manuscript writing.

Kreft Sophia: recruitment of patients, data acquisition, manuscript writing.

Kandolf Lidija: recruitment of patients, data acquisition, manuscript writing.

Dujovic Branko: recruitment of patients, data acquisition, manuscript writing.

Reinhardt Lydia: recruitment of patients, data acquisition

Tamara Etterlin: recruitment of patients, data acquisition, manuscript writing.

Márquez-Rodas Ivan: recruitment of patients, data acquisition, manuscript writing.

Fortuna Ana Raquel Ferraz Pereira: recruitment of patients, data acquisition, manuscript writing.

Nübling Alica: selection of methods

Niessner Heike: selection of methods, recruitment of patients, data acquisition, manuscript writing

Forschner Andrea: recruitment of patients, data acquisition, manuscript writing.

Garbe Claus: manuscript writing.

Popovic Aleksandar: recruitment of patients, data acquisition, manuscript writing.

Mirjana Balić: recruitment of patients, data acquisition, manuscript writing.

Meier Friedegund: recruitment of patients, data acquisition, manuscript writing.

Eigentler Thomas: recruitment of patients, interpretation of results, manuscript editing.

Leiter Ulrike: recruitment of patients, data acquisition, manuscript writing

Flatz Lukas: selection of methods, recruitment of patients, manuscript writing

Sinnberg Tobias: research concept, selection of methods, recruitment of patients, manuscript writing

Amaral Teresa: research concept, selection of methods, recruitment of patients, data acquisition, data analysis, interpretation of results, manuscript writing

8. Acknowledgements

This dissertation would not have been possible without the support of many individuals to whom I owe my deepest gratitude.

I extend my heartfelt thanks to my doctoral supervisor, Professor Dr. med. Lukas Flatz, for his invaluable guidance, mentorship, and continuous support throughout all stages of my doctoral studies.

I am especially grateful to Dr. Teresa Amaral and Dr. rer. nat. Tobias Sinnberg for their outstanding supervision, critical input, and consistent encouragement. Their expertise and dedication were instrumental in shaping both the scientific direction and integrity of this work.

I would also like to express my sincere appreciation to all the colleagues and collaborators from the various institutions who contributed to this research. Their scientific input and practical support were crucial in making this work possible.

A warm and special thank you goes to all the members of the laboratory, colleagues, and friends who provided both scientific and personal support.

Lastly, I dedicate this thesis to my family. I am deeply grateful for their unconditional love, encouragement, and patience throughout the entire course of my doctoral studies. Without their constant support, this achievement would not have been possible.