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MOLECULAR TUMOR BOARD DECISIONS BASED ON NEXT GENERATION SEQUENCING FOR ADVANCED GASTROINTESTINAL CANCERS

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LIST OF ABBREVIATIONS

5-FU	5-fluorouracil
ACMG	American College of Medical Genetics and Genomics
ADP	adenosine diphosphate
ATM	ataxia telangiectasia mutated
ATR	ataxia telangiectasia and Rad3-related
BCLC	Barcelona Clinic Liver Cancer
BICC1	BicC family RNA-binding protein 1
BRCA	breast cancer
BTC	biliary tract cancer
CAPOX	capecitabine plus oxaliplatin
CCC	cholangiocellular carcinoma
CDK	cyclin dependent kinase
CDKN	cyclin dependent kinase inhibitor
CHEK	checkpoint kinase
CIP/KIP	CDK interacting protein/ kinase inhibitory protein
CKI	cyclin dependent kinase inhibitor
CRC	colorectal carcinoma
CUP	cancer of unknown primary
DNA	deoxyribonucleic acid
DPYD	dihydropyrimidine dehydrogenase
DSB	double strand breaks
E2F	E2 promotor binding factor
EBV	Epstein-Barr virus
EGFR	epidermal growth factor receptor
ECOG	Eastern Cooperative Oncology Group
EGFR	epidermal growth factor receptor
FANCM	Fanconi anemia, complementation group M
FAP	Familial Adenomatous Polyposis
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor

FOLFOX	5-fluorouracil/leucovorin plus oxaliplatin
FOLFIRINOX	5-fluorouracil/leucovorin plus irinotecan plus oxaliplatin
G1	gap phase 1
G2	gap phase 2
G6PD	glucose-6-phosphate dehydrogenase
GBC	gall bladder cancer
GDP	guanosine diphosphate
GEF	guanine nucleotide exchange factor
GEJ	gastro-esophageal junction
GI	gastrointestinal
GIST	gastrointestinal stroma tumor
GTP	guanosine triphosphate
HCC	hepatocellular carcinoma
HER	human epidermal growth factor receptor
HNPCC	Hereditary Non-Polyposis Colorectal Cancer
HRAS	Harvey rat sarcoma virus
IARC	International Agency for Research on Cancer
INK4	inhibitor of CDK4
IPMN	intraductal papillary mucinous neoplasm
KRAS	Kirsten-rat sarcoma virus
LOH	loss of heterozygosity
M	mitosis
MAPK	mitogenic activated protein kinase
MAPKK	mitogenic activated protein kinase kinase
Mbp	Mega base pair
MCN	mucinous cystic neoplasm
MDM2	mouse double minute 2 homolog
MLH1	MutL homolog 1
MMR	mismatch repair
MRE11	meiotic recombination 11 homolog
MSH	MutS homolog
MSI	microsatellite instability

MTB	Molecular Tumor Board
mTOR	mammalian target of rapamycin
NBS1	Nijmegen Breakage Syndrome 1
NET	neuroendocrine tumor
NGS	next generation sequencing
NRAS	neuroblastoma RAS viral oncogene homolog
NRG	neuroregulin
NSCLC	non-small cell lung cancer
OS	overall survival
PALB2	partner and localizer of BRCA2
PanIN	pancreatic intraepithelial neoplasm
PARP	Poly (ADP-ribose) Polymerase
PC	pancreatic cancer
PD	progressive disease
PD-1	programmed death 1 receptor
PD-L1	programmed death ligand 1
PFS	progression free survival
PI3K	phosphoinositide 3-kinase
PIP3	Phosphatidyl Inositol triphosphate
PMS2	postmeiotic segregation increased 2
PR	partial response
PTEN	phosphatase and tensin homolog
RAF	rapidly accelerated fibrosarcoma
RAS	rat sarcoma virus
RB	retinoblastoma
S	synthesis phase
SD	stable disease
SOS	son of sevenless
SSB	single strand break
TKI	tyrosine kinase inhibitors
TMB	tumor mutational burden
UGC	upper gastrointestinal tract cancer

Var	variants
VEGF	vascular epidermal growth factor
VEGFR	vascular epidermal growth factor receptor
VUS	variant of uncertain significance
WES	whole exome sequencing
WGS	whole genome sequencing

1 INTRODUCTION

1.1 GASTROINTESTINAL TUMORS

Gastrointestinal (GI) tumors are a heterogenous group of diseases. They comprise cancer of the esophagus, the stomach, and the intestine as well as the liver, the pancreas, and the biliary system. In most instances, malignant GI tumors are carcinomas, thus cancer which derived from the epithelium of these organs. Since the stomach, the small intestine and the colon are lined with mucosal membrane, adenocarcinomas are found here. In the esophagus and the rectum both adenocarcinomas and squamous cell carcinoma can occur. Apart from that malignant GI tumors can, for example, be lymphomas, mesenchymal tumors, or neuroendocrine carcinomas.

Most types of GI cancers rank under the top ten of malignancies with the highest numbers of absolute years of life lost [1]. Especially pancreatic adenocarcinoma, adenocarcinoma of the biliary tract and gastric and esophageal carcinoma are among the tumors with a very low five-year survival rate [2].

1.1.1 Colorectal carcinoma

Colorectal carcinoma (CRC) is the most frequent of the malignant GI carcinomas. Globally, CRC was the third leading type of cancer for females and the fourth leading type of cancer for males in 2017 [1]. In Germany it ranges even on rank two for females and rank three for males in incidence [2]. The life-time risk for developing colorectal carcinoma is about 5.3% for females and 6.5% for males [2].

In most cases CRC occurs sporadically, although in a considerable part it develops within well-known hereditary cancer syndromes; up to 5% of CRC arise within Hereditary Non-Polyposis Colorectal Cancer (HNPCC), also known as Lynch syndrome [3]. A smaller percentage develops in association with other hereditary cancer syndromes like Familial Adenomatous Polyposis (FAP), or Peutz-Jeghers-Syndrome [4]. Also, the occurrence of CRC in family members is a risk factor for CRC in the individual, which indicates predisposing aspects of

occurrence of CRC in the individual [5]. Other risk factors for example are lifestyle and dietary factors as well as chronic inflammatory diseases of the bowel.

In about half of the cases CRC manifests in the rectum. The other half is allocated to the different parts of the colon, the further oral the less frequent.

There are substantial differences in the features of CRC regarding localization [6]. Site is distinguished between right and left, or proximal and distal, respectively [6].

Most colorectal carcinomas are adenocarcinomas. That leaves a minority of tumors with squamous cell carcinoma and other rarer histological types.

The majority of adenocarcinomas develops from adenomas, in the well-described adenoma-carcinoma sequence, while 15-30 % emerge from the serrated pathway [7].

The therapeutic regime consists of both surgical and pharmaceutical options. The adequate choice depends on the general condition of the patient, the localization of the carcinoma and the stage, i.e., the local extent and if metastatic spread is existent or not. The screening on adenomas leads to detection and removal of CRC in early stages (UICC 0-1). Though, when diagnosed at later stages and patients are already exhibiting metastatic disease (UICC4), prognosis is limited, with a 5-year survival rate in metastatic CRC of only 14% [7].

For metastatic cancer, chemotherapy with 5-fluorouracil/leucovorin plus oxaliplatin (FOLFOX) or 5-fluorouracil/leucovorin plus irinotecan (FOLFIRI) or even a triple combination (FOLFIRINOX) are first line treatment choices. There is the option of combination with the anti-vascular epidermal growth factor receptor (VEGFR) antibody Bevacizumab [7]. For patients having KRAS non-mutated tumors, anti-epidermal growth factor receptor (EGFR) antibodies Cetuximab or Panitumumab can be added [7]. Apart from that, immune checkpoint inhibitors are approved for the first- and second-line treatment of a subgroup of advanced CRC with mismatch repair deficiency or high microsatellite instability [8-10].

1.1.2 Carcinoma of the small intestine

Adenocarcinomas of the small intestine form a very small part of malignant GI tumors [11]. Of all malignant tumors located in the small intestine adenocarcinomas predominate, with another large part formed by neuroendocrine neoplasia [2, 11, 12]. Furthermore, gastrointestinal stroma tumors (GIST), other sarcomas or lymphomas can occur [2, 11, 12].

Risk factors apart from alcohol, tobacco smoke and dietary factors are chronic inflammatory diseases of the bowel as well as cystic fibrosis [2, 11]. Also, adenocarcinomas of the small intestine arise more frequently within hereditary tumor syndromes like FAP, HNPCC and Peutz-Jeghers syndrome [2, 11].

The therapeutic option in curative intention is chirurgical treatment with the aim of complete resection [12]. However, adenocarcinomas of the small intestine are commonly diagnosed at an advanced stage, in which R0 resection and thus curative treatment is not possible [12]. In these cases, pharmaceutical treatment regimens with, e.g., FOLFOX or capecitabine plus oxaliplatin (CAPOX) are applied, while randomized studies on benefit are lacking [11, 12]. 5-year survival rate for metastatic adenocarcinoma of the small intestine ranges between 3 and 5% [12].

1.1.3 Gastric cancer

Gastric cancer located in the cardia or at the gastro-esophageal junction (GEJ), respectively, must be distinguished from non-cardia gastric cancer [13].

Globally, the life-time risk for the occurrence of gastric cancer is about 1.2% for females and 3% for males [1]. While the incidence of malignant non-cardia gastric tumors constantly is decreasing in Germany as well as in other developed countries, the number of new cases shows a global increase [2, 14]. Globally, gastric cancer is the fifth most common cancer [13].

Important risk factors include the infection with the bacterium *Helicobacter pylori*, *Epstein-Barr virus* (EBV) infection, tobacco smoke, alcohol or an excessive level of nitrates in the consumed food [2]. The risk of developing gastric tumors is also elevated within HNPCC as well as by the presence of affected family members [15].

Surgical resection is a curative option in early stages. If diagnosed at the advanced stage, chemotherapy with either capecitabine or 5-fluorouracil (5-FU) plus cisplatin or oxaliplatin (possibly plus docetaxel) is the recommended fist line treatment for human epidermal growth factor receptor 2 (HER2) negative cancer [14]. In the case of HER2 positive status, the addition of the anti-HER2 antibody Trastuzumab improves the outcome and constitutes current standard of care for this subgroup [14, 16]. Overall survival still is poor, particularly for advanced HER2 negative gastric cancer [13, 17].

1.1.4 Esophageal cancer

In western countries up to 50 % of esophageal cancer are adenocarcinomas, showing an increasing frequency and mortality rates over the last years [2, 18, 19]. Adenocarcinomas generally are located in the lower parts of the esophagus and at the GEJ [2, 19]. The distinction between gastric and esophageal adenocarcinoma is not definite and commonly they are depicted as adenocarcinoma of the GEJ [19].

Important risk factors for adenocarcinoma are obesity and associated chronic reflux disease, resulting in Barrett's esophagus, which is considered as a precancerous condition [2, 18, 19].

Squamous cell carcinomas, making up also approximately 50%, are most located in the upper parts of the esophagus [19]. Risk factors for squamous cell carcinomas, at least in the Western World, are smoking and alcohol [2, 18, 19].

Incidence of esophageal cancer is more than three times higher in males than in females and increases with higher age [2].

Therapeutically there are options of endoscopic as well as surgical resection, when appropriate supported by perioperatively applied chemoradiotherapeutic regimes or adjuvant use of immune checkpoint inhibitors [8, 18]. Immunotherapy is also approved as a first line option for tumors with a certain level of PD-L1 expression [10]. At the metastatic stage the palliative treatment options comprise brachytherapy, platin and fluoropyrimidine containing chemotherapy, checkpoint inhibitors and best supportive care [18]. 5 year survival rate for metastatic esophageal cancer is below 5% [2].

1.1.5 Hepatic cancer

Hepatocellular carcinoma (HCC) is a malignant cancer arising from the hepatocytes or their progenitor cells [20]. It accounts for up to 90% of liver cancer [20]. Globally it is the sixth most common cancer type, and it occurs about three times more frequently in males than in females [20].

The most important risk factor is the prevalence of hepatic cirrhosis and its causes like chronic viral or fatty inflammation of the liver [20-22].

Positive family history is also elevating the likelihood for development of HCC, so most likely hereditary factors are influencing environmental risk factors [22].

The Barcelona Clinic Liver Cancer (BCLC) classification is central in the choice of the therapeutic options. Curative treatment options are resection and liver transplantation, but at diagnosis, two out of three patients have advanced disease that does not allow for this [20, 23, 24]. For intermediate stages, trans arterial chemoembolization (TACE) is preferred [20]. For advanced HCC, the multi kinase inhibitors Sorafenib and Lenvatinib, as well as the combination of anti-PD-L1 antibody Atezolizumab plus anti-VEGF antibody Bevacizumab or the combination of anti-PD-1 antibody Durvalumab plus anti-CTLA4 antibody Tremelimumab are approved in the first line therapy [25]. Following on treatment with Sorafenib, second line therapy options are the multi kinase inhibitors Regorafenib and Cabozantinib and the anti-VEGF antibody Ramucirumab for patients with high levels of alphafetoprotein [20, 23].

Whether systemic treatment can be implemented is mainly dependent on liver function and Child Pugh Score and performance status. In patients with Child-Pugh A and good performance status (ECOG 1-2), with systemic treatment a median overall survival of up to 19 months can be achieved [20].

1.1.6 Biliary tract cancer

Biliary tract cancer (BTC) is a rare disease and comprises cholangiocarcinoma (CCA) and gall bladder cancer (GBC) [26, 27].

CCA is a carcinoma of the bile ducts. It can be located intrahepatic or extrahepatic, including perihilar (Klatskin tumor) and location at the Papilla vateri [28, 29].

For all biliary tract cancers incidence increases with higher age [2], and there is also an increase in global incidence, at least for intrahepatic CAA [28, 30]. Risk factors are including chronic inflammatory conditions of the bile ducts, so as primary sclerosing cholangitis, as well as obesity, diabetes, alcohol and smoking, especially for western populations [2, 29, 31]. In developed countries the highest risk for carcinoma of the gall bladder exists in chronic cholecystitis due to gallstones [26].

Biliary tract cancers occur mostly sporadically [32], although there is an association of CCA with genetic predispositions and cancer syndromes, for example HNPCC [29].

The only therapeutic option with curative intention is surgery, but only about 25% to 35% of all cases of CCA, and even less cases of GBC, are eligible for this option [30, 32, 33]. First line therapy for advanced or metastasized CCA is gemcitabine plus cisplatin in combination with anti-PD-1 antibody Durvalumab, and a second line option is FOLFOX [29, 34, 35]. Apart from that, FGFR2 inhibiting treatments are approved for selected patient groups after at least one previous treatment [29]. A growing number of molecular-based therapies are currently tested in clinical trials [36].

For GBC systemic treatment options include oxaliplatin, cisplatin and capecitabine [33].

1.1.7 Pancreatic cancer

Globally pancreatic cancer (PC) takes up not only two percent of all types of cancer [1]. However, for Germany it was projected to be the second most common GI cancer regarding new cases in 2020 [37].

There are genetic predispositions for the development of PC and approximately 10 % of the cases arise within familial pancreatic cancer [38]. Other major risk factors are chronic pancreatitis and, associated with that, tobacco smoke, alcohol, obesity, and diabetes mellitus, primarily type 2 [39].

Pancreatic cancer is classified by its location in the caput, corpus, or cauda, with the caput as the most common site. Adenocarcinoma of the pancreas arises either from the ductal or the acinar cells, and ductal adenocarcinoma is the most common type of pancreatic cancer [39]. Other malignant cancer occurring in the pancreas is neuroendocrine neoplasia.

Pancreatic adenocarcinoma develops from precancerous lesions like pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN) or mucinous cystic neoplasm (MCN) [38].

The only curative intended therapeutic option is the complete resection of all tumor tissue. If compatible with the patient's general condition and organ functions surgery is supported by adjuvant chemotherapy.

At the time of diagnosis only less than 20% of the patients with PC have a tumor that can be fully resected and that allows the curative approach. Also, even if fully resected, recurrence of cancer is frequent [40].

Treatment options for advanced PC are gemcitabine in combination with nab-Paclitaxel, or FOLFIRINOX, with an overall survival of about 12 months [40, 41]. The mean five-year survival rate for all stages with <10 % is very limited [41]. In both females and males, pancreatic cancer is under the top five cancer types leading to the most deaths in Germany [2].

1.2 MOLECULAR PATHOGENESIS OF CANCER

A tumor, when understood as a synonym of neoplasia (gr neo = new, plastein = to form) defines the new formation of cells of a specific tissue, which results in the gain of this tissue. It is considered to be abnormal and of autonomic growth. In contrast to benign tumors a malignant tumor, i.e. cancer, harbors the features of infiltration and destruction of neighboring tissues, as well as the invasion in blood and lymph vessels and serous cavities, which can lead to metastatic spread [42].

For carcinogenesis, i.e., the development of a malignant tumor, features like uncontrolled proliferation and survival, as well as invasiveness and metastatic settlement has to be acquired by the cells [42]. Also, an altered cell metabolism as well as the ability to escape from the immune system is central [42].

Cell growth, division, survival, migration, and shape are controlled by complex biochemical signaling pathways [42, 43]. These pathways, when disturbed by mutations in the respective coding genes, can get abrogated or remain active in an uncontrolled way [43]. Thus, cancer is a genetic disease, and underlying are somatic mutations or, in the case of hereditary tumor syndromes, mutations of the germline [44-46].

To attain the malignant phenotype more than one cellular pathway must be altered, and the specific pattern of mutations and transformed cellular processes is determined by evolutionary principles [45].

The transformation of a cellular genome takes place in multiple subsequential steps [42, 47]. It goes along with the accumulation of mutations and epigenetic changes that, generally speaking, if leading to cancer each lead to a growth or survival advantage for the cell [42, 47]. Mutations also affect genes whose gene products have functions like the repair of DNA damage or replication errors, or the induction of cell death if DNA damage occurs [42]. Subsequently, genome instability is a frequent characteristic of cancer cells, and promotes further accumulation of mutations itself [42].

During carcinogenesis and tumor progression clonal, as well as subclonal, mutations arise [42, 44, 47]. Clonal mutations affect every cell in the tumor tissue, whereas subclonal alterations only affect a subset of the cancer cells [42, 44, 47]. This leads to heterogeneity within one single tumor [42]. Next to intratumoral heterogeneity, also a huge intertumoral heterogeneity exists. Nevertheless, there are some pathways that are abrogated or deregulated in the majority of cancer. Functional alteration of these pathways is central for the features of proliferation or survival and is found across different cancer types [42, 43, 46, 48].

1.2.1 The cell cycle and its regulation

For proliferation, a cell must progress through the cell cycle. In adult tissues, during cell cycle duplication of the DNA and the required cell substance takes place [49, 50]. The cell cycle consists of four phases, a gap phase (G1), which is followed by the S (synthesis) phase in which DNA is replicated, another gap phase (G2), and finally the M phase, where mitosis takes place and two progeny

cells arise [49-53]. Cell growth, duplication of cell mass and required substances take place during the gap phases in preparation for DNA replication in S phase (G1) and cell division in M phase (G2) [50, 51]. Alternative options to cell division are temporary or enduring cell rest [54].
Most of the cells of the human body are located in temporary rest, also called quiescence, or G0 [51, 52]. In those cells, presence of mitogenic signals, i.e., growth factors, is needed to enter the cell cycle [53, 55, 56]. Mitogenic signaling cascades will be described in more detail below.

Some of the central players in the cell cycle are the cyclin family, the family of cyclin dependent kinases (CDKs) and inhibitors of the cyclin-dependent kinases (CKIs). Cyclin dependent kinases (CDKs) are serine/ threonine kinases and act through phosphorylation of their substrates [52, 54]. Their status of activity is dependent on the presence and level of cyclins and highly increases when the CDK form a heterodimer with a matching cyclin [57, 58]. There are 20 different types of CDKs which function together with different types of the 29 types of cyclins [50]. The most important cyclins and CDKs for cell cycle control are Cyclin D1-3, that activate CDK4 and CDK6, and Cyclin E1 and E2, as well as Cyclin A2, that activate CDK2 [49, 55, 56, 58].
In simple terms, activity of CDK2 and CDK4/6 lead to phosphorylation of the Rb protein, which breaks up its binding of the transcription factor E2F [58]. Free E2F leads to the transcription of, among others, Cyclin E1 and E2, thereby promoting activity of CDK2 and further phosphorylation of Rb [49, 51, 53, 54, 56]. Via this positive feedback loop, the cell then enters a state where progression through the cell cycle is independent of growth signals [51-54, 56] and important gene products for DNA replication are synthesized [49-51, 58]. This point in the cell cycle is called ‘restriction point’ [51-54, 56].
However, acute cellular stress like DNA damage can still stop the cell cycle and there are more layers in the complex system of cell cycle regulation [53, 55].
Opponents of mitogenic signaling cascades are CDK-inhibitors (CKIs) [54]. There are basically two families of CKIs [54]. The Cip/Kip family, including p21, p27, p57, are inhibitors of both CDK1 and CDK2, but have ambivalent, also stabilizing

effects on CDK4/6 [53, 56]. The INK4 family consist of p16(INK4a), encoded by *CDKN2A*, and the related proteins p15(INK4b), p18(INK4c) and p19(INK4d), which are specific inhibitors of CDK4/6 [54, 55]. CKIs mainly act at the so-called cell cycle check points, of which one is the restriction point, and can arrest the cell cycle by inhibition of CDK4/6 and CDK2, respectively [59]. Other well established cell cycle check points are the G2/M and the mitotic spindle checkpoint [52, 60]. Reasons for cell cycle check point activation apart from the absence of growth factors at the restriction point are including occurrence of DNA damage or replication errors [60]. Activation of cell cycle checkpoints is central in the response to DNA damage or replication errors, which will be addressed below.

So, whether cell division and, consequently, proliferation is suitable and adequate for given circumstances is mainly decided during G1 phase by complex computation of several different signals from intra- and extracellular [49, 52, 54]. Under physiological conditions, mitogenic signals from extracellular are required for the decision in favor of continuing through the cell cycle and against alternative options [49, 58, 61]. Cells located in G0 only enter the cell cycle if there is a specific amount of external mitogenic signals that exceed the level of CKIs [50, 52, 55].

1.2.2 Mitogenic pathways

When growth factors bind to their cell receptors, they lead to proliferation via pathways that in the end result in expression of the proteins that enforce progression through the cell cycle [62-64].

Important growth factor receptors, also in the pathogenesis of cancer, are part of the ErbB family and the fibroblast growth factor receptor (FGFR) family.

Both receptor families are part of the superfamily of Receptor Tyrosine Kinases (RTK), transmembrane proteins that have a ligand-binding domain on their extracellular site and a tyrosine kinase domain on their cytoplastic site [65].

1.2.2.1 The FGFR signaling pathway

Members of the FGFR family are FRGFR1,2,3 and 4 [66, 67].

Their ligands are 18 members of the fibroblast growth factor (FGF) family, that activate FGFR1,2,3,4 via binding [66-68].
As a result of binding of the according ligand, FGFR gets activated due to receptor dimerization and autophosphorylation of tyrosine sites [67, 68]. This complies with the typical mechanism of receptor tyrosine kinase activation. Upon activation, downstream signaling cascades are initiated including the RAS and the PI3K pathway [66].

1.2.2.2 The ErbB signaling pathway

Members of the ErbB family are ErbB1, also known as human epidermal growth factor receptor 1 (HER1) or epidermal growth factor receptor (EGFR) [63, 69]; ErbB2, also known as HER-2; ErbB3; and ErbB4 [70].
Important ligands are the epidermal growth factor (EGF), binding to EGFR (HER1) and NRG1-4, binding to ErbB3 and ErbB4 [71, 72].
There is no ligand directly binding to HER2 but HER2 gets activated by heterodimerization with other ErbB members, EGFR and ErbB3 in particular [73, 74]. NRG1 is expressed on the cell surface and partly gets released by proteolytic processing [70, 71]. It thus mostly acts in a paracrine and juxtacrine way [70, 71]. Upon binding of NRG1, ErbB3, that itself provides an insufficient kinase activity, forms heterodimers with HER2 (ErbB2), EGFR (ErbB1), or ErbB4 [70, 73]. Downstream of ErbB2, particularly after heterodimerization with ErbB3, above all the mitogenic PI3K-AKT-mTOR pathway is activated, as will be described below [74, 75].

1.2.2.3 The RAS-RAF-MEK-ERK pathway

The RAS family consists of KRAS, NRAS and HRAS [62, 76]. RAS is present in its GDP-bound, inactive form and for activation GDP has to be exchanged with GTP [62]. This exchange is rendered by so-called guanine nucleotide exchange factors (GEFs); one of it, SOS, gets activated downstream of EGFR [62, 76, 77]. RAS activation conversely is limited by dephosphorylation of bound GTP. RAS acts as a GTPase and inactivates itself but only on a low activity level [62]. RAS GTPase activity is increased by proteins summarized as GAPs [62, 76, 78].

Activated RAS then activates the serine-threonine kinase BRAF [62, 79]. Subsequently the MAPK kinases (MAPKK) MEK 1 and MEK 2 are stimulated, activating the mitogenic activated protein kinase (MAPK), also known as ERK1 and ERK2, leading to the expression of D-type cyclins [62, 79]. The RAS-RAF-MEK-ERK pathway hereby carries the mitogenic information a cell receives in form of growth factors via various kinases up to the nucleus where the translation of proteins is stimulated [62]. Those proteins, as effectors themselves, promote cell cycle progression and thereby foster proliferation [62, 80].

1.2.2.4 The PI3K-AKT-mTOR pathway

Another central mitogenic pathway is the PI3K-AKT-mTOR pathway.

PI3K is activated by the receptor tyrosine kinase directly or via RAS and produces the second messenger PIP3 (PtdIns-3,4,5-P_3) [61, 62, 81].

PIP3 activates AKT, which has anti-apoptotic effects, also via subsequent activation of mTOR [61, 62]. The PI3K pathway is antagonized by PTEN, because PTEN dephosphorylates PIP3, thereby reducing the level of active second messenger [61, 62].

mTOR not only receives signals activating downstream from receptor tyrosine kinases, but also is inhibited if the nutrient status of the cell is on a low level [81]. Active mTOR leads to protein synthesis, affecting cell metabolism and promoting cell growth and angiogenesis [81].

1.2.3 DNA damage response pathways

As mentioned above, if DNA damage or replicative errors occur, physiologically the cell cycle is arrested through activation of cell cycle checkpoints [60]. Checkpoint activation basically works by the inhibition of CDK activity [60].

p53, also known as the ‘guardian of the genome’ is a transcription factor and mainly acts during G1 as part of the G1/S checkpoint, by initiation of transcription of *CDKN1A* encoding p21 [60, 82, 83]. p21 then, in its function of inhibitor of CDKs, leads to cell cycle arrest in G1 [82]. In case of irreparable DNA damage p53 can also induce apoptosis via transcription of proapoptotic factors and the resulting start of the apoptotic signaling cascade [83].

1.2.3.1 DNA damage repair system

Double strand breaks (DSB) are detected by a protein complex of MRE11, RAD50 and NBS1, called MRN complex, which also assembles further proteins of the pathway and interacts with cell cycle checkpoint [83]. Upon DSB, the MRN complex subsequently activates proteins ATM and ATR [83]. ATM activates CHEK2, which stabilizes p53 by phosphorylation [83]. This prevents the degradation of p53 by its negative regulator MDM2 [83]. ATM thereby is an important part of the G1/S checkpoint [83].

ATM and CHEK2 also lead to activation of PALB2 and BRCA1 and 2, that eventually recruit RAD51 [41]. *BRCA1* and *BRCA2* encode proteins that take part in repair of DNA DSB by homologues recombination [83, 84]. DNA damage in single-strand DNA, that results from environmental damaging agents but also subsequent of DSB repair, leads to activation of ATR [83]. ATR is part of the G2/M checkpoint, inhibiting CDK2 and CDK1 in response to DNA damage, thereby preventing transition to mitosis [83].

1.2.3.2 The mismatch repair system

After S phase the mismatch repair (MMR) system can recognize base pair mismatches that took place during replication or occurred due to damage or erroneous genetic recombination [85, 86]. *MLH1, MSH2, MSH6* and *PMS2,* among others, are coding for proteins that act in the mismatch repair system [85-88].

The MMR system acts in heterodimers of MSH2/MSH6 and MSH2/MSH3 that identify mismatches, in the case of MSH2/6 base substitutions and small mismatches of 1 or 2 nucleotides, whereas MSH2/MSH3 recognizes also errors regarding up to 10 nucleotides [85, 86]. MSH2/MSH6, or MSH2/MSH3, respectively, then bind the heterodimer of MLH1/PMS2 [85, 86]. This leads to assembling and guidance of further enzymes, like DNA exonuclease, polymerase, and ligase [85, 86].

Disfunction of this system inevitably results in genome instability and promotes the accumulation of somatic mutations [89].

Only aspects of the complex computation of proliferation signals and their effects on the cell are described here of course. Nevertheless, it becomes clear that the constant activation of mitogenic signaling pathways due to alteration of encoding genes or their expression leads to uncontrolled proliferation, a typical step in the development of cancer [90].

Defective DNA repair systems lead to the accumulation of mutations and hence give rise to other driver mutations in oncogenes or tumor suppressor genes, promoting tumor progression [42, 85]. On the other hand, excessive DNA damage also leads to senescence or cell death [91]. Therefore, additional mutations in genes encoding cell cycle checkpoint proteins must occur for maintenance of the malignant phenotype [91]. If cell cycle regulation is defective DNA damage and replication errors are passed through cell division and accumulate, leading to genome instability [83].

The abovementioned pathways are critical in cancer development, with mutations in the genes coding for the respective proteins being frequently found in tumors. Alterations of genes encoding players in cell cycle regulation and DNA repair are recurrently found in cancer, above all *TP53*, but also *ATM* and *CHEK2* [83].

Also, the proteins altered in their function in cancer cells partly provide as treatment rationale for targeted treatment options that are currently being tested in clinical trials or already have been approved.

1.2.4 Cancer genes

1.2.4.1 Oncogenes and tumor suppressor genes

So called cancer genes are those genes that encode the players in critical pathways or regulate cell processes by control and modulation of chromatin, RNA-splicing and epigenomics [44].

The term oncogene describes the altered version of a gene which physiologically encodes one, or more than one, protein that is part of biochemical processes in the cell [92]. The gene in its unaltered sequence is called proto-oncogene and its gene product often is a player in proliferative, mitogenic or anti-apoptotic pathways [92]. Proto-oncogenes or their gene products, respectively, that acquire a gain of function due to genetic alteration, become oncogenes [93]. The new or

altered function of the oncogene product shifts the balance between pro- and anti-proliferative signals in the cell to the proliferative side [94].

The effect of alterations that make proto-oncogenes oncogenes are often dominant, and one affected allele is considered sufficient to change the phenotype of the cell [93].

The mutational mechanisms regarding proto-oncogenes are (focal) amplifications, translocations or, when it comes to single nucleotide variants, base mutations that result in a gain of function in the means of an increased and dysregulated activation of the encoded protein [88, 92].

On the contrary, tumor suppressor genes physiologically hold functions that are anti-proliferative [42]. Genes that encode proteins of cell cycle checkpoints or that repair DNA are also considered as tumor suppressor genes [42]. Inactivation of a tumor suppressor gene due to genetic alteration with the consequence of loss of its gene product hence leads to proliferation, cell growth or other malignant features typical for a cancer cell [93]. As a result, in a tumor cell, inactivating mutations are most often found in tumor suppressor genes [44, 94].

Mutational mechanisms regarding tumor suppressor genes are copy number variants in the form of (focal) deletions or truncating single nucleotide variants [92]. The latter are, e.g., frameshift or nonsense variants that result in a stop codon. They thereby lead to a loss of function or missing expression of the gene product [92].

Mutations of tumor suppressor genes are normally recessive and both alleles must be affected to fully abolish the function of the tumor suppressor gene or of the encoded protein [45]. This biallelic inactivation can be caused by somatic mutations but also by an underlying predisposing inactivating variant of a tumor suppressor gene in the germline that leads to heterozygous deletion [45]. If it then comes to the somatic deletion of the remaining wildtype allele, loss of heterozygosity (LOH) results in homozygous deletion [92]. In consequence there is a loss of the gene product and the malignant phenotype arises, as it was described for *RB*, and the Rb protein, respectively in the two-hit hypothesis [95].

1.2.4.2 Driver mutations

For most cancers the associated genes are known and millions of somatic alterations have been detected today [48].

Driver mutations are those that lead to the stepwise acquisition of malignant features and that alter genes in a way that is sufficient for the initiation of tumor growth and progression [94]. In genetic diagnostics one has a retrospective view on carcinogenesis, finding multiple mutations that exist at the end of cancer development, in the probe of a primary cancer tissue or even metastasis. This leads to the question which of the found mutations are driver mutations and which are passenger mutations [44]. Driver mutations normally comprise only a small subset of the alterations found in the respective tumor tissue [44, 45, 94].

Through widespread approaches of genomic profiling, for a huge number of cancer types driver mutations have already been identified, in the aim to find new treatment options to counter the limited outcomes of patients with advanced tumor disease [45, 96].

1.3 PRECISION ONCOLOGY AND MOLECULAR TUMOR BOARDS

1.3.1 Precision Oncology

The investigation of mutations that lead to cancer is constantly closing knowledge gaps of carcinogenesis and led to the discovery of causal genetic alterations in cancers [44]. In the end the knowledge about key processes in the development of cancer especially is important to develop novel and effective therapeutic agents [43]. The understanding of the features cells and tumors have to acquire to become malignant cells has led to new treatment options [43].

Precision Medicine or Personalized Medicine, as it is also called, means the process of understanding pathogenesis and finding adequate biomarkers in order to better prevent, diagnose, or treat diseases [43].

The term “individualized Medicine” or “Personalized Medicine” first came up in an article 1979 and is used more commonly in particular since 1999/2000 [97].

Because it has often been used since then but was still very vague, a precising definition was given in 2013 by Schleidgen et al. in their review of 683 articles about individualized medicine. It says that Precision Medicine “seeks to improve

stratification and timing of health care by utilizing biological information and biomarkers on the level of molecular disease pathways, genetics, proteomics as well as metabolomics" [97].

The concept of Precision Medicine is applied in clinical oncology since cancer still is a disease which is hard to treat. The average response rate of patients with cancer on available drugs ranges on a lower level compared to other patient groups [98].

The aim of Precision Oncology is the identification of optimal anti-cancer treatments and the administration of the right therapy for each patient [99-101]. Providing the 'right' treatment results in an improvement of the patient's outcome and also has a safer profile of side-effects [43, 100, 102-104]. Knowledge about pathogenesis and biomarkers must be collated with the findings in each tumor tissue. Thereby, the patient's individuality and, since there is a huge intertumoral heterogeneity, the individuality of the tumor particularly is addressed [103, 105]. To reach this aim recommended treatments are often beyond standard of care therapy [106].

Targeted therapies mean therapeutic agents that target specific altered proteins that lead to uncontrolled activity or abrogated regulation of a pathway [43, 107]. These proteins are encoded by genes harboring driver mutations, though not every driver mutation also is suitable as target for therapy [62].

Often, targeted therapies are inhibitors of oncogene products, in order to impede the according aberrantly activated signaling pathway [107]. This mechanism is called 'oncogene addiction', implying that the malignant cell is dependent on the activity of one main pathway [107]. It already has become clear though, that in many cases the inhibition of one single oncogene is not sufficient, due to the heterogeneity of a single tumor and the emergence of resistant clones [107]. Thus, the feasibility and effect of combination therapy is being investigated and supporting data has been reported [103].

Another mechanism recurrently utilized is exploiting 'synthetic lethality' [91, 108]. 'Synthetic lethal' means that the coincident loss of function of two gene products leads to the death of the cell, whereas loss of function of only one of the gene products does not [107]. Cell death can therefore be therapeutically induced by

inhibition of one gene product, if, due to mutation, there already is the loss of another in the respective pathway [108].

The number of approved targeted therapies is constantly growing [43, 109, 110]. A prominent example for an established targeted agent is the anti-HER2 antibody Trastuzumab, recommended for cancer in the case of HER2 positive status [13, 107].

Besides finding targetable mutations, focus lies on the identification of alterations that serve as biomarkers and can predict outcomes on specific treatments [111]. As an example, assessing the KRAS status is essential for treatment decisions in advanced CRC, because KRAS mutations are predicting resistance to anti-EGFR antibodies [7, 111].

An essential part of Precision Oncology is sequencing cancer genomes [112]. The use of novel sequencing technologies is being implemented in the clinical management of cancer and forms a basis for diagnostics and treatment decisions [43, 111]. By ascertaining the mutational landscapes of tumors, important biomarkers are supposed to be unveiled that not only are causal in pathogenesis or progression of cancer but also have a prognostic or predictive value [43, 98, 111, 113]. That is, they are determining treatment options or serve as targets for specific therapeutic agents [43, 98, 111, 113].

1.3.2 Sequencing technologies

The Human Genome Project (HGP), a research project started in 1990, coordinated by the U.S. Department of Energy and the National Institutes of Health of the U.S., had the aim to fulfill a complete sequencing of the human DNA and thereby identify all human genes and determine the whole sequence of the base pairs [114, 115]. The task officially was completed in 2004, providing information about an estimated count of 20,000 to 25,000 protein-coding genes and leaving only about 1% of the euchromatine yet to be ascertained utilizing more advanced technologies in the future [114, 115].

As the identification of protein-coding genes consequently led to the hope of understanding the pathogenesis and hereditary factors of various diseases other genome projects focused on special types of maladies [116].

Since 2006 a project called The Cancer Genome Atlas (TCGA) exists, initiated and funded by the NIH, to determine all alterations (genomic and epigenetic) in cancer [117].

The first sequencing approaches were performed by Sanger sequencing, which was expensive and time-consuming [44]. With Next Generation Sequencing (NGS) a cost and time-effective option for molecular profiling is provided [118]. It facilitates the sequencing of a large quantity of genes in a high quality, also from limited tissue samples [118, 119]. This makes it accessible for medical institutions and laboratories [100, 120]. NGS advanced to be the standard technology for sequencing panels or whole genome or exome sequencing [118]. With NGS sequencing diagnostics, somatic and germline alterations can be detected, as well as single nucleotide variants, copy number variants or structural rearrangements [100, 111, 121]. Because the entire coding sequence of a gene is investigated, common and rare mutations are revealed, and somatic allele frequencies, and thereby intratumoral heterogeneity can be assessed [111, 118, 119].

The new information that are maintained by using NGS lead to a more advanced understanding of cancer [111, 118, 121]. Hereby the clinical practice in oncology is changed, but also by the higher complexity in the interpretation and working with the data [102, 106, 119, 121].

1.3.3 Molecular Tumor Boards

Nowadays the performance of molecular profiling of cancers in order to be able to give comprehensive treatment recommendations is in a way established in clinical oncology care [103, 112, 122].

In Tübingen, the Molecular Tumor Board (MTB) was formed in 2016. It was founded among various others in different hospitals in Germany and around the world [103, 104, 106, 112, 120, 122-124].

A Genomic or Molecular Tumor Board is a multidisciplinary group of clinicians and scientists with expertise in, among others, medical and translational oncology, radiology, genomics, pathology, biology and bioinformatics [101, 103, 104, 106, 111, 124]. It is often affiliated to the cancer center of a hospital to guide

diagnostic and treatment decision in the era of Personalized Medicine [103, 104, 106, 111]. MTBs were formed for the implementation of Precision Oncology in the clinical practice and can be regarded as a bridge between new technological achievements and physicians caring for patients [98, 101, 106, 111]. The great amount of genomic data produced within the approach of Precision Oncology must be integrated into clinical decision making [104, 122]. MTBs are a result of the complexity of the approach and it has been shown that the formation of a board is needed to address the challenges coming along with the implementation of Precision Oncology in clinical practice [101, 104, 106, 122]. They face tasks like decision on whether and at which point in the patient's history indication is given for sequencing tumor tissue, and which tissues are supposed to be sequenced, as well as on the choice of the sequencing technique [109, 111]. Furthermore, within patients' presentation to the MTB query of sequencing results is included, and conceivably also management of reimbursement of testing and, later on, administered off-label therapies [106].

Above all, it is patients with advanced tumor diseases and lack of further standard of care treatment that are currently presented to the MTB, whose tumor genomes are sequenced and that are provided with novel treatment options [103, 122, 125]. It has been shown that the approach of MTBs is feasible in everyday practice and that additional treatment options can be identified for a part of the presented patients [103, 122].

1.4 AIM OF THE RESEARCH

The major aims of this study included to investigate the clinical course of patients with GI cancers that were presented to the MTB at the University Hospital Tübingen and that were treated in accordance with the recommendations given by the MTB. This study also aims to document the applied molecular diagnostic procedures, to identify clinically relevant altered signal transduction pathways and to analyze MTB-guided decisions and attribute them to these altered pathways.

Parts of this work have been published previously by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]. For further information on authorship regarding distinct aspects of the work, please also consult the 'Erklärungen zum Eigenanteil' (declaration of own contribution).

After the start of the MTB at Tuebingen University in April 2016, a new quality and amount of clinical data was documented for all cancer patients that were discussed within this interdisciplinary board. As the MTB can be regarded as a bridge between rapidly developing complex diagnostic procedures and physicians caring for patients [101], there was a need of new tools to meet this challenge. Tools had to be developed and applied to evaluate the diagnostic procedures, the recommendations given by the MTB and the clinical outcomes of patients that were treated accordance with these recommendations. At the same time, one of the major goals of the MTB work is to constantly improve the quality of MTB recommendations [101].

To this end, patients with GI cancers were chosen as a first group of patients that was further investigated after the start of the MTB. We chose these tumors, because to our knowledge there had not been detailed reports with regard to comprehensive sequencing data, subsequent recommendations by an MTB, the implementation of molecular guided treatments and documented outcome with similar cohort sizes so far [101].

This work includes patients that were discussed at the MTB between April 2016 and February 2018.

2 MATERIAL AND METHODS

Information on the Materials and Methods are also part of the publication of the cohort by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]. For further information on authorship regarding distinct aspects of the work, please also consult the 'Erklärungen zum Eigenanteil' (declaration of own contribution).

The local ethics committee of the Medical Faculty reviewed and approved this retrospective study (511/2018BO) [101].

2.1 PATIENT COLLECTIVE

96 patients with GI tumors that were presented at the Molecular Tumor Board (MTB) at the University Hospital Tübingen between initiation of the MTB and end of February 2018 are included in this study.

Of 25 patients who received a treatment based on MTB presentation and recommendation, 20 patients were available for best response analysis [101]. All 25 patients were available for follow-up until death or data cut-off on March 31, 2019 (Fig. 9).

2.2 DATA COLLECTION AND INTERPRETATION

Data for the analysis were obtained from the electronic MTB platform, which will be further described below, as well as from patient history documentation in the SAP system. In case of missing data in the documentation, access to further information was given in collaboration with the Tübingen Center for Personalized Medicine, the attending physicians, and the cancer registry Baden-Württemberg. This especially pertained information on treatment duration and survival of patients that had been presented to the MTB but lived and thus were treated in the regions of their hometowns.

Genetic test results including somatic alterations, alterations in the germline, tumor mutational burden and examined tumor specimen were obtained from the MTB platform, the MTB reports or directly from the reports sent by the sequencing

institution (for further information on the involved sequencing institutions see also 2.4).

MTB recommendation and treatment implementation were documented on the MTB platform or otherwise information was given by the MTB reports, the documentation of patient's histories in the SAP system or the attending physicians.

Information on treatment response was taken from radiologic imaging reports within the follow-up that were documented within the clinical patient files. Based on radiological imaging studies best response was assessed, this was in line with RECIST 1.1 criteria [126], iRECIST criteria in case of treatment with Immune Checkpoint Inhibitors [127], or mRECIST criteria for HCC [24, 101].

Progression free survival (PFS) was calculated from the day of initiation of the MTB-recommended treatment until radiographic progression, or death [101]. Overall survival (OS) was calculated from the day of initiation of the MTB-recommended treatment until death, irrespective of its cause, or data cut-off on March 31, 2019 [101].

2.3 ORGANIZATION OF THE MTB

The MTB Tübingen is coordinated by the Tübingen Center for Personalized Medicine [101]. The MTB comprises an interdisciplinary team which includes next to experts in clinical and translational oncology, radiology, and pathology also experts in molecular biology, bioinformatics, and human genetics [101]. The MTB team assembled weekly in presence meetings. For introduction of patients to the MTB team, provision of necessary information for preparation and follow-up as well as subsequent documentation, an electronic Web-based platform (MTB platform) was set up [101]. Beside findings and clinical data, meta- and structured-parametric data could be stored and prepared in conformity with IT-security regulations [101]. There was no data analysis of the sequencing results by the version of MTB platform used in the observation period [101]. Equally, there were no treatment options suggested by the MTB platform itself [101]. Interdisciplinary expert discussion and agreement in the presence meetings resulted in identification of targets and target prioritization [101].

Based on the identified targetable molecular alterations there was a recommendation for available clinical studies or in-label, off-label, or matched experimental treatments given by the MTB [101]. As also depicted in in our publication, Bitzer, Ostermann et al, 2020, “off-label use refers to the administration of an FDA/European Medicines Agency-approved drug outside its approved indication. For recommended off-label therapies, an application for reimbursement was submitted to the patient’s health insurance. Experimental individual treatment describes an individualized therapy (Heilversuch) in patients with exhausted standard therapeutic options according to §34 Arzneimittelgesetz (German Pharmacy Law). Medications used within the scope of a Heilversuch do not need to be FDA/EMA approved. Patients treated within a Heilversuch have been registered at the local authority, in this case the Regierungspräsidium Tübingen.” [101]

2.4 GENETIC ANALYSIS

Tissue for genome sequencing was obtained either from former surgical resection within previous therapeutic regimes or biopsies performed for diagnosis or from biopsies performed with the purpose of tumor genome sequencing.

Matching germline DNA was received either from normal tissue or from peripheral blood [101]. Before sequencing, the tumor type was validated. Also, in the sections and microsections of the analyzed tumor areas, the percentage of tumor cells was determined by a pathologist. The genetic investigation of tumor and normal tissues was performed by NGS panel sequencing of full coding sequences or by whole exome sequencing [101].

For NGS panel sequencing there was DNA extraction from FFPE-embedded tissues [101]. NGS Panel sequencing was carried out by the Institute of Medical Genetics and Applied Genomics Tübingen or CeGaT GmbH and Praxis für Humangenetik Tübingen. Across both institutes there were 4 different panel versions. Genes represented in the different panel versions are shown in Table 1. By NGS panel sequencing there was coverage of the whole coding sequence of all genes that were included in the respective panel [101]. All technical steps

and the analysis of raw data were performed by these diagnostic partners. Technical details are also given in the publication of this cohort [101].

6 patients received more than one panel sequencing analysis during the observation period and 4 patients received additional diagnostics subsequently to their presentation at the MTB, in an observation time frame extended until end of 06/2018, that also are included in this analysis.

	Version V3 (649 genes)	**Version V4 (710 genes)**	**Version V2 (337 genes)**	**Version V3 (678 genes)**
ABCB1	ABCB1		ABCB1	ABCB1
ABCC2	ABCC2			ABCC2
ABCC4				ABCC4
ABCG2	ABCG2			ABCG2
ABL1	ABL1	ABL1	ABL1	ABL1
ABL2	ABL2	ABL2	ABL2	ABL2
ACD	ACD	ACD		
ACE			ACE	
ACO1				ACO1
ACTB				ACTB
ACVR1	ACVR1			
ACVR1B	ACVR1B		ACVR1B	ACVR1B
ACVR2A				ACVR2A
ADAM10				ADAM10
ADAMTS18				
ADCY1				ADCY1
ADGRA2			ADGRA2	ADGRA2
ADH1A			ADH1A	
ADH1B			ADH1B	
ADH1C			ADH1C	
ADRB1			ADRB1	
ADRB2			ADRB2	
AHR			AHR	
AIP		AIP		
AJUBA	AJUBA	AJUBA		AJUBA
AKAP9				AKAP9
AKT1	AKT1	AKT1	AKT1	AKT1
AKT2	AKT2	AKT2	AKT2	AKT2
AKT3	AKT3	AKT3	AKT3	AKT3
ALDH1A1			ALDH1A1	
ALK	ALK	ALK	ALK	ALK
ALOX12B				ALOX12B

ALOX5			ALOX5	
AMER1	AMER1	AMER1	AMER1	AMER1
ANK3				ANK3
ANKRD26		ANKRD26		
APAF1				APAF1
APC	APC	APC	APC	APC
APCDD1				APCDD1
AR	AR	AR	AR	AR
ARAF	ARAF	ARAF	ARAF	ARAF
ARFRP1	ARFRP1		ARFRP1	ARFRP1
ARHGAP35	ARHGAP35	ARHGAP35		ARHGAP35
ARHGEF6				ARHGEF6
ARID1A	ARID1A	ARID1A	ARID1A	ARID1A
ARID1B	ARID1B	ARID1B	ARID1B	ARID1B
ARID2	ARID2	ARID2	ARID2	ARID2
ARID5B	ARID5B	ARID5B		ARID5B
ARNT	ARNT			
ASXL1	ASXL1	ASXL1	ASXL1	ASXL1
ASXL2		ASXL2		ASXL2
ATF1	ATF1			
ATG2B		ATG2B		
ATM	ATM	ATM	ATM	ATM
ATP1A1	ATP1A1	ATP1A1		ATP1A1
ATP5B	ATP5B			
ATR	ATR	ATR	ATR	ATR
ATRX	ATRX	ATRX	ATRX	ATRX
AURKA	AURKA	AURKA	AURKA	AURKA
AURKB	AURKB	AURKB	AURKB	AURKB
AURKC	AURKC	AURKC		
AXIN1	AXIN1	AXIN1	AXIN1	AXIN1
AXIN2	AXIN2	AXIN2		AXIN2
AXL	AXL	AXL		AXL
AZGP1	AZGP1			
B2M	B2M	B2M		B2M
BACH1				BACH1
BAP1	BAP1	BAP1	BAP1	BAP1
BARD1	BARD1	BARD1	BARD1	BARD1
BBC3				BBC3
BCL10	BCL10	BCL10		
BCL11A	BCL11A	BCL11A		
BCL11B	BCL11B	BCL11B		
BCL2	BCL2	BCL2	BCL2	BCL2
BCL2A1			BCL2A1	
BCL2L1	BCL2L1		BCL2L1	BCL2L1

BCL2L11				BCL2L11
BCL2L2	BCL2L2		BCL2L2	BCL2L2
BCL3	BCL3	BCL3		
BCL6	BCL6	BCL6	BCL6	BCL6
BCL9	BCL9	BCL9		
BCLAF1				BCLAF1
BCOR	BCOR	BCOR	BCOR	BCOR
BCORL1	BCORL1	BCORL1		BCORL1
BCR	BCR	BCR		BCR
BIRC2	BIRC2	BIRC2		
BIRC3	BIRC3	BIRC3		
BIRC5	BIRC5	BIRC5		
BLM	BLM	BLM		BLM
BMPR1A	BMPR1A	BMPR1A		BMPR1A
BRAF	BRAF	BRAF	BRAF	BRAF
BRCA1	BRCA1	BRCA1	BRCA1	BRCA1
BRCA2	BRCA2	BRCA2	BRCA2	BRCA2
BRD3		BRD3		
BRD4	BRD4	BRD4	BRD4	BRD4
BRE	BRE			
BRIP1	BRIP1	BRIP1	BRIP1	BRIP1
BTG1				BTG1
BTK	BTK	BTK	BTK	BTK
BTNL2	BTNL2	BTNL2		
BUB1B	BUB1B	BUB1B		BUB1B
C11ORF30	C11ORF30	C11ORF30		CAD
CALR		CALR		
CAMK2G		CAMK2G		
CARD11	CARD11	CARD11	CARD11	CARD11
CARM1				CARM1
CASP8	CASP8	CASP8		CASP8
CAST				CAST
CBFB	CBFB	CBFB	CBFB	CBFB
CBL	CBL	CBL	CBL	CBL
CBLB		CBLB		CBLB
CBLC		CBLC		CBLC
CCAR1				CCAR1
CCDC6	CCDC6	CCDC6		
CCND1	CCND1	CCND1	CCND1	CCND1
CCND2	CCND2	CCND2	CCND2	CCND2
CCND3	CCND3	CCND3	CCND3	CCND3
CCNE1	CCNE1	CCNE1	CCNE1	CCNE1
CD1D	CD1D			CD1D
CD274	CD274	CD274		CD274

CD276				CD276
CD38		CD38	CD38	
CD52		CD52		
CD58		CD58		
CD70	CD70			CD70
CD79A	CD79A	CD79A	CD79A	CD79A
CD79B	CD79B	CD79B	CD79B	CD79B
CD82	CD82	CD82		
CDC27	CDC27			CDC27
CDC73	CDC73	CDC73	CDC73	CDC73
CDH1	CDH1	CDH1	CDH1	CDH1
CDH2	CDH2	CDH2	CDH2	
CDH11		CDH11		
CDH20			CDH20	CDH20
CDH5			CDH5	
CDK12	CDK12	CDK12	CDK12	CDK12
CDK2				CDK2
CDK4	CDK4	CDK4	CDK4	CDK4
CDK6	CDK6	CDK6	CDK6	CDK6
CDK8	CDK8	CDK8	CDK8	CDK8
CDKN1A	CDKN1A	CDKN1A	CDKN1A	CDKN1A
CDKN1B	CDKN1B	CDKN1B	CDKN1B	CDKN1B
CDKN1C		CDKN1C		
CDKN2A	CDKN2A	CDKN2A	CDKN2A	CDKN2A
CDKN2B	CDKN2B	CDKN2B	CDKN2B	CDKN2B
CDKN2C	CDKN2C	CDKN2C	CDKN2C	CDKN2C
CDX2	CDX2			
CEBPA	CEBPA	CEBPA	CEBPA	CEBPA
CEP57	CEP57	CEP57		
CHD1		CHD1		
CHD2	CHD2	CHD2	CHD2	
CHD3				CHD3
CHD4	CHD4	CHD4	CHD4	CHD4
CHD8				CHD8
CHEK1	CHEK1	CHEK1	CHEK1	CHEK1
CHEK2	CHEK2	CHEK2	CHEK2	CHEK2
CHUK				CHUK
CIC	CIC	CIC	CIC	CIC
CIITA		CIITA		
CKS1B	CKS1B	CKS1B		
CLTC				CLTC
CNOT1				CNOT1
CNOT3		CNOT3		
CNTNAP1				CNTNAP1

COL1A1	COL1A1	COL1A1		COL1A1
COL3A1				COL3A1
COMMD1		COMMD1		
COMT			COMT	COMT
CRBN				CRBN
CREB1	CREB1	CREB1		CREB1
CREBBP	CREBBP	CREBBP	CREBBP	CREBBP
CRKL	CRKL	CRKL	CRKL	CRKL
CRLF2			CRLF2	CRLF2
CRTC1	CRTC1	CRTC1		CRTC1
CRTC2		CRTC2		
CRTC3				CRTC3
CSDE1				CSDE1
CSF1R	CSF1R	CSF1R	CSF1R	CSF1R
CSF2		CSF2		
CSF3R		CSF3R	CSF3R	
CSMD1		CSMD1		
CSNK1A1		CSNK1A1		
CTCF	CTCF	CTCF	CTCF	CTCF
CTLA4		CTLA4		CTLA4
CTNNA1	CTNNA1	CTNNA1	CTNNA1	CTNNA1
CTNNB1	CTNNB1	CTNNB1	CTNNB1	CTNNB1
CTNND1				CTNND1
CTTN				CTTN
CUL1				CUL1
CUL3	CUL3			
CUL4A				CUL4A
CUL4B	CUL4B	CUL4B		CUL4B
CUX1	CUX1	CUX1	CUX1	CUX1
CXCR4		CXCR4		
CYLD	CYLD	CYLD	CYLD	CYLD
CYP1A2	CYP1A1		CYP1A2	
CYP1A2	CYP1A2			
CYP17A1				CYP17A1
CYP1B1				CYP1B1
CYP2A6	CYP2A6		CYP2A6	
CYP2A7		CYP2A7		
CYP2B6	CYP2B6		CYP2B6	
CYP2C19	CYP2C19		CYP2C19	CYP2C19
CYP2C8	CYP2C8		CYP2C8	CYP2C8
CYP2C9	CYP2C9		CYP2C9	CYP2C9
CYP2D6	CYP2D6		CYP2D6	CYP2D6
CYP2E1	CYP2E1		CYP2E1	
CYP2J2			CYP2J2	

CYP3A4	CYP3A4		CYP3A4	CYP3A4
CYP3A5	CYP3A5		CYP3A5	CYP3A5
DAXX	DAXX	DAXX	DAXX	DAXX
DCC	DCC	DCC		
DCUN1D1				DCUN1D1
DDB2	DDB2	DDB2		DDB2
DDIT3	DDIT3			
DDR1		DDR1		DDR1
DDR2	DDR2	DDR2	DDR2	DDR2
DDX11		DDX11		
DDX3X	DDX3X	DDX3X		DDX3X
DDX41		DDX41		
DDX5				DDX5
DEK	DEK	DEK		
DHFR		DHFR		
DIAPH1	DIAPH1			DIAPH1
DICER1	DICER1	DICER1		DICER1
DIDO1				DIDO1
DIS3	DIS3	DIS3		DIS3
DIS3L2		DIS3L2		
DKC1		DKC1		
DLL1			DLL1	
DLL3			DLL3	
DLL4			DLL4	
DMD				DMD
DNMT1	DNMT1	DNMT1		DNMT1
DNMT3A	DNMT3A	DNMT3A	DNMT3A	DNMT3A
DNMT3B				DNMT3B
DOT1L	DOT1L	DOT1L	DOT1L	DOT1L
DPYD	DPYD	DPYD	DPYD	DPYD
DRD2			DRD2	
DST	DST			
E2F3				E2F3
EBP		EBP		
EED			EED	EED
EEF1A1			EEF1A1	EEF1A1
EGFL7				EGFL7
EGFR	EGFR	EGFR	EGFR	EGFR
EGLN1		EGLN1		
EGR2		EGR2		
EGR3	EGR3	EGR3		EGR3
EIF1AX				EIF1AX
EIF4A2				EIF4A2
ELAC2	ELAC2	ELAC2		

ELANE		ELANE		
ELF3	ELF3	ELF3		ELF3
EML4	EML4	EML4		EML4
ENG	ENG			
EP300	EP300	EP300	EP300	EP300
EPAS1		EPAS1		
EPCAM	EPCAM	EPCAM		EPCAM
EPHA2	EPHA2	EPHA2		EPHA2
EPHA3	EPHA3	EPHA3	EPHA3	EPHA3
EPHA4		EPHA4		
EPHA5	EPHA5		EPHA5	EPHA5
EPHA6			EPHA6	EPHA6
EPHA7	EPHA7		EPHA7	EPHA7
EPHB1	EPHB1		EPHB1	EPHB1
EPHB2				EPHB2
EPHB4	EPHB4	EPHB4	EPHB4	
EPHB6	EPHB6	EPHB6	EPHB6	EPHB6
EPHX1	EPHX1			
EPPK1				EPPK1
ERBB2	ERBB2	ERBB2	ERBB2	ERBB2
ERBB3	ERBB3	ERBB3	ERBB3	ERBB3
ERBB4	ERBB4	ERBB4	ERBB4	ERBB4
ERCC1	ERCC1	ERCC1		ERCC1
ERCC2	ERCC2	ERCC2	ERCC2	ERCC2
ERCC3	ERCC3	ERCC3		ERCC3
ERCC4	ERCC4	ERCC4		ERCC4
ERCC5	ERCC5	ERCC5		ERCC5
ERG	ERG	ERG	ERG	ERG
ERRFI1	ERRFI1	ERRFI1	ERRFI1	
ESR1	ESR1	ESR1	ESR1	ESR1
ESR2		ESR2	ESR2	ESR2
ETNK1		ETNK1		
ETS1	ETS1	ETS1		
ETV1	ETV1	ETV1		ETV1
ETV4	ETV4	ETV4		ETV4
ETV5	ETV5	ETV5		ETV5
ETV6	ETV6	ETV6		ETV6
EWSR1	EWSR1	EWSR1		EWSR1
EXO1		EXO1		
EXT1	EXT1	EXT1		EXT1
EXT2	EXT2	EXT2		EXT2
EZH1	EZH1	EZH1		EZH1
EZH2	EZH2	EZH2	EZH2	EZH2
F5			F5	

FAM175A	FAM175A	FAM175A		FAM175A
FAM46C	FAM46C	FAM46C	FAM46C	FAM46C
FAN1		FAN1		
FANCA	FANCA	FANCA	FANCA	FANCA
FANCB	FANCB	FANCB		
FANCC	FANCC	FANCC	FANCC	FANCC
FANCD2	FANCD2	FANCD2	FANCD2	FANCD2
FANCE	FANCE	FANCE	FANCE	FANCE
FANCF	FANCF	FANCF	FANCF	FANCF
FANCG	FANCG	FANCG	FANCG	FANCG
FANCI	FANCI	FANCI		FANCI
FANCL	FANCL	FANCL	FANCL	FANCL
FANCM	FANCM	FANCM		FANCM
FAS	FAS	FAS		FAS
FAT1	FAT1	FAT1		FAT1
FAT3				FAT3
FBN1				FBN1
FBXO11				FBXO11
FBXW7	FBXW7	FBXW7	FBXW7	FBXW7
FES	FES	FES		FES
FGF10	FGF10	FGF10		FGF10
FGF12				FGF12
FGF14	FGF14	FGF14		FGF14
FGF19	FGF19	FGF19		FGF19
FGF2		FGF2		
FGF23	FGF23	FGF23		FGF23
FGF3	FGF3	FGF3		FGF3
FGF4	FGF4	FGF4		FGF4
FGF5		FGF5		
FGF6	FGF6	FGF6		FGF6
FGF7				FGF7
FGFBP1	FGFBP1	FGFBP1		FGFBP1
FGFR1	FGFR1	FGFR1	FGFR1	FGFR1
FGFR2	FGFR2	FGFR2	FGFR2	FGFR2
FGFR3	FGFR3	FGFR3	FGFR3	FGFR3
FGFR4	FGFR4	FGFR4	FGFR4	FGFR4
FH	FH	FH		FH
FKBP1A		FKBP1A		
FLCN	FLCN	FLCN	FLCN	FLCN
FLI1	FLI1	FLI1		
FLT1	FLT1	FLT1	FLT1	FLT1
FLT3			FLT3	FLT3
FLT4	FLT4	FLT4	FLT4	FLT4
FN1	FN1			FN1

FOXA1	FOXA1	FOXA1		FOXA1
FOXA2	FOXA2	FOXA2		FOXA2
FOXE1	FOXE1	FOXE1		FOXE1
FOXL2	FOXL2	FOXL2	FOXL2	FOXL2
FOXO1	FOXO1	FOXO1		
FOXO3	FOXO3	FOXO3		
FOXP1	FOXP1	FOXP1	FOXP1	FOXP1
FOXP4			FOXP4	
FOXQ1	FOXQ1	FOXQ1		FOXQ1
FRK		FRK		
FRS2	FRS2	FRS2		
FUBP1	FUBP1	FUBP1	FUBP1	FUBP1
FUS	FUS	FUS		
FYN		FYN		
G6PD	G6PD	G6PD	G6PD	
GABRA6	GABRA6	GABRA6	GABRA6	GABRA6
GALNT12	GALNT12	GALNT12		
GATA1	GATA1	GATA1	GATA1	GATA1
GATA2	GATA2	GATA2	GATA2	GATA2
GATA3	GATA3	GATA3	GATA3	GATA3
GATA4	GATA4	GATA4		
GATA6	GATA6	GATA6	GATA6	
GDNF	GDNF			
GID4	GID4			GID4
GLA				GLA
GLDN		GLDN		
GLI1	GLI1	GLI1		
GLI2		GLI2		
GNA11	GNA11	GNA11	GNA11	GNA11
GNA13	GNA13	GNA13		GNA13
GNAI1				GNAI1
GNAQ	GNAQ	GNAQ	GNAQ	GNAQ
GNAS	GNAS	GNAS	GNAS	GNAS
GOLGA5				GOLGA5
GOT1	GOT1			GOT1
GPC3	GPC3	GPC3		
GPER1		GPER1		
GPR124	GPR124	GPR124		
GPS2				GPS2
GPX1				GPX1
GREM1		GREM1		GREM1
GRIN2A	GRIN2A	GRIN2A		GRIN2A
GRM3	GRM3	GRM3		GRM3
GSK3A		GSK3A		

GSK3B	GSK3B			GSK3B
GSTM1	GSTM1			
GSTP1	GSTP1		GSTP1	GSTP1
GSTT1	GSTT1		GSTT1	
GUCY1A2			GUCY1A2	GUCY1A2
GUSB	GUSB			GUSB
H3F3A	H3F3A	H3F3A	H3F3A	H3F3A
H3F3B	H3F3B			
H3F3C				H3F3C
HCFC1				HCFC1
HCK		HCK		
HERC1				HERC1
HGF	HGF	HGF	HGF	HGF
HIF1A	HIF1A	HIF1A		HIF1A
HIST1H1C				HIST1H1C
HIST1H2BD				HIST1H2BD
HIST1H3B	HIST1H3B	HIST1H3B		HIST1H3B
HLA-A	HLA-A	HLA-A		HLA-A
HLA-B	HLA-B	HLA-B		HLA-B
HLA-C	HLA-C	HLA-C		
HLA-DPA1		HLA-DPA1		
HLA-DPB1		HLA-DPB1		
HLA-DQA1		HLA-DQA1		
HLA-DQB1		HLA-DQB1		
HLA-DRA		HLA-DRA		
HLA-DRB1		HLA-DRB1		
HLF	HLF	HLF		
HMGA2	HMGA2	HMGA2		
HMGCR			HMGCR	
HMGN1		HMGN1		
HMOX2		HMOX2		
HNF1A	HNF1A	HNF1A	HNF1A	HNF1A
HNF1B	HNF1B	HNF1B		
HOXA3			HOXA3	
HOXA9	HOXA9			
HOXB13	HOXB13	HOXB13		
HOXD8		HOXD8		
HRAS	HRAS	HRAS	HRAS	HRAS
HSD3B1	HSD3B1	HSD3B1		HSD3B1
HSP90AA1	HSP90AA1	HSP90AA1	HSP90AA1	
HSP90AB1	HSP90AB1	HSP90AB1		HSP90AB1
HSPA8				HSPA8
ICOSLG				ICOSLG
ID3		ID3		

IDH1	IDH1	IDH1	IDH1	IDH1
IDH2	IDH2	IDH2	IDH2	IDH2
IFNGR1		IFNGR1		IFNGR1
IFNGR2		IFNGR2		
IGF1				IGF1
IGF1R	IGF1R	IGF1R	IGF1R	IGF1R
IGF2	IGF2	IGF2		IGF2
IGF2R	IGF2R	IGF2R	IGF2R	IGF2R
IKBKB	IKBKB	IKBKB		
IKBKE	IKBKE	IKBKE	IKBKE	IKBKE
IKZF1	IKZF1	IKZF1	IKZF1	IKZF1
IKZF3		IKZF3		
IL10				IL10
IL1B		IL1B		
IL1RN		IL1RN		
IL2	IL2	IL2		
IL21R	IL21R	IL21R		
IL6		IL6		
IL6ST	IL6ST	IL6ST		
IL7R	IL7R	IL7R	IL7R	IL7R
ING1	ING1			ING1
ING4	ING4	ING4		
INHBA	INHBA		INHBA	INHBA
INPP4B	INPP4B	INPP4B		INPP4B
INPPL1	INPPL1	INPPL1		INPPL1
INSR			INSR	INSR
IRF1		IRF1		
IRF2	IRF2			
IRF4	IRF4			IRF4
IRF6	IRF6			
IRS1				IRS1
IRS2	IRS2	IRS2	IRS2	IRS2
IRS4				
ITGB2	ITGB2			
ITK	ITK	ITK		
JAG1			JAG1	JAG1
JAG2			JAG2	JAG2
JAK1	JAK1	JAK1	JAK1	JAK1
JAK2	JAK2	JAK2	JAK2	JAK2
JAK3	JAK3	JAK3	JAK3	JAK3
JUN	JUN	JUN	JUN	JUN
KALRN				KALRN
KAT6A	KAT6A	KAT6A		KAT6A
KCNH2			KCNH2	KCNH2

KCNJ11			KCNJ11	
KCNJ5	KCNJ5			
KCNQ1				KCNQ1
KDM5A	KDM5A	KDM5A	KDM5A	KDM5A
KDM5C	KDM5C	KDM5C	KDM5C	KDM5C
KDM6A	KDM6A	KDM6A	KDM6A	KDM6A
KDR	KDR	KDR	KDR	KDR
KEAP1	KEAP1	KEAP1	KEAP1	KEAP1
KEL	KEL			KEL
KIAA1549	KIAA1549	KIAA1549		
KIT	KIT	KIT	KIT	KIT
KLF2		KLF2		
KLF4	KLF4	KLF4		KLF4
KLF5				KLF5
KLF6	KLF6			
KLHDC8B		KLHDC8B		
KLHL6	KLHL6	KLHL6		KLHL6
KMT2A	KMT2A	KMT2A	KMT2A	KMT2A
KMT2B	KMT2B	KMT2B		KMT2B
KMT2C	KMT2C	KMT2C	KMT2C	KMT2C
KMT2D	KMT2D	KMT2D	KMT2D	KMT2D
KRAS	KRAS	KRAS	KRAS	KRAS
LAMA2				LAMA2
LAMP1	LAMP1			
LATS1	LATS1	LATS1		LATS1
LATS2	LATS2	LATS2		LATS2
LCK	LCK	LCK		
LCP1				LCP1
LDLR				LDLR
LGI1	LGI1			
LIFR	LIFR			LIFR
LIG4	LIG4	LIG4		
LIMK2		LIMK2		
LMNA				LMNA
LMO1	LMO1	LMO1	LMO1	LMO1
LPP	LPP			LPP
LRP1B	LRP1B	LRP1B	LRP1B	LRP1B
LRP6			LRP6	
LRRK2	LRRK2	LRRK2		LRRK2
LTK	LTK	LTK	LTK	
LYL1	LYL1			
LYN	LYN	LYN	LYN	LYN
LZTR1	LZTR1	LZTR1		LZTR1
MAD2L2		MAD2L2		

MAF	MAF			
MAFB	MAFB	MAFB		
MAGEA1	MAGEA1	MAGEA1		
MAGI1	MAGI2	MAGI1		
MAGI2		MAGI2	MAGI2	
MALAT1				MALAT1
MALT1	MALT1			
MAML1	MAML1	MAML1		
MAML2				MAML2
MAP2K1	MAP2K1	MAP2K1	MAP2K1	MAP2K1
MAP2K2	MAP2K2	MAP2K2	MAP2K2	MAP2K2
MAP2K3		MAP2K3		
MAP2K4	MAP2K4	MAP2K4	MAP2K4	MAP2K4
MAP2K5		MAP2K5		
MAP2K6		MAP2K6		
MAP2K7		MAP2K7		MAP2K7
MAP3K1	MAP3K1	MAP3K1	MAP3K1	MAP3K1
MAP3K13				MAP3K13
MAP3K14		MAP3K14		
MAP3K3		MAP3K3		MAP3K3
MAP3K4		MAP3K4		MAP3K4
MAP3K6	MAP3K6	MAP3K6		
MAP4K1				MAP4K1
MAP4K3				MAP4K3
MAPK1	MAPK1	MAPK1		MAPK1
MAPK11		MAPK11		
MAPK12		MAPK12		
MAPK3		MAPK3		
MAPK8	MAPK8			
MAPK8IP1	MAPK8IP1	MAPK8IP1		MAPK8IP1
MAX	MAX	MAX		MAX
MBD1	MBD1	MBD1		MBD1
MC1R	MC1R	MC1R		MC1R
MCL1	MCL1	MCL1	MCL1	MCL1
MDC1		MDC1		MDC1
MDM2	MDM2	MDM2	MDM2	MDM2
MDM4	MDM4	MDM4	MDM4	MDM4
MECOM	MECOM	MECOM		MECOM
MED1				MED1
MED12	MED12	MED12	MED12	MED12
MED17				MED17
MED23				MED23
MEF2A				MEF2A
MEF2B	MEF2B	MEF2B		MEF2B

MEN1	MEN1	MEN1	MEN1	MEN1
MET	MET	MET	MET	MET
MGA	MGA	MGA		MGA
MGMT		MGMT		
MITF	MITF	MITF	MITF	MITF
MLH1	MLH1	MLH1	MLH1	MLH1
MLH3	MLH3	MLH3		MLH3
MLLT10	MLLT10	MLLT10		MLLT10
MLLT3	MLLT3	MLLT3		MLLT3
MMP2	MMP2			MMP2
MN1	MN1	MN1		MN1
MNDA				MNDA
MOB1A	MOB1A			
MOB1B	MOB1B			
MPL	MPL	MPL	MPL	MPL
MPO	MPO			MPO
MRE11A	MRE11A	MRE11A	MRE11A	MRE11A
MS4A1		MS4A1	MS4A1	
MSH2	MSH2	MSH2	MSH2	MSH2
MSH3	MSH3	MSH3		
MSH4		MSH4		
MSH5		MSH5		
MSH6	MSH6	MSH6	MSH6	MSH6
MSR1	MSR1	MSR1		
MST1R		MST1R		
MTHFR	MTHFR	MTHFR	MTHFR	MTHFR
MTOR	MTOR	MTOR	MTOR	MTOR
MTR	MTR			
MTRR	MTRR	MTRR		
MUC1	MUC1	MUC1	MUC1	
MUC16		MUC16		
MUTYH	MUTYH	MUTYH	MUTYH	MUTYH
MXI1	MXI1	MXI1		
MYB	MYB	MYB	MYB	MYB
MYBPC3				MYBPC3
MYC	MYC	MYC	MYC	MYC
MYCL	MYCL	MYCL	MYCL	MYCL
MYCN	MYCN	MYCN	MYCN	MYCN
MYD88	MYD88	MYD88	MYD88	MYD88
MYH11	MYH11	MYH11		
MYH7				MYH7
MYH9	MYH9	MYH9		MYH9
MYL2				MYL2
MYL3				MYL3

MYLK				MYLK
MYOD1				MYOD1
NAT1	NAT1			
NAT2	NAT2		NAT2	
NAV3				NAV3
NBN	NBN	NBN		NBN
NCOA1	NCOA1	NCOA1		
NCOA2	NCOA2			
NCOA3	NCOA3	NCOA3		NCOA3
NCOR1	NCOR1	NCOR1		NCOR1
NCOR2				NCOR2
NEDD4L				NEDD4L
NF1	NF1	NF1	NF1	NF1
NF2	NF2	NF2	NF2	NF2
NFE2L2	NFE2L2	NFE2L2	NFE2L2	NFE2L2
NFKB1	NFKB1	NFKB1		NFKB1
NFKB2	NFKB2	NFKB2		NFKB2
NFKBIA	NFKBIA	NFKBIA		NFKBIA
NFKBIE		NFKBIE		
NIN	NIN	NIN		NIN
NIPBL				NIPBL
NKX2-1	NKX2-1		NKX2-1	NKX2-1
NKX3-1	NKX3-1			
NLRC5		NLRC5		
NOP10		NOP10		
NOTCH1	NOTCH1	NOTCH1	NOTCH1	NOTCH1
NOTCH2	NOTCH2	NOTCH2	NOTCH2	NOTCH2
NOTCH3	NOTCH3	NOTCH3	NOTCH3	NOTCH3
NOTCH4		NOTCH4	NOTCH4	NOTCH4
NPM1	NPM1	NPM1	NPM1	NPM1
NQO1		NQO1	NQO1	NQO1
NR1I2			NR1I2	
NR1I3		NR1I3		
NR4A2				NR4A2
NRAS	NRAS	NRAS	NRAS	NRAS
NRG2		NRG2		
NSD1	NSD1	NSD1	NSD1	NSD1
NT5C2		NT5C2	NT5C2	
NTHL1		NTHL1		
NTN4				NTN4
NTRK1	NTRK1	NTRK1	NTRK1	NTRK1
NTRK2	NTRK2	NTRK2	NTRK2	NTRK2
NTRK3	NTRK3	NTRK3	NTRK3	NTRK3
NUMA1	NUMA1	NUMA1		NUMA1

NUP93	NUP93			NUP93
NUP98	NUP98	NUP98		NUP98
NUTM1				NUTM1
OR5A1				OR5A1
OTC				OTC
P2RY1			P2RY1	
P2RY12			P2RY12	
PABPC1				PABPC1
PAK1				PAK1
PAK3	PAK3	PAK3	PAK3	PAK3
PAK7				PAK7
PALB2	PALB2	PALB2		PALB2
PALLD	PALLD	PALLD		
PARK2	PARK2	PARK2	PARK2	PARK2
PARP1		PARP1		PARP1
PARP2		PARP2		PARP2
PARP3				PARP3
PARP4		PARP4		PARP4
PAX3	PAX3	PAX3		
PAX5	PAX5	PAX5	PAX5	PAX5
PAX7	PAX7	PAX7		
PAX8				PAX8
PBK		PBK		
PBRM1	PBRM1	PBRM1	PBRM1	PBRM1
PBX1	PBX1	PBX1		
PCBP1	PCBP1			PCBP1
PCSK9				PCSK9
PDCD1		PDCD1		PDCD1
PDCD1LG2	PDCD1LG2	PDCD1LG2		
PDF		PDF		
PDGFA		PDGFA		
PDGFB	PDGFB	PDGFB		
PDGFC		PDGFC		
PDGFD		PDGFD		
PDGFRA	PDGFRA	PDGFRA	PDGFRA	PDGFRA
PDGFRB	PDGFRB	PDGFRB	PDGFRB	PDGFRB
PDK1	PDK1	PDK1		PDK1
PDPK1				PDPK1
PER1	PER1			
PGR		PGR	PGR	
PHF6	PHF6	PHF6	PHF6	PHF6
PHLPP2			PHLPP2	
PHOX2B	PHOX2B	PHOX2B		PHOX2B
PIAS4		PIAS4		

PIGA		PIGA		
PIK3C2A		PIK3C2A		
PIK3C2B	PIK3C2B	PIK3C2B		PIK3C2B
PIK3C2G		PIK3C2G		PIK3C2G
PIK3C3				PIK3C3
PIK3CA	PIK3CA	PIK3CA	PIK3CA	PIK3CA
PIK3CB	PIK3CB	PIK3CB		PIK3CB
PIK3CD	PIK3CD	PIK3CD		PIK3CD
PIK3CG	PIK3CG	PIK3CG	PIK3CG	PIK3CG
PIK3R1	PIK3R1	PIK3R1	PIK3R1	PIK3R1
PIK3R2	PIK3R2	PIK3R2		PIK3R2
PIK3R3		PIK3R3		
PIM1	PIM1	PIM1		PIM1
PIP5K1A				PIP5K1A
PKHD1	PKHD1	PKHD1	PKHD1	
PKP2				PKP2
PLCG1	PLCG1	PLCG1	PLCG1	PLCG1
PLCG2	PLCG2	PLCG2		PLCG2
PLK2				PLK2
PMAIP1				PMAIP1
PML	PML	PML		PML
PMS1	PMS1	PMS1		PMS1
PMS2	PMS2	PMS2	PMS2	PMS2
PNRC1				PNRC1
POLD1	POLD1	POLD1	POLD1	POLD1
POLE	POLE	POLE	POLE	POLE
POLH	POLH	POLH		
POLQ	POLQ	POLQ		POLQ
POLR3B				POLR3B
POT1	POT1	POT1		
POU2AF1	POU2AF1			
POU2F2	POU2F2			POU2F2
POU5F1	POU5F1			
PPM1D	PPM1D	PPM1D		PPM1D
PPP2R1A	PPP2R1A		PPP2R1A	PPP2R1A
PPP6C				PPP6C
PRAM1			PRAM1	
PREX2				
PRDM1	PRDM1	PRDM1	PRDM1	PRDM1
PRDM16	PRDM16	PRDM16		
PREX2	PREX2	PREX2		
PRF1	PRF1	PRF1		
PRKACA	PRKACA			
PRKAG2				PRKAG2

PRKAR1A	PRKAR1A	PRKAR1A		PRKAR1A
PRKCA		PRKCA		
PRKCI	PRKCI			
PRKD1	PRKD1	PRKD1		
PRKDC	PRKDC	PRKDC	PRKDC	PRKDC
PROM2		PROM2		
PRPF8				PRPF8
PRSS1	PRSS1	PRSS1		
PRSS8	PRSS8			PRSS8
PRX	PRX	PRX		PRX
PSIP1	PSIP1	PSIP1		PSIP1
PSMB1		PSMB1		
PSMB10		PSMB10		
PSMB2		PSMB2		
PSMB5		PSMB5		
PSMB8		PSMB8		
PSMB9		PSMB9		
PSMC3IP		PSMC3IP		
PSPH	PSPH	PSPH		
PTCH1	PTCH1	PTCH1	PTCH1	PTCH1
PTCH2		PTCH2	PTCH2	
PTEN	PTEN	PTEN	PTEN	PTEN
PTGIS			PTGIS	
PTGS2	PTGS2	PTGS2	PTGS2	PTGS2
PTK2		PTK2		
PTK7		PTK7		
PTPN11	PTPN11	PTPN11	PTPN11	PTPN11
PTPRC	PTPRC	PTPRC		PTPRC
PTPRD	PTPRD	PTPRD	PTPRD	PTPRD
PTPRS				PTPRS
PTPRT	PTPRT	PTPRT		PTPRT
QKI	QKI			QKI
RAC1	RAC1	RAC1	RAC1	RAC1
RAC2		RAC2		
RAD21	RAD21	RAD21		RAD21
RAD50	RAD50	RAD50		RAD50
RAD51	RAD51	RAD51		RAD51
RAD51B	RAD51B	RAD51B		RAD51B
RAD51C	RAD51C	RAD51C		RAD51C
RAD51D	RAD51D	RAD51D		RAD51D
RAD52				RAD52
RAD54B		RAD54B		
RAD54L		RAD54L		RAD54L
RAF1	RAF1	RAF1	RAF1	RAF1

RALGDS	RALGDS	RALGDS		
RANBP2				RANBP2
RARA	RARA	RARA	RARA	RARA
RARB		RARB		
RARG		RARG		
RASA1	RASA1	RASA1		RASA1
RASAL1	RASAL1	RASAL1		
RB1	RB1	RB1	RB1	RB1
RBM10	RBM10	RBM10	RBM10	RBM10
RBM15	RBM15			
RBMX				RBMX
RECQL	RECQL			
RECQL4	RECQL4	RECQL4		RECQL4
REL	REL	REL		REL
RET	RET	RET	RET	RET
RFC2		RFC2		
RFWD2				RFWD2
RFX5		RFX5		
RGL1			RGL1	
RGL2			RGL2	
RHBDF2		RHBDF2		RHBDF2
RHEB	RHEB	RHEB		RHEB
RHOA	RHOA	RHOA		RHOA
RHOH	RHOH			
RICTOR	RICTOR	RICTOR	RICTOR	RICTOR
RINT1	RINT1	RINT1		
RIPK1		RIPK1		
RIT1		RIT1		RIT1
RNASEL	RNASEL	RNASEL		
RNF2	RNF2	RNF2		
RNF43	RNF43	RNF43		RNF43
ROS1	ROS1	ROS1	ROS1	ROS1
RPA1				RPA1
RPGR				RPGR
RPL22	RPL22	RPL22		RPL22
RPL5	RPL5			RPL5
RPS15				RPS15
RPS20		RPS20		
RPS6KA4				RPS6KA4
RPS6KB1		RPS6KB1		
RPS6KB2				RPS6KB2
RPTOR	RPTOR	RPTOR	RPTOR	RPTOR
RRM1	RRM1			
RSF1		RSF1		

RUNX1	RUNX1	RUNX1	RUNX1	RUNX1
RUNX1T1	RUNX1T1		RUNX1T1	RUNX1T1
RUNX3				RUNX3
RXRA	RXRA			RXRA
RYBP				RYBP
RYR1	RYR1	RYR1		RYR1
SACS	SACS	SACS		SACS
SALL4			SALL4	
SAMHD1		SAMHD1		
SAV1	SAV1	SAV1		SAV1
SBDS	SBDS	SBDS		SBDS
SCG5		SCG5		
SCN11A				SCN11A
SCN5A			SCN5A	SCN5A
SDHA	SDHA	SDHA		SDHA
SDHAF2	SDHAF2	SDHAF2		SDHAF2
SDHB	SDHB	SDHB		SDHB
SDHC	SDHC	SDHC		SDHC
SDHD	SDHD	SDHD		SDHD
SEC23B		SEC23B		
SELP	SELP			
SEMA4A	SEMA4A	SEMA4A		
SEPT9	SEPT9			
SETBP1	SETBP1	SETBP1		SETBP1
SETD2	SETD2	SETD2	SETD2	SETD2
SETDB1	SETDB1	SETDB1		SETDB1
SF3B1	SF3B1	SF3B1	SF3B1	SF3B1
SGK1	SGK1	SGK1		SGK1
SH2B1		SH2B1		
SH2B3		SH2B3		SH2B3
SH2D1A	SH2D1A	SH2D1A		SH2D1A
SHFM1		SHFM1		
SHH		SHH		
SHQ1				SHQ1
SIK2		SIK2		
SIN3A	SIN3A	SIN3A		SIN3A
SIRT1		SIRT1		
SKP2	SKP2	SKP2		
SLC15A2	SLC15A2			
SLC19A1			SLC19A1	SLC19A1
SLC1A3	SLC1A3			
SLC22A1	SLC22A1			
SLC22A2	SLC22A2			SLC22A2
SLC22A6	SLC22A6			

SLC26A3	SLC26A3	SLC26A3		SLC26A3
SLCO1B1	SLCO1B1		SLCO1B1	
SLCO1B3	SLCO1B3			SLCO1B3
SLIT2	SLIT2	SLIT2		
SLX4	SLX4	SLX4		
SMAD2	SMAD2		SMAD2	SMAD2
SMAD3	SMAD3	SMAD3	SMAD3	SMAD3
SMAD4	SMAD4	SMAD4	SMAD4	SMAD4
SMARCA1				SMARCA1
SMARCA4	SMARCA4	SMARCA4	SMARCA4	SMARCA4
SMARCB1	SMARCB1	SMARCB1	SMARCB1	SMARCB1
SMARCD1				SMARCD1
SMARCE1	SMARCE1	SMARCE1		
SMC1A	SMC1A	SMC1A		SMC1A
SMC3	SMC3	SMC3		SMC3
SMO	SMO	SMO	SMO	SMO
SMUG1	SMUG1			
SNCAIP	SNCAIP			SNCAIP
SOCS1	SOCS1	SOCS1	SOCS1	SOCS1
SOD2				SOD2
SOS1	SOS1			SOS1
SOX10	SOX10		SOX10	SOX10
SOX11	SOX11	SOX11		
SOX17	SOX17			SOX17
SOX2	SOX2	SOX2	SOX2	SOX2
SOX9	SOX9	SOX9	SOX9	SOX9
SPEN	SPEN	SPEN		SPEN
SPINK1	SPINK1	SPINK1		
SPOP	SPOP	SPOP	SPOP	SPOP
SPOPL				
SPRED1	SPRED1	SPRED1		
SPTA1	SPTA1	SPTA1		SPTA1
SPTAN1				SPTAN1
SRC	SRC	SRC	SRC	SRC
SRD5A2	SRD5A2	SRD5A2		
SRGAP1		SRGAP1		
SRP72		SRP72		
SRSF2	SRSF2	SRSF2	SRSF2	SRSF2
SSTR1		SSTR1		
SSTR2		SSTR2		
SSTR3		SSTR3		
SSTR5		SSTR5		
SSX1	SSX1	SSX1		
STAG1	STAG2	STAG1		

STAG2		STAG2	STAG2	STAG2
STAT1		STAT1		
STAT3	STAT3	STAT3	STAT3	STAT3
STAT4	STAT4			STAT4
STAT5A		STAT5A		
STAT5B	STAT5B	STAT5B		
STK11	STK11	STK11	STK11	STK11
STK19				
STK3	STK3			
STK31				
STK4	STK4			
STK40				STK40
SUFU	SUFU	SUFU	SUFU	SUFU
SULT1A1	SULT1A1		SULT1A1	SULT1A1
SUZ12	SUZ12	SUZ12		SUZ12
SYK	SYK	SYK	SYK	SYK
SYNE1				SYNE1
TAF1	TAF1	TAF1		TAF1
TAF15	TAF15	TAF15		
TAL1	TAL1			
TAP1	TAP1	TAP1		TAP1
TAP2		TAP2		
TBK1		TBK1		
TBL1XR1	TBL1XR1	TBL1XR1		TBL1XR1
TBX22			TBX22	
TBX3	TBX3	TBX3		TBX3
TCF12				TCF12
TCF3	TCF3	TCF3		TCF3
TCF7L1	TCF7L1			
TCF7L2	TCF7L2	TCF7L2		TCF7L2
TCL1A	TCL1A	TCL1A		
TEK		TEK		
TERC	TERC	TERC		
TERF2IP	TERF2IP	TERF2IP		
TERT	TERT	TERT	TERT	TERT
TET1	TET1	TET1		TET1
TET2	TET2	TET2	TET2	TET2
TFDP1				TFDP1
TFE3	TFE3	TFE3		
TGFBR1				TGFBR1
TGFBR2	TGFBR2	TGFBR2	TGFBR2	TGFBR2
TGIF1				TGIF1
THBS1	THBS1			
TIMP3	TIMP3			

TIPARP				TIPARP
TJP2				TJP2
TLR4	TLR4	TLR4		TLR4
TLX1	TLX1	TLX1		
TLX3	TLX3			
TMEM127	TMEM127	TMEM127		TMEM127
TMEM43				TMEM43
TMPRSS2	TMPRSS2			TMPRSS2
TNF	TNF	TNF		TNF
TNFAIP3	TNFAIP3	TNFAIP3	TNFAIP3	TNFAIP3
TNFRSF11A		TNFRSF11A		
TNFRSF13B		TNFRSF13B		
TNFRSF14	TNFRSF14	TNFRSF14		TNFRSF14
TNFRSF1A		TNFRSF1A		
TNFRSF1B		TNFRSF1B		
TNFRSF25		TNFRSF25		
TNFRSF8		TNFRSF8		
TNFSF11		TNFSF11		
TNK2	TNK2	TNK2		
TNKS			TNKS	
TNKS2			TNKS2	
TNNI3				TNNI3
TNNT2				TNNT2
TNPO1				TNPO1
TOM1				TOM1
TOP1	TOP1	TOP1	TOP1	TOP1
TOP2A	TOP2A	TOP2A	TOP2A	TOP2A
TP53	TP53	TP53	TP53	TP53
TP53BP1	TP53BP1	TP53BP1		TP53BP1
TP63				TP63
TPMT	TPMT		TPMT	TPMT
TPX2	TPX2	TPX2		TPX2
TRAF2		TRAF2		
TRAF3	TRAF3	TRAF3		TRAF3
TRAF5		TRAF5		
TRAF6		TRAF6		
TRAF7	TRAF7	TRAF7		TRAF7
TRIM24	TRIM24			
TRIM28				
TRIO				TRIO
TRRAP	TRRAP	TRRAP		TRRAP
TSC1	TSC1	TSC1	TSC1	TSC1
TSC2	TSC2	TSC2	TSC2	TSC2
TSHR	TSHR	TSHR	TSHR	TSHR

TTK				TTK
TUBA4A		TUBA4A		
TUBB		TUBB		
TXNIP				TXNIP
TYMS	TYMS	TYMS	TYMS	TYMS
U2AF1	U2AF1	U2AF1	U2AF1	U2AF1
UBE2T		UBE2T		
UBR5	UBR5	UBR5		UBR5
UGT1A1	UGT1A1		UGT1A1	UGT1A1
UGT2B15	UGT2B15	UGT2B15		
UGT2B17	UGT2B17			
UGT2B7	UGT2B7	UGT2B7		
UIMC1	UIMC1	UIMC1		
UNG		UNG		
UPF3B				UPF3B
USP34		USP34		
USP9X	USP9X	USP9X	USP9X	USP9X
VDR			VDR	
VEGFA	VEGFA	VEGFA	VEGFA	
VEGFB		VEGFB		
VHL	VHL	VHL	VHL	VHL
VKORC1	VKORC1	VKORC1	VKORC1	
VTCN1				VTCN1
WAS		WAS		
WASF3	WASF3	WASF3		WASF3
WHSC1	WHSC1	WHSC1		WHSC1
WHSC1L1				WHSC1L1
WISP3	WISP3	WISP3		WISP3
WNK1				WNK1
WRN	WRN	WRN		WRN
WT1	WT1	WT1	WT1	WT1
WWTR1	WWTR1			
XIAP		XIAP		XIAP
XPA	XPA	XPA		XPA
XPC	XPC	XPC		XPC
XPO1	XPO1	XPO1		XPO1
XRCC1	XRCC1	XRCC1		
XRCC2	XRCC2	XRCC2		XRCC2
XRCC3		XRCC3		XRCC3
XRCC5		XRCC5		
XRCC6		XRCC6		
YAP1	YAP1	YAP1		YAP1
YES1				YES1
ZBTB2	ZBTB2			

ZFHX3	ZFHX3	ZFHX3	ZFHX3
ZFP36L1			ZFP36L1
ZFP36L2			ZFP36L2
ZHX3		ZHX3	
ZMYM2			ZMYM2
ZMYM3			ZMYM3
ZNF217	ZNF217	ZNF217	ZNF217
ZNF703			ZNF703
ZNF750			ZNF750
ZNF814			ZNF814
ZNRF3		ZNRF3	
ZNF703	ZNF703		
ZRSR2		ZRSR2	ZRSR2

Table 1. Genes represented in different panel versions

Applied gene panel versions for next generation sequencing (NGS) comprised different combinations of 337, 649, 678 or 710 genes.

This table has been already published by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]

For Whole Exome Sequencing (WES) DNA was obtained from fresh frozen tissue sections [101]. Exome wide sequencing was performed at the Institute of Medical Genetics and Applied Genomics for 4 additional patients.

As also referred to in the publication of this cohort, “one patient with pancreatic cancer (PC3) received exome and transcriptome sequencing in the Molecularly Aided Stratification for Tumor Eradication Research (MASTER) Precision Oncology program at the National Center for Tumor Diseases in Heidelberg, Germany.” [72, 101]

Somatic single nucleotide variants (SNVs) and copy number variants (CNVs) were reported, as well as small insertions and deletions (INDELs) and selected fusions [101]. Reported results also included the estimated tumor mutational burden (TMB), as well as microsatellite analysis in some cases [101].

Detected germline variants were included in the study and germline variants that were considered relevant, e.g., for patient or family management or for therapy, were reported [101].

For CNV calling computation of the deviation of the sample's normalized coverage profile from the expected coverage was realized [101]. For this purpose, a model of the expected coverage was created from reference samples [101]. If there was a significant deviation from the expected coverage, the respective genomic region was called as a CNV [101]. High quality reads were used for computation that were uniquely mapping and non-duplicate [101].

Definition of TMB was set as the number of somatic variants (SNVs, InDel-changes and essential splicing changes) per complete coding region on exome level [128]. It was reported as the number of variants per one million coding bases (Var/Mb) [128]. For TMB calculation out of panel sequencing analysis, within all sequenced genes all somatic synonymous and non-synonymous variants that affect the protein-coding region were counted [128]. Variant frequency had to be 10% or higher [128]. Passenger mutations then were distinguished from driver mutations and their number was extrapolated to gene count of the whole exome [128]. Because driver mutations were considered to be limited to tumor associated genes included in the panel, their number was maintained unchanged [128]. Subsequently, computation of TMB was performed by normalization of the total count of driver and passenger mutations obtained to the size if the complete coding exome [128]. For those values which could not be converted, an estimated number of 100 or more somatic alterations on exome level was considered as high. If values for TMB, indicated as variants per mega base pair, were given, the tumor mutational burden was considered as high if it was 10 Var/Mbp or higher, or if it was 7.5 Var/Mbp or higher in case of tumor types known to have a generally low TMB.

In case of detection of relevant germline alterations patients were offered a genetic counseling [101]. Before giving their consent on sequencing all patients were informed by a clinical genetics' expert. Germline variants were ranked in accordance with a 5-tiered classification recommended by the American College of Medical Genetics and Genomics (ACMG), using the terms "pathogenic" (class 5), "likely pathogenic" (class 4), "likely benign" (class 2), "benign" (class 1) and "variant of uncertain significance" (class 1) [101, 129]. The current ACMG guideline on classifying germline variants is based on 27 different criteria, that

are either “very strong”, “strong”, “moderate” or “supporting” criteria for pathogenicity or “stand alone”, “strong” or “supporting” criteria for benignancy [129]. Combination of fulfilled criteria for each detected germline variant in compliance with pre-set rules leads to classification of the variant [129]. If the met criteria are contradictory regarding evidence for pathogenicity and benignancy or if they do not allow to classify the variant applying the rules, the variant is depicted as “variant of uncertain significance” [129].

2.5 STASTICAL ANALYSIS

SPSS and Excel were used for statistical analysis. Results were obtained from descriptive statistics by performing frequency distribution and cross tables. As also described in the publication of this cohort, “progression-free survival (PFS) and overall survival (OS) were analyzed with the Kaplan-Meier method dependent on the treatment response (SD and PR v progressive disease (PD)) compared by log-rank testing.” [101] There were 5 patients that could not be included in the Kaplan-Meier analysis due to lacking response monitoring and early drop-out [101].

3 RESULTS

The results of this analysis have been in part published by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]. For further information on authorship regarding distinct aspects of the work, please also consult the 'Erklärungen zum Eigenanteil' (declaration of own contribution).

3.1 CHARACTERISTICS OF THE PATIENTS COLLECTIVE

Between April 2016 and March 2018 96 patients with advanced GI cancers were presented at the molecular tumor board (MTB) Tübingen, 10 in 2016, 74 in 2017 and 12 in January and February of 2019.

Of the 96 patients, there were 22 patients (22.9%) with adenocarcinoma of the pancreas (PC), 19 (19.8%) with cancer of the biliary tract (BTC), 11 (11.5%) with hepatocellular carcinoma (HCC) and 9 patients (9.4%) with cancer of the upper gastrointestinal tract (UGC) (Fig 1A). Patients with UGC included 4 patients with esophageal cancer, 3 patients with gastric cancer and 2 patients with cancer of the small intestine (Fig. 1B). For further analysis they were grouped together as UGC because of the small sample size of the single entities.

32 patients (33%) had colorectal adenocarcinoma (CRC) (Fig. 1A). 9 of them showed cancer of the right sided intestine, defined as adenocarcinoma of the cecum, the ascending colon or the right colic flexure. 21 showed cancer of the left sided intestine, defined as adenocarcinoma of the left colic flexure, the descending colon, the sigmoid colon, the rectosigmoid transition zone or the rectum. One patient showed both, adenocarcinoma of the cecum and the rectum and one patient had an adenocarcinoma of the transverse colon (Fig. 1C).

3 patients had neuroendocrine tumors (NET), 2 of them with localization in the pancreas, and one patient had a NET with manifestation in the os sacrum (Fig 1A).

The median age when initially diagnosed was 58 years, ranging from 17 to 85 years. 86 of 96 patients (89.6%) had metastatic disease when presented to the MTB. 2 additional patients had suspected metastasis of the peritoneum and the

lung, respectively, and for one other patient, peritoneal carcinomatosis could not be precluded. 6 patients were without metastasis at the time of MTB presentation, while one of them had had metastasis which had been completely resected. For one patient no information about metastatic status could be obtained. When presented to the MTB, patients had a median number of systemic pretreatments of 3, ranging from 0 to 7 (Fig. 2) [101].

The cohort included all important entities within the spectrum of GI cancers with smaller numbers of patients with UGC and neuroendocrine tumors. The average number of pretreatments as well as the high proportion of metastatic disease was typical for a study population of patients with advanced GI cancers.

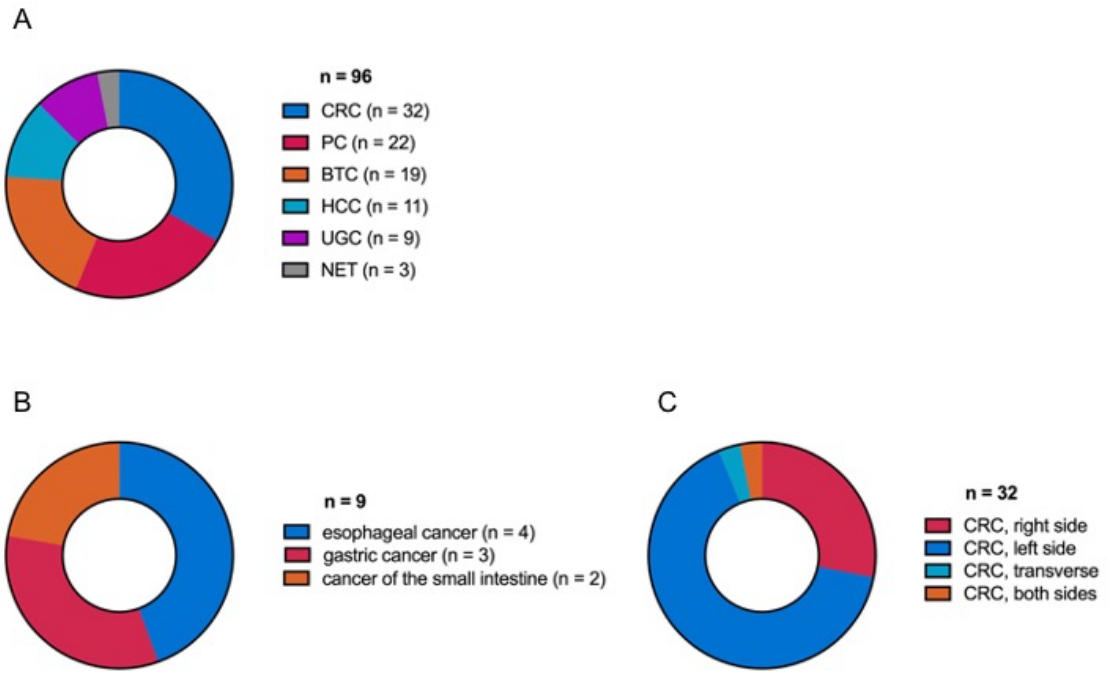

Figure 1. Characteristics of the patients collective.

A: number of patients with different tumor types. B: number of patients with upper gastrointestinal tract cancer. C: location of colorectal carcinoma; right side, adenocarcinoma of the cecum, the ascending colon or the right colic flexure; left side, adenocarcinoma of the left colic flexure, the descending colon, the sigmoid colon, the rectosigmoid transition zone or the rectum. BTC, biliary tract cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; NET, neuroendocrine tumor; PC, pancreatic cancer; UGC, upper gastrointestinal tract cancer.

This figure has been in part already published by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]

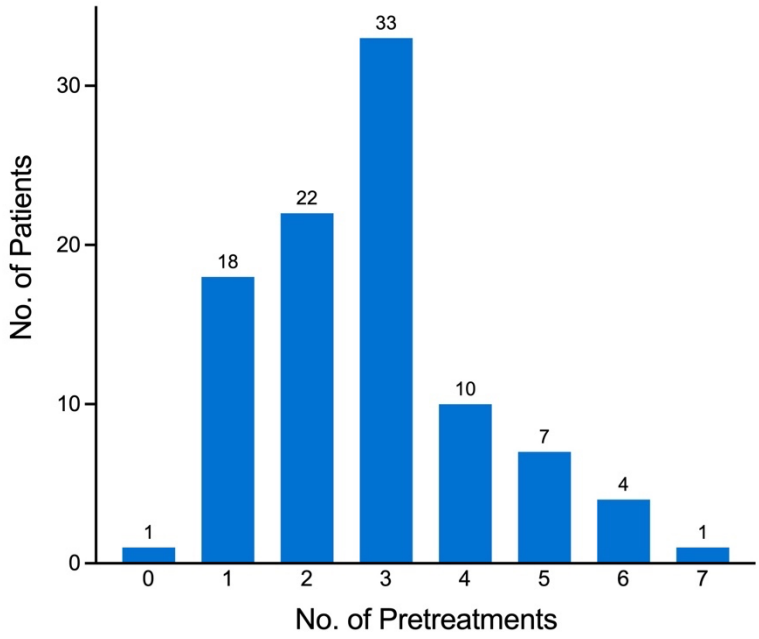

Figure 2. Number of systemic pretreatments before presentation at the molecular tumor board.

This figure has been already published by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]

3.2 DIAGNOSTICS

Patients that were presented at the MTB received molecular genetic analysis of their tumors and matched normal DNA from normal tissue or peripheral blood in order to identify clinically relevant alterations in the investigated signal transduction pathways as molecular rationales for targeted therapies.

Patients received panel analysis of 337 to 710 genes, whole exome or transcriptome sequencing (Fig. 3). The genetic testing was performed at the Institute of Medical Genetics and Applied Genomics Tübingen, at the Praxis für Humangenetik Tübingen CeGaT or within the DKTK-Master-trial in Heidelberg.

6 patients received more than one sequencing analysis during the observation period, and 4 patients received additional diagnostics subsequently to their presentation at MTB. Those are also included in this analysis.

40 patients received a gene panel analysis of at least 649 genes and 49 patients a gene panel analysis of 710 genes at CeGaT (Fig. 3). One patient received panel diagnostic of 337 genes and one patient received panel diagnostic of 678 genes at the Institute of Medical Genetics and Applied Genomics Tübingen (Fig. 3).
4 patients that were included in the so called e:Med trial (NCT02372162) received whole exome sequencing and one patient received whole exome sequencing within the DKTK Master Trial, Heidelberg (Fig. 3).
By performing these tests patients received a comprehensive genetic profiling of their tumors that generated extensive additional data on their diagnose. Data included mainly somatic variants and tumor mutational burden as well as secondary findings of germline variants. The genetic test results as well as their interpretation and implications are described below.

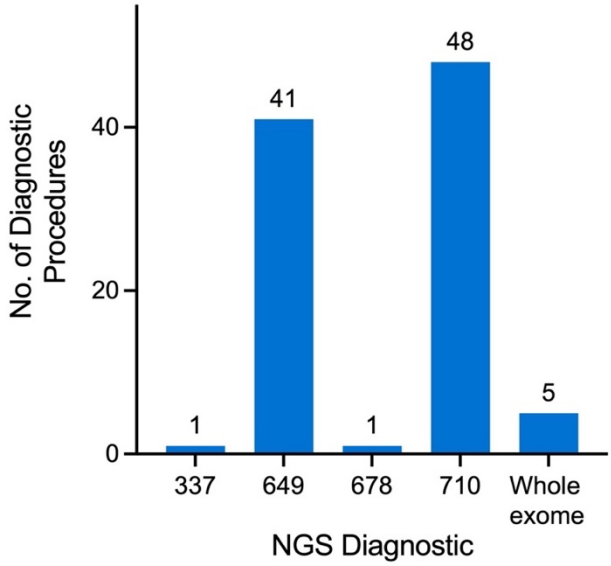

Figure 3. Performed diagnostic tests.

96 patients received genetic panel testing of 337, 649, 678 or 710 genes or whole exome sequencing.

This figure has been already published by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]

3.3 SECONDARY GERMLINE FINDINGS

For 37 patients of the cohort, by sequencing matched tumor with normal specimens, germline alterations were found, either in panel diagnostic or in whole exome or transcriptome sequencing. The number of germline alterations in the cohort in total was 55.

All germline alterations of the investigated cohort are summarized in Table 2. In order to assess their clinical utility and relevance the incidentally found germline alterations were estimated in accordance with a 5-tiered classification recommended by the ACMG, see also 2.4 [101].

In total, there were ten germline variants classified as “pathogenic” (class 5), seven germline variants classified as “likely pathogenic” (class 4), and 33 germline variants classified as “variant of uncertain significance” (class 3) in the cohort (Fig. 4). 33 patients (34.4%) had at least one relevant germline variant, defined as class 3 to 5, with 10 patients showing more than one class 3 to 5 germline variant. The mean number of classes 3 to 5 germline alterations in the complete cohort ranged from 0 to 4 germline alterations per patient. 10 patients (10.4%) had a “pathogenic” and 6 patients (6.3%) had at least one “likely pathogenic” alteration. In one patient with gastric cancer, 2 likely pathogenic variants were detected, in *PALB2* and *FANCM* (Fig. 4A).

In 19 patients (19.8%), variants of uncertain significance were detected, while two of these patients (one patient with colorectal cancer and one patient with neuroendocrine tumor located in the os sacrum) had an additional pathogenic germ line alteration of a different gene (Fig. 4B). So, 17 patients (17.7%) only had one or more variants of uncertain significance.

For 3 patients, pharmacologically relevant germline alteration could be identified. 2 patients had the same variant in *DPYD*, one patient with colorectal cancer and one patient with biliary tract cancer. Another patient with colorectal cancer had a pharmacologically relevant variant in *G6PD*.

In one patient with one germline variant, the detected germline variant could not be classified.

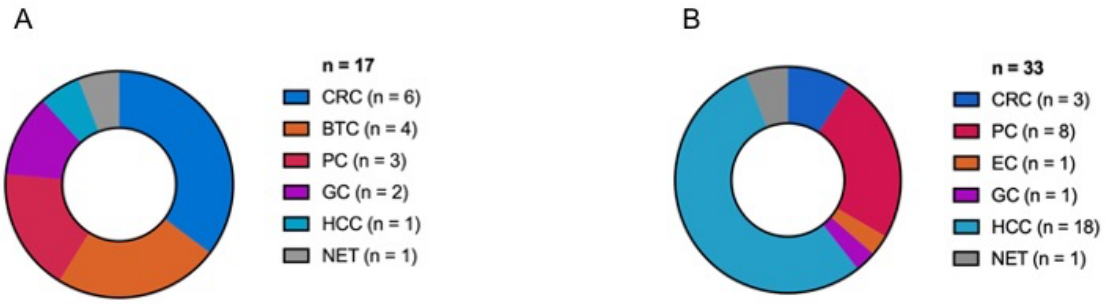

Figure 4. Number of relevant germline alterations per tumor type.

A: Number of pathogenic or likely pathogenic germline variants per tumor type. There were 17 pathogenic or likely pathogenic germline variants in the cohort. 16 patients had at least one pathogenic or likely pathogenic germline variant; in one patient with gastric cancer two likely pathogenic germline variants were detected. B: Number of germline variants of uncertain significance per tumor type. There were 33 variants of uncertain significance in 19 different patients of the cohort; two of these patients had an additional pathogenic germline alteration in a different gene. BTC, biliary tract cancer; CRC, colorectal cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; NET, neuroendocrine tumor, PC, pancreatic cancer.

This figure has been in part already published by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]

Looking at the level of affected genes, we found that germline variants classified as “pathogenic”, “likely pathogenic” or “VUS” were found in 36 different genes, most frequently in *BRCA2* (4), *CHEK2* (4) and *MSH6* (3) (Fig. 5). 3 patients had the same *CHEK2* variant, p.Ile157Thr, one patient with CRC, one with HCC and one with PC. This variant was classified as variant of uncertain significance. Two patients with PC had the same pathogenic *BRCA2* variant, p.Ala938Profs*21.

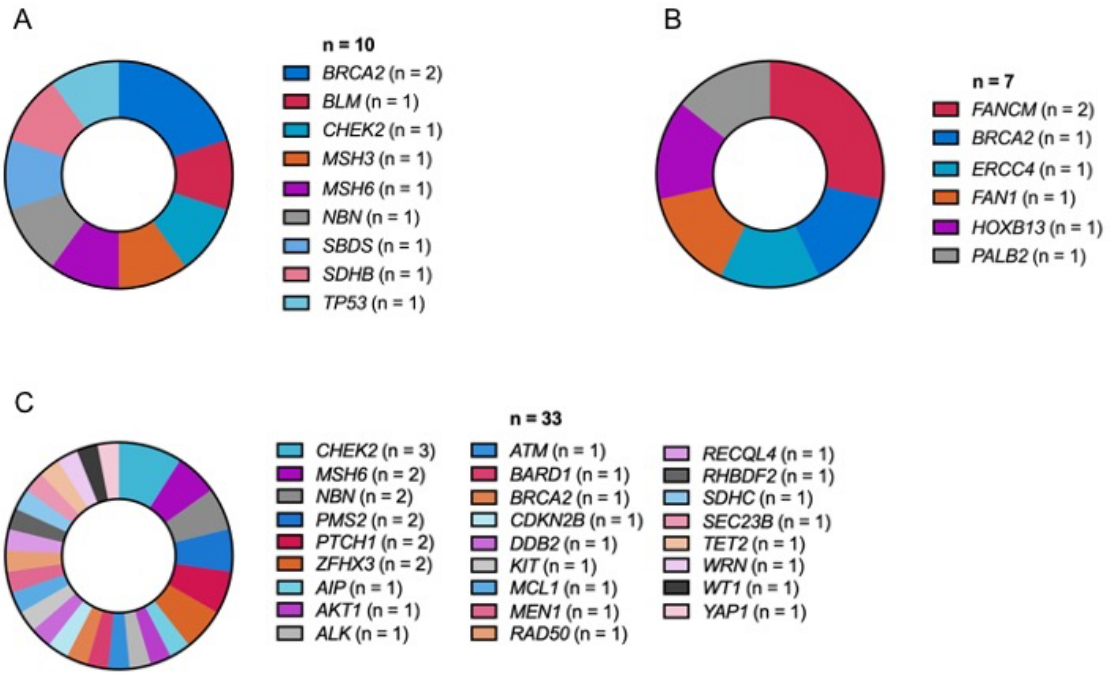

Figure 5. Genes with pathogenic and likely pathogenic variants.

*A: Pathogenic alterations were found in 9 different genes. There were 10 pathogenic germline variants in the cohort; two patients with pancreatic cancer had the same pathogenic BRCA2 variant, p.Ala938Profs*2. B: Likely pathogenic alterations were found in 6 different genes. There were 7 likely pathogenic germline variants in the cohort; two patients had the same likely pathogenic FANSM variant, c.5101C>T; p.Gln1701*, one patient with biliary tract cancer and one patient with gastric cancer. C: 33 Variants of uncertain significance were found in 26 different genes.*

This figure has been in part already published by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]

One important question considers the role of germ line mutations for therapy recommendations of the MTB. Of note, for 5 patients (5.2%) germline mutations were identified as targets, and the detected germline variant was the rationale for an MTB recommendation (Table 2). In 3 cases of PC this was a truncating *BRCA2* variant (p.A938Pfs*21, classified as “pathogenic”, in two cases and p.E2198*, classified as “likely pathogenic”, in one case), which led to a treatment recommendation for Poly (ADP-ribose) Polymerase (PARP) inhibition with

Olaparib. In one case of CRC the truncating MSH6 variant p.I1313Sfs*7, classified as “pathogenic”, led to a treatment recommendation for PD-1/PD-L1 immune checkpoint inhibition with Pembrolizumab and in another case of colorectal cancer the *CHEK2* p.I175T variant, classified as “VUS”, in combination with 2 somatic *ATM* truncations, led to a treatment recommendation for *ATR* inhibition and carboplatin (Table 2).

Tumor type	Patient	Gene	Alteration	Changes	Category of sequence variant	Identified as target
CRC*	CRC5	*CHEK2*	frameshift	c.1100delC; p.Thr367Metfs*15	pathogenic	no
		BRCA2	frameshift	c.10176delA; p.Glu3393Asnfs*34	uncertain significance	no
CRC	CRC6	*DPYD*	missense	c.2846A>T; p.Asp949Val	relevant for pharmacogenomics	no
CRC	CRC8	*MSH6*	missense	c.3961A>G; p.Arg1321Gly	uncertain significance	no
CRC	CRC13	*G6PD*	inframe deletion	c.655_657delTCC; p.Ser219del	relevant for pharmacogenomics	no
CRC	CRC18	*BLM*	stop gained	c.1108C>T; p.Gln370*	pathogenic	no
CRC	CRC19	*HOXB13*	missense	c.251G>A; p.Gly84Glu	likely pathogenic	no
CRC	CRC20	*CHEK2*	missense	c.470T>C; p.Ile157Thr	uncertain significance	yes
CRC	CRC25	*MSH6*	frameshift	c.3934_3937dupGTTA; p.Ile1313Serfs*7	pathogenic	yes
CRC	CRC26	*SBDS*	essential splice site	c.258+2T>C; p.?	pathogenic	no
CRC	CRC29	*FAN1*	missense	c.149T>G; p.Met50Arg	likely pathogenic	no
BTC	BTC12	*NBN*	frameshift	c.1142delC; p.Pro381Glnfs*23	pathogenic	no
BTC	BTC14	*ERCC4 (XPF)*	copy number	heterozygous deletion	likely pathogenic	no
BTC	BTC15	*FANCM*	stop gained	c.5101C>T; p.Gln1701*	likely pathogenic	no

BTC	BTC16	*DPYD*	missense	c.2846A>T; p.Asp949Val	relevant for pharmacogenomics	no
BTC	BTC18	*SDHB*	essential splice site	c.287-2A>G; p.?	pathogenic	no
HCC	HCC1	*DDB2*	missense	c.59G>A; p.Arg20Lys	uncertain significance	no
HCC	HCC5	*MSH3*	essential splice site	c.2319-1G>A; p.?	pathogenic	no
HCC*	HCC6	*CHEK2*	missense	c.470T>C; p.Ile157Thr	uncertain significance	no
		BARD1	missense	c.2306C>T; p.Ser769Phe	uncertain significance	no
		RAD50	missense	c.695C>A; p.Ala232Asp	uncertain significance	no
		RECQL4	missense	c.1892G>A; p.Arg631His	uncertain significance	no
HCC*	HCC8	*PTCH1*	missense	c.4033C>T; p.Arg1345Cys	uncertain significance	no
		AIP	missense	c.47G>A; p.Arg16His	uncertain significance	no
		AKT1	missense	c.138C>A; p.Asp46Glu	uncertain significance	no
HCC*	HCC9	*SDHC*	intron	c.242-5651G>T; p.?	uncertain significance	no
		PTCH1	missense	c.3257C>T; p.Pro1286Leu	uncertain significance	no
		RHBDF2	missense	c.611G>A; p.Arg204Lys	uncertain significance	no
		SEC23B	missense	c.770C>T; p.Thr257Ile	uncertain significance	no
HCC*	HCC10	*PMS2*	missense	c.595C>A; p.Arg199Cys	uncertain significance	no
		ATM	missense	c.7390T>C; p.Cys2464Arg	uncertain significance	no
HCC*	HCC11	*ALK*	missense	c.3133G>A; p.Val1045Met	uncertain significance	no
		PMS2	missense	c.706-3C>A; p.?	uncertain significance	no
		WRN	splice region and intron	c.1899-19C>A; p.?	uncertain significance	no
		YAP1	splice region and intron	c.689-11C>A; p.?	uncertain significance	no
GC*	UGC3	*PALB2*	stop gained	c.3441T>A; p.Cys1147*	likely pathogenic	no

		FANCM	stop gained	c.5101C>T; p.Gln1701*	likely pathogenic	no
GC	UGC5	*KIT*	stop gained	c.1540G>T; p.Glu514*	uncertain significance	no
NET*	NET1	*TP53*	missense	c.743G>A; p.Arg248Gln	pathogenic	no
		TET2	nonsense	c.1292T>G; p.Leu431*	uncertain significance	no
NET	NET3	*MEN1*	missense	c.1064G>A; p.Arg355Gln	uncertain significance	no
EC	UGC6	*POLQ*	stop gained	c.1156G>T; p.Glu386*	*not classified*	no
EC*	UGC9	*IL7R*	frameshift	c.898_902delCCTGA; p.Pro300Lysfs*9	*not classified*	no
		CDKN2B	missense	c.256G>A; p.Asp86Asn	uncertain significance	no
PC	PC6	*WT1*	splice region and intron	c.647-6C>A	uncertain significance	no
PC*	PC7	*ZFHX3*	inframe deletion	c.2328_2333delGGTGGC; p.Val777_Ala778del	uncertain significance	no
		ZFHX3	missense	c.7298C>T; p.Ala2433Val	uncertain significance	no
PC	PC9	*BRCA2*	frameshift	c.2808_2811delACAA; p.Ala938Profs*21	pathogenic	yes
PC	PC10	*BRCA2*	frameshift	c.2808_2811delACAA; p.Ala938Profs*21	pathogenic	yes
PC	PC12	*MSH6*	missense	c.2561A>T; p.Lys854Met	uncertain significance	no
PC	PC16	*CHEK2*	missense	c.470T>C; p.Ile157Thr	uncertain significance	no
PC	PC18	*BRCA2*	stop gained	c.6592G>T; p.Glu2198*	likely pathogenic	yes
PC	PC20	*MCL1*	copy number	focal amplification, 4 copies	uncertain significance	no

PC*	PC21	*NBN*	missense	c.511A>G; p.Ile171Val	uncertain significance	no
		NBN	missense	c.643C>T; p.Arg215Trp	uncertain significance	no

Table 2. Patients with germline alterations classified as pathogenic, likely pathogenic, of uncertain significance or relevant for pharmacogenomics.

*In two cases variants that could not be classified. BTC, biliary tract cancer; CRC; colorectal cancer; EC, esophageal cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; NET, neuroendocrine tumor; PC, pancreatic cancer. * more than one germline variant per patient*

This table has been already published by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]

3.4 TUMOR MUTATIONAL BURDEN AND MICROSATELLITE INSTABILITY

Tumor mutational burden and microsatellite instability are important parameters for the recommendation of checkpoint inhibitors.

3.4.1 Tumor mutational burden

Tumor mutational burden (TMB) could be assessed for 88 patients (91.7%) (Fig. 6). For 8 patients there was no TMB result given, either because it could not be estimated due to bad specimen quality or because the estimated TMB, indicated as the number of missense variants projected on the whole exome, could not be converted into the number of variants per mega base pair (Mbp).

For 8 patients more than one TMB result was present due to repeated sequencing of different specimens. In these cases, the estimated TMB values differed only slightly between different tissue samples (Table 3).

The median assessed TMB ranged from 1 Var/Mbp for NET to 6.3 Var/Mbp for CRC (Fig. 6) [101]. 17 patients (17.7%) had a TMB considered as high, among them 8 with a TMB of 10 Var/Mbp or higher (Fig. 6). High values for TMB were found in all examined tumor types except NET (Fig. 6). For 2 additional patients TMB was considered as high, based on 119 somatic alterations found in a patient with HCC (HCC1) and approximately 430 missense variations projected on the whole exome found in a patient with BTC (BTC12).

For 10 patients high TMB also led to recommendation for treatment with immune checkpoint inhibitors.

3.4.2 Microsatellite Instability

Of the 96 patients 63 (65.6 %) were also tested on microsatellite instability (MSI). For 59 of the 63 patients (92.1 %) no microsatellite instability could be detected, while 2 out of 63 (3.2 %) had one out of five microsatellite markers, defined as MSI-low and 2 of the tested patients (3.2%) had more than one positive marker and were classified as MSI-high.

Of the 17 patients that were found to have tumors with high TMB, 8 were tested on MSI. Surprisingly, only 2 patients (5%) were found to have an MSI-high tumor. Of those 2 patients with high MSI, one patient with CRC (CRC25) had a TMB of 117,9 Var/Mb and one patient with BTC (BTC 12) had a TMB of approximately 430 missense variants projected on the whole exome. So, all patients showing MSI-high also showed high TMB and patients with MSI-high were among the patients with the highest TMB values of the whole cohort.

For both patients a recommendation for immune checkpoint inhibitors was given also due to high TMB, and the MSI-high status further underpinned this decision.

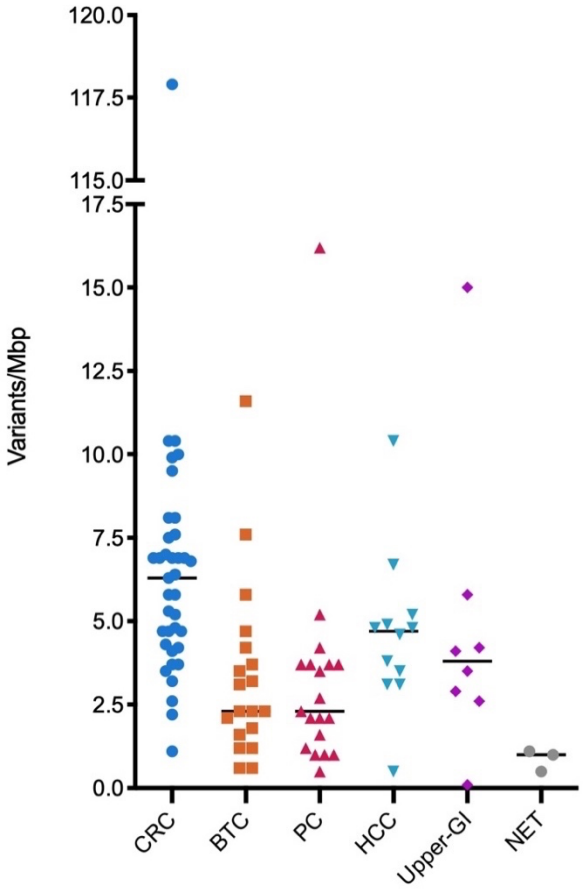

Figure 6. Tumor mutational burden per tumor type.

Tumor mutational burden was calculated for 88 patients. Two patients had 3 and six patients had 2 different calculations of separate tissue samples during the observation period, which are included in the analysis. The median is shown for each tumor type [101]. BTC, biliary tract cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; NET, neuroendocrine tumor; PC, pancreatic cancer; UGC, upper gastrointestinal tract cancer

This figure has been in part already published by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]

tumor type	patient number	tissue	sampling date	TMB (Var/Mbp)
CRC				
	CRC13	metastasis	Jan 17	4,7
		metastasis	Jul 17	6,3
		metastasis	Jan 18	6,9
	CRC18	metastasis	Jun 17	4,7
		metastasis	Apr 18	2,6
	CRC21	primary disease	N/A	4,2
		metastasis	Mar 18	6,9
	CRC26	cell-free DNA	Oct 17	1,1
		metastasis	Oct 17	2,2
	CRC29	primary disease	Jul 17	3,7
		metastasis	Nov 17	5,3
HCC				
	HCC9	primary disease (bevor start of treatment)	Aug 17	4,8
		primary disease (progress on treatment with Sorafenib)	Oct 17	5,2
	HCC10	primary disease (bevor start of treatment)	Apr 16	4,6
		primary disease (progress on treatment with Sorafenib)	Jun 16	4,8
		metastasis	Jul 17	3,8
BTC				
	BTC9	metastasis	Jan 17	0,6
		recurrent disease	Apr 18	3,7

Table 3. Patients with more than one TMB result due to repetitive testing.

8 patients had more than one TMB result due to repeated sequencing of different specimens. The estimated TMB values differed only slightly between different tissue samples. BTC, biliary tract cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma. N/A, not accessible. Var/Mbp; variants per mega base pair.

3.5 IDENTIFICATION OF TARGETS

One of the major aims of the MTB is to define potential targets for therapeutic approaches.

For 47 (49%) of the 96 presented patients at least one molecular target could be identified (Table 4) [101]. There was an identification of a “second priority” target for a possible therapy implementation in the further clinical course for 5 patients. Target identification was possible in 74% of BTC (14 out of 19 patients), in 56% of UGC (5 out of 9), in 50% of PC (11 out of 22 patients), in 44% of CRC (14 out of 32 patients), in 27% of HCC (3 out of 11 patients) and in 0% of NET (0 out of 3 patients) (Fig. 7).

For 10 patients high TMB was the target, as depicted above (Table 4).

The most frequently identified target gene was *CDKN2A* in 10 patients, in 6 patients in combination with another detected alteration in *CDKN2B* and in 2 patients in combination with amplification of *CDK6*. One patient had two alterations in *CDKN2A* in cis. Alterations in *FGF19,2,3* and *FGFR1,2,4* as well as *FGFR2* fusion genes were identified as targets in 6 patients. For 4 patients a *BRCA2*-Mutation and for another 4 patients an *IDH1*-Mutation was identified as a relevant target (Table 4).

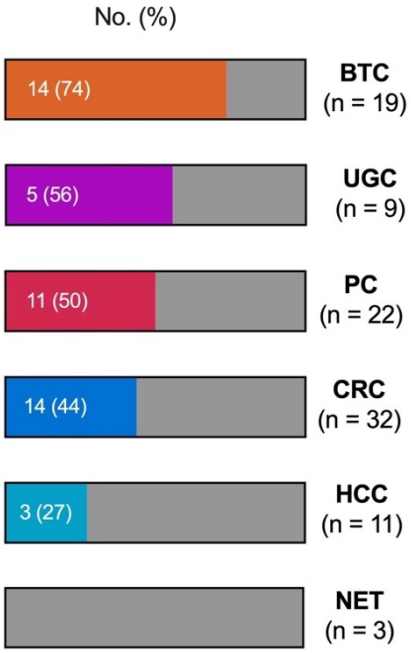

Figure 7. Frequency of target identification per tumor type.

A molecular target could be identified for 47 out of 96 patients. BTC, biliary tract cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; NET, neuroendocrine tumor; PC, pancreatic cancer; UGC, upper gastrointestinal tract cancer.

This figure has been in part already published by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]

3.6 TREATMENT RECOMMENDATIONS

For 6 patients, a molecular target could be found but no recommendation for the implementation of the therapy could be given because those patients had either complete tumor resection (2 patients) or already had a sustained disease control (4 patients) [101]. Those targets would have been *CDKN2A/B* deletion in a patient with CRC, IDH1 mutation in one patient with UGC and in one patient with HCC and *FGFR2-BICC1* fusion gene in two patients with BTC, in one of the cases plus an *FGFR2-PRKCQ* fusion and high TMB in another patient with BTC (Table 4).

For the remaining 41 patients (42.7%) a recommendation for treatment implementation was given by the MTB. All recommended therapies and the respective targets are also depicted in Table 4. Recommended targeted drugs

were immune checkpoint inhibitors in 9 cases, with inhibition of PD-1/PD-L1 via Pembrolizumab or Nivolumab in 8 cases and combined inhibition of PD-1/PDL-1 and CTLA4 with Nivolumab plus Ipilimumab in another case. Therapy with the CDK4/6 inhibitor Palbociclib was recommended in another 9 cases due to deleterious alterations or deletion of *CDKN2A* or *CDKN2B* or both, partially combined with amplification of *CDK6*. There was a recommendation for Poly (ADP-ribose) polymerase (PARP) inhibition with Olaparib for 5 patients with deletion of *BRCA1* or *BRCA2* or deleterious alteration of *BRCA2* in the germline and for ATR inhibition combined with carboplatin in 2 cases of *ATM* mutation. For 5 patients that showed upregulation of FGFR signaling, a therapy with tyrosine kinase inhibitors (TKI) like Ponatinib, Lenvatinib, or Regorafenib was recommended and for 3 patients with upregulation of ErbB signaling, Trastuzumab plus Lapatinib or Pertuzumab plus Erlotinib was recommended.
Other recommended drugs were the combination of BRAF and MEK inhibitor with Dabrafenib plus Trametinib in 2 cases due to *BRAF* alteration, IDH1 inhibitor in 2 cases due to *IDH1* mutation, BET-inhibitor in 2 cases due to *BRD4* mutation, Aurora-A Kinase inhibitor in one case due to *MYC* amplification and FLT inhibition in one case due to *FLT1/3* amplification.

NGS sequencing of GI tumors revealed molecular targets in 49% and treatment options in 43% of all cases of our cohort. Treatment recommendations could be given across all examined tumor types except NET. The highest percentage of target identification was found in patients with BTC. Next to a high TMB the most frequent targets involved the pathways of cell cycle regulation and mitogenic signaling.

Tumor type	Patient	No. of pre-treatments	Identified target	Variant	Board recommendation	Treatment
CRC						
	CRC1	3	*BRD4*	p.N576Kfs*2	BET inh.	BET-inhibitor I-BET
	CRC2	2	*MLH1* high TMB (10.4 Var/Mbp)	p.T117M	PD-1 inh.	Pembrolizumab
	CRC5	3	*BRAF, KRAS*	*BRAF* p.I463T, *KRAS* p.Q61H	BRAF and MEK inh.	Dabrafenib + Trametinib
	CRC6	5	*FGF3,4,19* amplification	approx. 5 copies	FGFR pathway inh.	Regorafenib
	CRC9	6	high TMB (8.1 Var/Mbp)	N/A	PD-1 inh.	Pembrolizumab
	CRC11	6	*MYC* amplification	approx. 3 copies	Indirect Myc inh.	Aurora-A-kinase inhibitor MLN-8237
	CRC17	6	*ERBB2* amplification	> 10 copies	ERBB2 inh.	Trastuzumab + Lapatinib
	CRC20	5	*ATM, CHEK2* (germline), *TP53BP1*	*ATM* p.Leu516* and p.Gln1579*, *CHEK2* p.Ile157Thr, *TP53BP1* p.Glu82*	ATR inh.	ATR inhibitor AZD6738 + Carboplatin
	CRC21	3	*CDKN2A/B* deletion	*CDKN2A* focal del. (homozygous), *CDKN2B* focal del. (heterozygous)	no recommendation for treatment due to disease control under standard treatment with trifluiride/ tipiracil	
	CRC22	3	*ERBB2* amplification	> 10 copies	ERBB2 inh.	Trastuzumab + Lapatinib (FOLFIRI)
	CRC23	6	high TMB (8.1 Var/Mbp)	N/A	PD-1 inh.	Pembrolizumab
	CRC24	3	*ATM*	*ATM* p.Ala1426Glnfs*25 (frameshift) (del 1 Bp)	ATR inh.	ATR inhibitor AZD6738 + Carboplatin
	CRC25	2	*MSH6* (germline), high TMB (118 Var/Mbp)	*MSH6* p.Ile1313Serfs*7 (frameshift) (dup 4 Bp)	PD-1 inh.	Pembrolizumab

	CRC29	2	*FLT1* and *FLT3* amplification	> 8 copies	FLT1/3 inh.	TKI with FLT1-/FLT3 inhibitory activity (e.g., Nintedanib)
PC						
	PC1	3	*CDKN2A/B* deletion	del. (homozygous)	CDK4/6 inh.	Palbociclib
	PC2	4	*CDKN2A* mutation and deletion	p.Trp15Gly and focal del. (in cis)	CDK4/6 inh,	Palbociclib
	PC3	3	2 *NRG1* fusion genes and increased *ERBB3* and *NRG3* expression	*TMEM66-NRG1, NRG1-CDH6,* focal dup. Chr.1p (increased expression in transcriptome analysis)	Inh. of ERBB1 and ERBB2	Pertuzumab + Erlotinib
	PC6	3	*CDK6* amplification and *CDKN2A* deletion	*CDKN2A* p.Leu32_Leu37del	CDK4/6 inh.	Palbociclib
	PC8	5	high TMB (16.2 Var/Mbp)	N/A	PD-1 inh.	Pembrolizumab
	PC9	5	*BRCA2 (*germline)	*BRCA2* p.Ala938Profs *21 and LOH in tumor cells	PARP inh.	Olaparib
	PC10	2	*BRCA2 (*germline)	*BRCA2* p.Ala938Profs *21 and LOH in tumor cells	PARP inh.	Olaparib
	PC12	3	*CDKN2A/B* deletion	del. (homozygous)	CDK4/6 inh.	Palbociclib
	PC18	1	*BRCA2 (*germline)	*BRCA2* p.Glu2198* and LOH in tumor cells	PARP inh.	Olaparib
	PC19	3	*CDKN2A/B* deletion	del. (homozygous)	CDK4/6 inh.	Palbociclib
	PC20	3	*CDKN2A/B* deletion	focal del. (heterozygous)	CDK4/6 inh.	Palbociclib
BTC						
	BTC1	3	*BRCA1* deletion	del. (heterozygous)	PARP inh.	Olaparib
	BTC2	3	high TMB (11.6 Var/Mbp)	N/A	PD-1 / PD-L1 inh.	PD-1/PD-L1 checkpoint inhibitor
	BTC6	2	high TMB (7.6 Var/Mbp)	N/A	PD-1 inh.	Pembrolizumab
	BTC8	2	*CDKN2A*	c.151-2A>G; p.? (essential splice site)	CDK4/6 inh.	Palbociclib

	BTC9	1	*IDH1*	p.R132C	IDH1 inh.	IDH1 inhibitor
	BTC10	2	*FGFR* fusion	*FGFR2-BICC1, FGFR2-PRKCQ*	no recommendation for treatment due to disease control under standard treatment with gemcitabine plus cisplatin	
	BTC12	1	high TMB	N/A	no recommendation for treatment due to complete tumor resection	
	BTC13	3	*FGFR* fusion	*FGFR2-BICC1*	FGFR pathway inh.	Ponatinib
	BTC14	3	*FGFR2* fusion gene	*FGFR2-AHCYL2*	FGFR pathway inh.	Lenvatinib
	BTC15	3	*IDH1*	p.Trp245*	IDH1 inh.	IDH1 inhibitor
	BTC16	0	*BRCA2* deletion	del. (heterozygous)	PARP inh.	Olaparib
	BTC17	1	*BRAF*	*BRAF* p.Val600Glu	BRAF and MEK inh.	Dabrafenib +Trametinib
	BTC18	3	*FGFR1 and FGFR4 amplification*	approx. 5 copies	FGFR pathway inh.	Ponatinib
	BTC19	3	*FGFR* fusion	*FGFR2-BICC1*	no recommendation for treatment due to disease control under standard treatment with FOLFIRI	
UGC						
	UGC1	2	*FGF3* and *FGF4* amplification	approx. 6 copies	FGFR pathway inh.	TKI with FGFR inhibitory activity
	UGC2	1	*IDH1*	p.Arg132Cys	no recommendation for treatment due to complete tumor resection	
	UGC3	2	high TMB (15 Var/Mbp)	N/A	PD-1 and CTLA4-inh.	Nivolumab + Ipilimumab
	UGC4	3	*CDK6* amplification and *CDKN2A* deletion	*CDK6* > 5 copies *CDKN2A* del. (heterozygous)	CDK4/6 inh.	Palbociclib
	UGC6	3	*CDKN2A/B* deletion	focal del. (homozygous)	CDK4/6 inh.	Palbociclib
HCC						
	HCC1	1	high TMB (119 Var/exome)	N/A	PD-1 inh.	Nivolumab
	HCC6	3	*MYC* amplification	> 4 copies	BET inh.	Brd4 inhibitor

HCC11	1	*IDH1*	p.Arg249fs (frameshift) (del 17 Bp) and p.His248Leu	no recommendation due to complete remission under treatment with FOLFOX

Table 4. Target identification and treatment recommendation by the Molecular Tumor Board.

BTC, biliary tract cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; NET, neuroendocrine tumor; PC, pancreatic cancer; UGC, upper gastrointestinal tract cancer. Inh., inhibitor; N/A, not applicable; TKI, tyrosine kinase inhibitor.

This table has been in part already published by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]

3.7 TREATMENT IMPLEMENTATION AND OUTCOME

Within the aim of providing additional treatment options according to the MTB recommendation for patients with advanced GI cancers a major challenge addresses the drug reimbursement or study inclusion to implement the treatment. For 16 patients the recommended treatment could not be implemented. This was due to rapid clinical deterioration and a poor performance status at the time when therapy could have been started (Fig. 8).

25 patients (61 %), however, received the recommended treatment (Fig. 8). These were 11 patients (34%) of all 32 patients with CRC, 7 patients (32%) of all 22 patients with PC, 4 patients (21%) of all 19 patients with BTC, 2 patients (22%) of all 9 patients with UGC and 1 patient (9%) of all 11 patients with HCC (Table 5) [101].

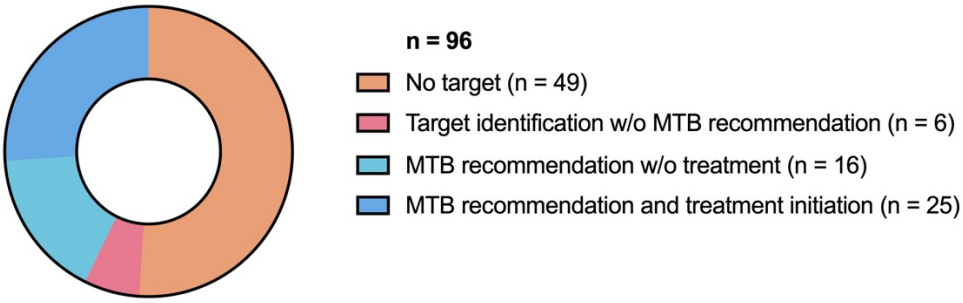

Figure 8. Frequency of potential target identification by the MTB, patients with target identification but no treatment and patients with MTB-recommended treatment initiation within the cohort of 96 patients with advanced gastrointestinal tumors.

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All implemented treatments and their duration as well as the outcomes are depicted in Table 5. A treatment with an immune checkpoint inhibitor was administered in 8 cases (6 Pembrolizumab, 1 Nivolumab, 1 Nivolumab plus Ipilimumab), 3 patients received Olaparib and 3 received Palbociclib. Therapies with Ponatinib, Lenvatinib or Regorafenib were implemented in one case each, Trastuzumab plus Lapatinib in two cases and in one case Pertuzumab plus Erlotinib. Two patients received treatment with ATR inhibition, one patient with a BET inhibitor, one patient with an Aurora-A-kinase inhibitor and one patient with an IDH1 inhibitor.
The respective applied drugs were either provided via a clinical study, in-label, off-label, or for a matched experimental treatment (Table 5) [101].

20 patients that were treated were also evaluable for analysis of best response according to radiological imaging studies that were documented within the clinical patient files [101]. For 9 of them a disease control could be reached, with partial response (PR) in 3 cases (15%) and stable disease (SD) in 6 cases (30%). 11 patients (55%) showed tumor progression (progressive disease (PD)) despite treatment (Table 5) [101]. So, for those 20 patients the disease control rate was 45% [101].

The disease control rate per tumor type was 67% for BTC (2 out of 3 patients), 50% for CRC (5 out of 10 patients) and 20 % for PC (1 out of 5 patients). Only one patient with HCC and one patient with UGC received treatment and were available for follow-up (Table 5).

Within the patients who only showed PD as best response, applied treatments included checkpoint inhibition due to high TMB, Olaparib due to germline alteration of *BRCA2*, BRD4 inhibition due to *BRD4* mutation, Regorafenib due to *FGFR* alteration, aurora A kinase inhibition due to *MYC* amplification, ATR-inhibitor with carboplatin due to *ATM* mutation, or Palbociclib due to *CDKN2A/B* deletion (Table 5) [101].

Patients who showed SD received treatment with Pembrolizumab (in 2 cases due to high TMB in CRC, in one of these cases there was also a germline variant of *MSH6* and an MSI high status) or Nivolumab (due to high TMB in HCC), ATR inhibitor plus carboplatin (due to a *CHEK2* germline variant in CRC), Trastuzumab plus Lapatinib (due to *ERBB2* amplification in CRC) or IDH1 inhibitor (due to *IDH1* mutation in BTC) (Table 5) [101].

Patients who showed PR received treatment with Pembrolizumab (due to high TMB in CRC), Pertuzumab plus Erlotinib (due to NRG1 fusion in PC) or Lenvatinib (due to *FGFR2* fusion in BTC) (Table 5) [101].

5 patients were not available for follow-up, either because of a lack of data or because they suffered a rapid deterioration of their performance status and therapy had to be stopped soon after initiation (Table 5).

PFS was calculated from the day of the initiation of the MTB-recommended treatment until radiographic progression or death and was assessable for 19 patients [101]. Median PFS for those 19 patients was 2.8 months and ranged from 29 days (1.0 month) in a patient with CRC who received BET-inhibitor to 275 days (9.0 months) in a patient with BTC who received Lenvatinib (Fig. 9A).

OS was calculated from the day of the initiation of the MTB-recommended treatment until death, irrespective of its cause, or data cut-off on March 31, 2019 [101]. OS could be assessed for all 25 patients in who treatment was implemented. OS ranged from 4 days (0.1 month) in a patient with UGC who

received Palbociclib until treatment was stopped after 4 days due to rapid clinical deterioration and 549 days in a patient with CRC who received continued treatment with pembrolizumab after progression in addition to individual peptide vaccination and was still alive at data cut-off (not reached). The median OS was 5.2 months (Fig. 9B) [101].

In total, 4 patients were still alive at data cut-off, 3 with CRC and one with BTC.

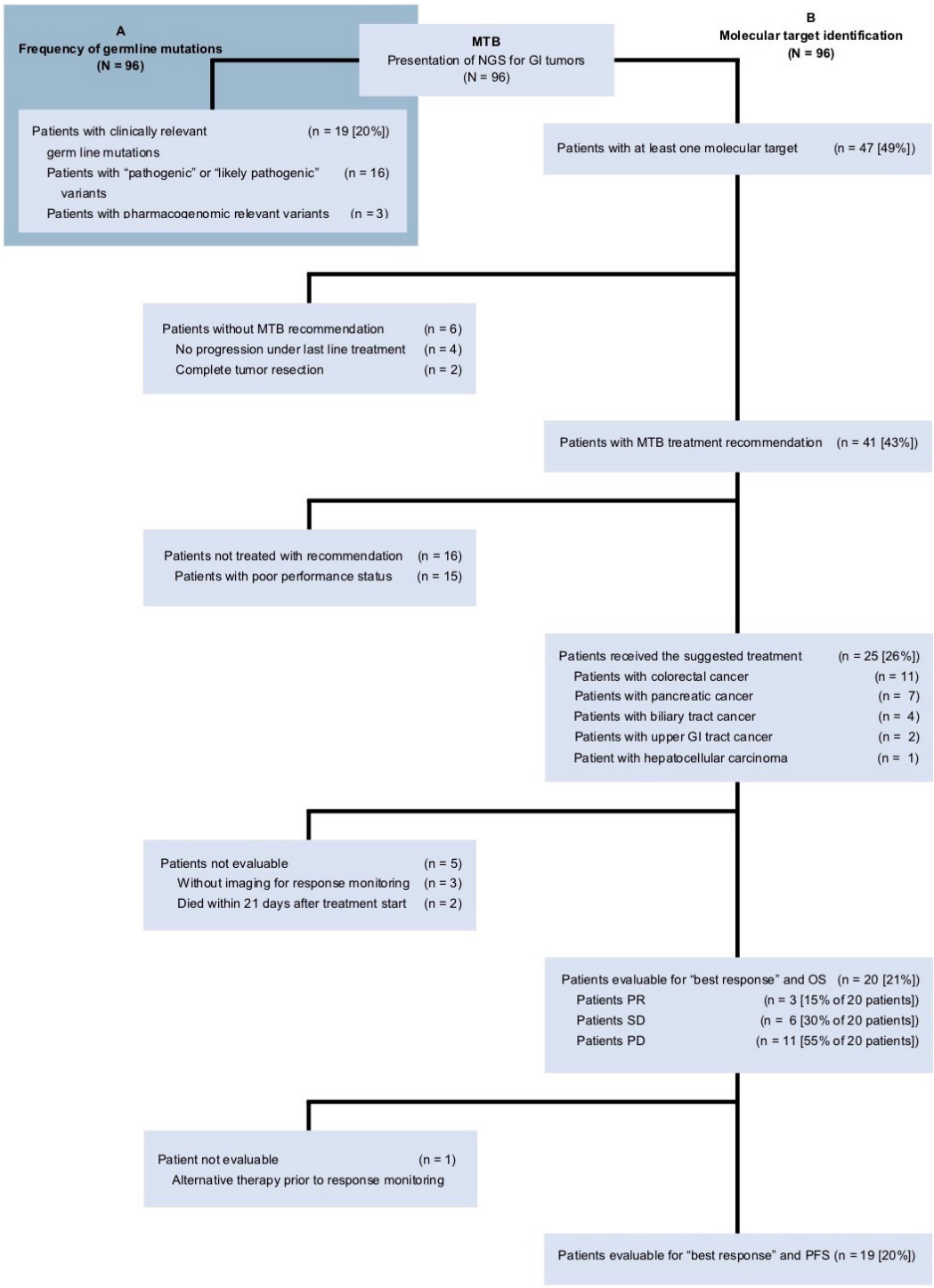

Figure 9. Course of patients after initiating next generation sequencing (NGS).

A: Frequency of clinically relevant germline variants. B: Molecular target identification and course of patients. MTB, molecular tumor board; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

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Tumor type	Pat	No. of pre-treatments	Treatment	Duration (d)	Drug availability	Best Response	PFS (d)	OS (d)
CRC								
	CRC1	3	BET-inhibitor molibresib	35	Individualized exp. treatment	PD	29	103
	CRC2	2	Pembrolizumab	113	off-label	PD	113	127
	CRC6	5	Regorafenib	65	approved	PD	66	103
	CRC9	6	Pembrolizumab	516	off-label	SD	257	549+
	CRC11	6	Aurora-A-kinase inhibitor MLN-8237	41	individualized exp. treatment	PD	41	68
	CRC17	6	Trastuzumab + Lapatinib	90	off-label	no F/U	no F/U	158
	CRC20	5	ATR inhibitor AZD6738 + Carboplatin	197	individualized exp. treatment	SD	112	276
	CRC22	3	Trastuzumab + Lapatinib (FOLFIRI)	258	off-label	SD	258	537+
	CRC23	6	Pembrolizumab	175	off-label	PR	175	319
	CRC24	3	ATR inhibitor AZD6738 + Carboplatin	89	individualized exp. treatment	PD	84	305
	CRC25	2	Pembrolizumab	238	off-label	SD	238	486+
PC								
	PC3	3	Pertuzumab + Erlotinib	148	off-label	PR	146	284
	PC8	5	Pembrolizumab	71	off-label	PD	69	148
	PC9	5	Olaparib	70	off-label	no F/U	no F/U	84
	PC10	2	Olaparib	43	off-label	PD	42	46

	PC12	3	Palbociclib	51	off-label	PD	51	59
	PC18	1	Olaparib	134	off-label	PD	86	186
	PC19	3	Palbociclib	20	off-label	no F/U	no F/U	36
BTC								
	BTC6	2	Pembrolizumab	89	off-label	PD	85	116
	BTC9	1	IDH1 inhibitor BAY 1436032	88	clinical study	SD	N/A	547
	BTC14	3	Lenvatinib	275	off-label	PR	275	398+
	BTC18	3	Ponatinib	10	off-label	no F/U	no F/U	21
UGC								
	UGC3	2	Nivolumab + Ipilimumab	63	off-label	PD	61	305
	UGC6	3	Palbociclib	4	off-label	no F/U	no F/U	4
HCC								
	HCC1	1	Nivolumab	217	off-label	SD	254	420

Table 5. Patients treated according to MTB recommendation.

Treatment was implemented in 25 patients. The applied drugs were either used in-label, off-label, via a clinical study, or supplied for a matched individualized experimental treatment. Five patients were not available for assessment of best response. For one additional patient time of progression was not assessable. Data cut-off was March 31st, 2019. OS, overall survival; PFS, progression free survival; d, days. N/A, not assessable; no F/U, no follow-up; PD, progressive disease; PR, partial response; SD, stable disease.

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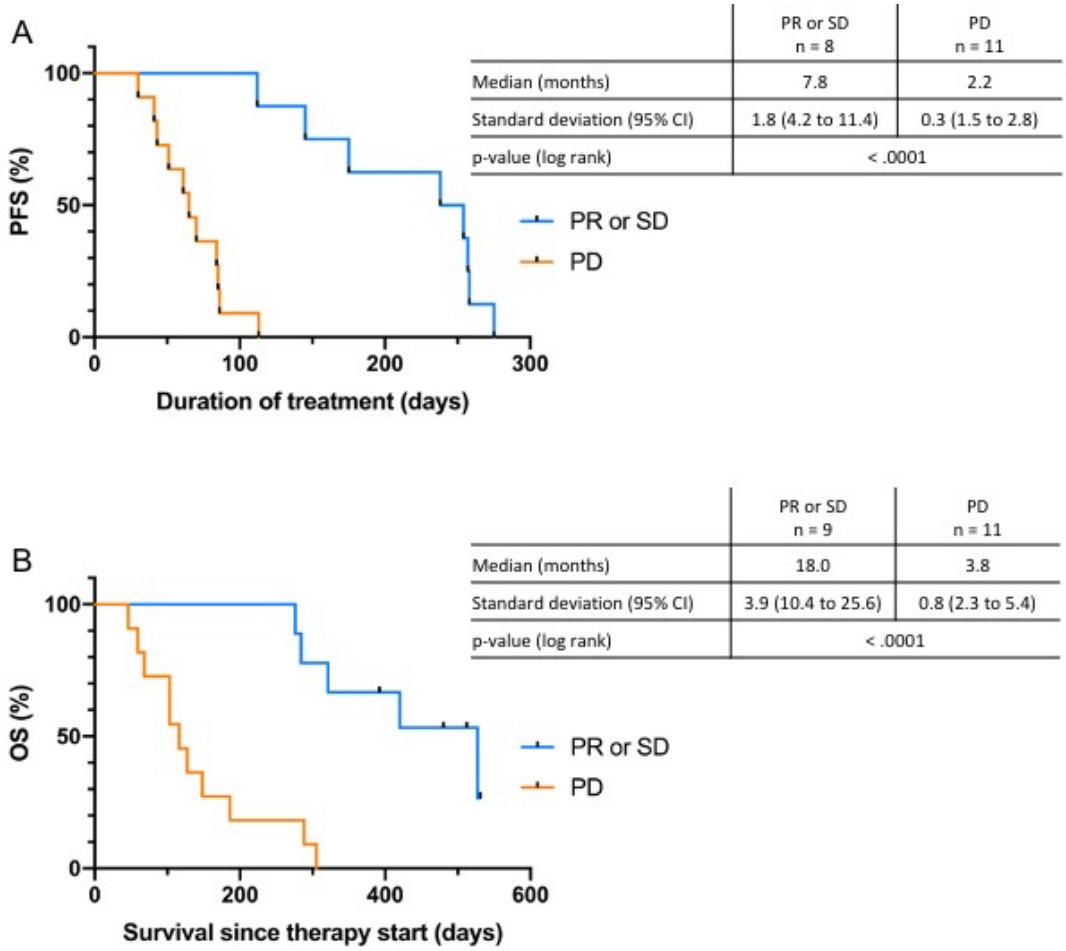
To assess whether patients had a benefit from the MTB-recommended therapy beyond best response, a comparison of OS and PFS between patients who reached disease control (PR or SD) for at least 3 months and patients who reached PD was performed using the Kaplan-Meier method [101].

It was shown that the median PFS of patients who reached disease control (SD or PR) was significantly longer with 7.8 months (95% CI, 4.2 to 11.4 months) versus 2.2 months (95% CI, 1.5 to 2.8 months; P < .0001) in patients with PD [101]. The same could be shown for the median OS with 18.0 months (95% CI, 10.4 to 25.6 months) in patients with disease control versus 3.8 months (95% CI, 2.3 to 5.4 months; P < .0001) in patients with PD [101]. All patients who showed OS of at least 12 months had reached disease control with either SD or PR [101].



	PR or SD n = 8	PD n = 11
Median (months)	7.8	2.2
Standard deviation (95% CI)	1.8 (4.2 to 11.4)	0.3 (1.5 to 2.8)
p-value (log rank)	< .0001	

	PR or SD n = 9	PD n = 11
Median (months)	18.0	3.8
Standard deviation (95% CI)	3.9 (10.4 to 25.6)	0.8 (2.3 to 5.4)
p-value (log rank)	< .0001	

Figure 10. Outcome of patients treated according to recommendations of the Molecular Tumor Board.

A: Kaplan-Meier analysis of progression free survival (PFS) in evaluable patients according to best response. B: Kaplan-Meier analysis of overall survival in evaluable patients according to best response. PD, progressive disease; PR, partial response; SD, stable disease.

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4 DISCUSSION

Parts of this work have been published previously by Bitzer, Ostermann, et al., *JCO Precision Oncology;* https://doi.org/10.1200/po.19.00359 (2020) [101]. For further information on authorship regarding distinct aspects of the work, please also consult the 'Erklärungen zum Eigenanteil' (declaration of own contribution).

4.1 PATIENT COLLECTIVE WITH GI CANCERS

The cohort of this study comprises 96 patients with advanced gastrointestinal cancers (Fig. 1A). At the time point of study initiation, previous reports of the implementation of Precision Oncology at different hospitals included patients with advanced solid tumors from quite different disease entities [104, 106, 122]. Within these cohorts the fraction of GI tumors often only accounted for small numbers of patients [104, 106, 122].

90% of the patients of our cohort had metastatic disease at the time of presentation to the MTB. Of 198 patients that were presented to the MTB affiliated to the University of Freiburg, 74% had metastatic solid tumors [106]. The MTB at the Dartmouth-Hitchcock Medical Center referred to a small number of patients presented, of which > 90% had recurrent, metastatic, or advanced disease [104]. Across all 96 patients of our cohort, they had received a mean number of 2.8 systemic pretreatments against their cancer disease at the time of presentation to the MTB (Fig. 2) [101]. Only one patient had not had any systemic treatment before presentation to the MTB and one patient had received 7 different previous systemic therapy lines (Fig. 2). This is in line with the characteristics of the aforementioned patient populations, showing a mean number of 2 pretreatments, ranging from 1 to 7, and 1 to 11, respectively [104, 106]. Taken together, to our knowledge, our cohort of advanced GI cancers presents the largest reported approach of implementation of Precision Oncology in this entity group to that time that was analyzed in such detail [101]. In line with previous reports, our cohort included patients with advanced cancers [109, 110] and exhausted standard therapies [130].

4.2 RELEVANCE OF SECONDARY GERMLINE FINDINGS IN GI CANCER

4.2.1 Implications of matched tumor and normal tissue sequencing

In 37 of 96 patients with advanced GI tumors, we found genomic alterations of the germline by performing comprehensive NGS panel sequencing or WES of tumor and matched normal tissue samples, respectively [101].

For the accurate identification of molecular targets, the sequencing of both tumor and matched normal tissue is considered an important procedure [101, 102, 121]. Recently, it has even been suggested that the sequencing of the tumor tissue and matched normal tissue sample should become standard of practice [100, 112]. Since most cancer genomes harbor various mutations, both somatic and germline [100, 131], identifying somatic mutations from sequencing the tumor genome alone is in principle possible but prone to error [131, 132]. Moreover, it is often difficult to distinguish driver mutations or alterations that offer therapeutical options from other alterations that are clinically less relevant [44, 133]. The sequencing of matched samples from tumor and normal tissues, like blood, enables a more precise differentiation of somatic and germline variants [100, 102, 131, 133-135].

However, in this context the question of how to deal with secondary germline findings adds another level of complexity to this diagnostic approach [100, 119, 134, 135].

First and foremost, in the setting of sequencing both tumor and normal tissue patients need to be properly informed about the potential of findings that implicate to be inheritable and thus also involve the lives of their relatives [100, 134, 135]. Still, finding hereditary changes also holds opportunities for a better clinical management of the concerned patients and their family members [136, 137]. That is, for example, early detection, also of metachronous cancers, or adequate, risk-adapted treatment regimens [100].

Nevertheless, for the appropriate management of secondary germline findings, their clinical relevance has to be assessed correctly, which is quite challenging, for example because of benign germline polymorphisms [133]. In general, there is a considerable genetic diversity in humankind and many of the found variants

may have no clinical or prognostic relevance [138]. Moreover, a general characteristic of secondary findings is that the prior likelihood that those variants are pathogenic is low [129].

For giving clear advise to patients, the knowledge about pathogenicity or benignancy of the germline variant is essential [139]. The applied ACMG classification system for germline variants provides a framework for the interpretation of their clinical relevance [129].

4.2.2 Findings of pathogenic and likely pathogenic germline variants

By applying the ACMG classification system to our study, germline variants classified as pathogenic and likely pathogenic could be identified in as many as 16 patients (17%) (Fig. 4A). We did not expect such a high number at the beginning of the study.

The ACMG classification system was also utilized in a recent investigation that reported the finding of pathogenic or likely pathogenic germline variants in 7.9 % of 10,389 patients across 33 cancer types [101, 140]. In 8.8% of 1,796 patients with GI tumors pathogenic or likely pathogenic germline variants were found [101, 140]. They found rates of pathogenic or likely pathogenic germline mutations that differed widely between cancers of different types, also between different types of GI cancers, with 14.1% for pancreatic cancer (PC) to only 2.2% for cholangiocarcinoma [101, 140].

Similar results were obtained when 1,000 patients were tested for germline variants in 72 genes in total [134]. While there were 826 patients whose examined tumors exhibited one or more nonsynonymous germline variants, in 7.8 % of all patients pathogenic or likely pathogenic variants were found [134]. Our numbers are even higher, and one reason for this could be the smaller sample size in our study [101].

Another earlier approach found germline variants considered to be pathogenic in only approximately 3% of the examined tumor samples [102]. However, the applied gene panel comprised a small number of genes and the criteria on classification of pathogenicity of the detected variants differed from the ACMG classification system [102].

From the beginning of investigating matched tumor and normal samples it was suggested that the numbers of detected germline variants seem to exceed the numbers found by single gene testing [121]. However, how many germline variants, especially likely pathogenic variants, and VUS, are identified is dependent on the panel size, or whether WES/ WGS has been performed [134]. Mezina et al. tested 217 patients with HCC on predisposing germline alterations with a gene panel of 134 genes and found pathogenic or likely pathogenic variants in 11.5% of patients. Interestingly, *MSH3*, the only gene with a pathogenic/ likely pathogenic germline alteration within HCC in our cohort was not found to be altered in that study (Table 2) [22]. Of note, the VUS in *CHEK2*, p.Ile157Thr, found in HCC6, was also found by Mezina et al. and was considered to be pathogenic, but with low penetrance (Table 2) [22].

Taken together, our cohort shows an unexpected high incidence of germline alterations [140]. Such observations in cancer patients do not reflect frequencies in the general population. Furthermore, the sequencing method as well as the applied classification system are important variables that can lead to divergent results [134]. These issues should be considered by comparing different studies.

4.2.3 Treatment options due to germline findings

Identifying cancer predisposing germline variants may also help decipher processes of pathogenesis and possible reasons for the development of resistance [101, 141]. Apart from that, pathogenic and likely pathogenic germline variants can also hold treatment options [101, 134, 135]. Here, I will discuss the importance of germline findings for therapeutic opportunities for several observed alterations in our cohort.

4.2.3.1 BRCA2 germline variants in pancreatic cancer

In pancreatic cancer (PC), two pathogenic and one likely pathogenic BRCA2 germline variants led to therapy recommendations (Table 4).

A subgroup of PC can be considered as a BRCA-associated cancer type and in about 4% to 7% of patients with PC germline alterations in *BRCA* are found [142, 143].

The POLO study tested the effect of a maintenance therapy with the PARP inhibitor Olaparib in patients with germline *BRCA* mutations [143]. In this trial treatment with Olaparib was compared to placebo in patients that had not shown progress on a prior treatment with platinum-based chemotherapy of at least 4 months [143]. Treatment with Olaparib led to a significantly longer PFS [143].

PARP inhibitors like Olaparib target Poly(ADP-ribose) polymerase (PARP) 1 and 2, two enzymes that work within the DNA damage response system [108]. PARP inhibitors prevent the PARP enzymes from leaving damaged DNA, which results in their malfunction [41, 108, 144]. Hereby, a halt of replication forks is caused, which leads to DSB [41, 108, 145].

BRCA2, a tumor suppressor gene, is encoding a protein that is part of the homologous recombination repair (HRR) system that, for example, takes affect after occurrence of DNA DSB [84, 91, 143]. Deleterious alterations in *BRCA2* lead to a defective HRR system, which makes cells sensitive to DNA damaging agents [91]. Treatment with PARP inhibitors can therefore result in an accumulation of DSB in cells with defective BRCA1/2, which will lead to cell death or senescence [91]. This concept is called conditional synthetic lethality [91, 108].

For 2 of the patients who received the PARP inhibitor Olaparib due to germline *BRCA2* alteration best response was only PD (PC10 and PC18), and for one patient response monitoring could not be performed (PC9). The latter received treatment for 70 days and had an OS of 84 days (Table 5). Those results raise the question of mechanisms of resistance to PARP inhibitors. For ovarian and breast cancer it has been suggested that *BRCA2* loss of function needs to be homozygous, otherwise there would be primary resistance to DNA damaging agents [91]. There was a somatic missense *BRCA2* variant in PC9 (p.Gly132Cys), a somatic truncating *BRCA2* variant in PC10 (p.Glu120*), and a somatic heterozygous small deletion of *BRCA2* in PC18 (g.13:32913178_32952054). For all 3 patients LOH was found in the tumor cells (Table 4). A mechanism of secondary resistance can be the restoration of the

DDR by subsequent mutations of *BRCA1/2*, or also *TP53*, thereby disabling the cell cycle checkpoint that induces apoptosis [91]. A somatic missense variant of TP53 was found in PC10 (p.C176Y), which has been demonstrated to be inactivating [146, 147].

The concept of conditional synthetic lethality is transferable to other DNA damaging agents. For example, platinum-based chemotherapy is causing DNA damage, therefore, patients with impairing germline mutations of BRCA are supposed to show better response to platinum-based chemotherapy [148]. It has been suggested that sensitivity to prior platinum-based therapy could serve as a predictive biomarker but also that secondary resistance can occur after platinum-based therapy [144]. PC10 had been previously treated with 5 cycles of FOLFIRINOX, PC18 only received cisplatin during 3 cycles of pressurized intraperitoneal aerosol chemotherapy (PIPAC). However, in an investigation by Kaufman et al. no difference in response to PARP inhibition could be shown between patients that had received prior treatment with platinum-based therapy and patients that had not [145].

In conclusion, the lack of benefit on treatment with a PARP inhibitor in 2 cases of PC might be mainly explained by mechanisms of secondary resistance that are not evident by the available data.

4.2.3.2 Colorectal cancer and germline variants in MSH6

In colorectal cancer, one pathogenic *MSH6* germline variant led to treatment recommendation of treatment with Pembrolizumab for one patient (CRC25) (Table 4).

MSH6 is one of the genes encoding the proteins that act within the mismatch repair system [88]. It can be affected within HNPCC, although *MLH1* or *MSH2* are affected more often [3, 88, 89, 149].

The tumor of CRC25 also revealed high TMB and high MSI and the patient received Pembrolizumab. Association between TMB, MSI, MMR defects and response to immune checkpoint inhibition will be discussed below. For CRC25 disease control could be achieved with SD as best response with a PFS of 238 days (7.8 months) (Table 5). The implementation of treatment based on

molecular alterations on the level of the germline in this case can be considered successful, though it cannot be determined which of the found rationales in this patient was the pivotal biomarker.

All in all, the availability of NGS made it feasible to implement both sequencing of larger gene panels and of matched tumor and normal tissues. The more involving detection of germline variants by performing NGS beyond the testing of patients with positive family histories can reveal predisposing variants that would not have been found otherwise [134]. But still, the clinical utility of those findings must be further investigated [134]. Likewise, the pathogenicity and clinical relevance of germline variants in genes not associated with the investigated tumor type has been questioned [101, 102]. Other data on the contrary, is promoting the implementation of targeted therapy due to germline variants also beyond association with the entity [150]. Though there was successful implementation of treatment based on alteration in the germline in our cohort, numbers are simply too small to draw further conclusions on the benefit of this approach.
In any case, the correct interpretation of the precise locus and accompanying somatic variants is central for assessing the implications for treatment recommendations [91, 142]. It is therefore essential that the MTB comprises of experts in clinical genetics and that the prospect of secondary germline findings is appropriately addressed [101].

4.3 IMMUNOTHERAPY IN GASTROINTESTINAL CANCER

4.3.1 Implementation of immunotherapy in our cohort

Immune checkpoint inhibitors represent an additional approach to targeted therapies. 8 patients were treated with checkpoint inhibitors due to high TMB, and the disease control rate (DCR) was 50% with a duration of at least 4.8 months [101]. Disease control with SD or PR was reached by 3 patients with CRC who received Pembrolizumab and one patient with HCC who received Nivolumab (Table 4, 5). 4 other patients with CRC, PC, BTC and UGC, respectively, showed

PD in the first follow-up. They were treated with Pembrolizumab, or, in the case of UGC, with Nivolumab plus Ipilimumab (Table 5).

Nivolumab and Pembrolizumab are monoclonal IgG4 antibodies against PD-L1, while Ipilimumab is a monoclonal IgG1 anti-CTLA-4-antibody [151-153]. The programmed death 1 (PD-1) and the cytotoxic t-lymphocyte-associated antigen 4 (CTLA-4) pathway, the so called immune-checkpoint pathways, are important in the mechanisms of peripheral tolerance of the immune system towards the body's own tissues [154, 155]. They mainly prevent autoreactivity and autoimmune disease [154, 155]. Cancer cells get recognized by the immune system. By utilizing the immune-checkpoint pathways to attenuate the immune response on the cancer cells they dispose of mechanisms of immune escape [156-161]. The inhibition of each pathway thus increases the immune response on the tumor [153, 155, 157, 162].

Immune checkpoint inhibitors have become important options in the treatment particularly of melanoma and non-small cell lung cancer (NSCLC) [151, 152, 163-170]. In recent years, however, the area of admission expanded with convincing results achieved from different phase I to III studies for the treatment of various other entities, also within GI cancers [17, 23, 171-173].

Efficacy of Nivolumab and Pembrolizumab could be demonstrated for subgroups of CRC, esophageal cancer, or adenocarcinoma of the stomach and the GEJ [17, 171-173].

The combination of immune checkpoint inhibitors targeting CTLA-4 and PD-L1 acts complementary on both checkpoint pathways [170]. Of note, one patient of our cohort, CRC25, with high TMB (118 Var/Mb), high MSI and MSH6 germline variant showed progression under PD-1 inhibition after 7.8 months but even responded after the subsequent addition of CTLA4 inhibition [101].

Nevertheless, 50% of the patients treated with immune checkpoint inhibitors did not respond to the treatment.

Currently, numerous mechanisms of resistance to immune checkpoint inhibitors are being discussed and identified [161], as non-response (primary resistance) and secondary resistance to immune checkpoint inhibitors is a frequently observed problem [153].

To name only a few, mechanisms of resistance can be the malfunction of recognition of tumor cells by the immune system, for example by impairment of antigen presentation in tumor tissues [161, 174], or of the effector function of T cells that ought to kill tumor cells [174]. On the molecular level, this can be due to the presence of alterations in other cell pathways [161, 175], or due to epigenetic alterations [153, 161, 174].

Within our cohort it was mainly primary resistance that was observed. At the time of treatment application, the molecular analysis did not indicate known signs for potential resistance in the 4 patients that only showed tumor progression under checkpoint inhibition [101]. Knowledge on this, however, has expanded since then, and still is expanding rapidly.

4.3.2 Biomarkers for Immunotherapy in GI cancer

TMB was the main rationale for the recommendation of treatment with immune checkpoint inhibitors in our approach.

It is defined as the load of variants in the coding genome [128, 176, 177]. For the assessment of TMB, panel sequencing seems to be as qualified as whole exome sequencing, as long as the panel is including a sufficient number of genes [89, 132, 178]. As can be taken from literature, for the reliable calculation of TMB there is a suggested minimum size of tumor panels of at least 300 genes, or 1.5 Mb of the target region, more specifically, which both is met in our approach [101, 132, 160, 178].

TMB values in our cohort varied between the tumor types, as it has been reported in further studies [89, 96]. It has also been reported that nearly every entity included tumors showing high TMB [89]. Despite in NET, where TMB ranged between 0.5 and 1.1 Var/Mbp, also in our cohort high TMB of > 10 Var/Mbp was found in every tumor type (4 CRC, 1 UGC, 1 PC, 1 BTC, 1 HCC) (Fig. 6).

This means that high TMB can be a rationale for therapy recommendation across various entities and provide additional treatment options for a wide range of patients within the MTB approach. In the US, pembrolizumab has been approved for solid tumors with a TMB value > 10 Var/Mbp [9]. However, still, it is in question

whether TMB is a good predictive marker for the treatment success with immune checkpoint inhibitors.

Studies could show a correlation between response to immunotherapy and high TMB [153, 179, 180]. In a cohort of 1662 patients across 10 tumor types, there was a significant association between high TMB and improved OS after therapy with both anti-PD-1/PD-L1 and anti-CTLA-4 therapy, as well as with combination of both [181]. When examined for each tumor type, at least for CRC, next to bladder, head and neck and non-small lung cancer, significant association between HR and high TMB was found [181]. In a recent report a high TMB of 175 or more mutations per exome was shown to predict benefit on Pembrolizumab monotherapy across different entities [182].

On the other hand, for some tumors an association between TMB and response to immune checkpoint inhibition could not be found, and also for responders, TMB in general has been shown to be a weak predictor [177, 183]. Apart from that, a major issue of discussion for TMB as a biomarker is the lack of a generally valid cut-off for the definition of high TMB [176].

In conclusion, it can be stated that there is an association between higher TMB and benefit from anti-PD-1/PD-L1 or anti-CTLA-4 therapy in certain tumor entities [153], though it still is under debate how reliably TMB can predict the outcome on treatment with checkpoint inhibitors [175, 176, 184].

The application of immune checkpoint agents is also linked to other biomarkers, e.g. the status of PD-L1 positivity [8, 10]. As an example, interrelation between efficacy of anti-PD-1-antibodies and level of PD-L1 expression could be demonstrated for progression free survival or overall survival in previously untreated, advanced esophageal squamous cell carcinoma in the KEYNOTE-590 study, which supported the admission [173]. However, response on immune checkpoint inhibiting agents could also be obtained in other studies regardless of the PD-L1 status [153], for example for study populations of NSCLC with high TMB [185] or MSI high or mismatch repair (MMR) deficient CRC [186]. Furthermore, high TMB is shown to have a higher association with response to

PD-1/PD-L1 checkpoint inhibition than any IHC test on PD-1/PD-L1 expression [89, 160].
All in all, at least within the entity of esophageal cancer patients with PD-L1 expressing tumors seem to benefit from anti-PD-L1 treatment [17, 173]. However, especially in the cases of response to immune-checkpoint agents regardless of PD-L1 status, it is important to find those biomarkers that enable a reasonable administration of immunotherapy in patients beyond those who's tumors are exhibiting expression of PD-L1 in IHC.

Other well-established biomarkers for the indication for Nivolumab plus Ipilimumab or Pembrolizumab for patients with advanced CRC are a status of high MSI or MMR deficiency [8, 10].
Microsatellites are short repetitive DNA sequences that are located at various sites of the genome [86]. Microsatellite instability occurs in MMR deficient cancers [7, 87, 88]. MMR deficiency can be sporadic, which it is in most cases and then usually is caused by hypermethylation of the promotor region of *MLH1*, which leads to a loss of the gene product [88]. If MMR deficiency occurs hereditarily, then *MLH1*, *MSH2* or, less frequently, *MSH6* or *PMS2* are affected and cause the tumor syndrome HNPCC [88, 89, 149]. It has shown to be a good predictive marker and association between MMR deficiency and the response to immune checkpoint inhibitors have been reported [154, 177].
Since MSI results from MMR deficiency, it is tested to assess the functionality of the MMR system and is also used as a predictive biomarker for the response to immune checkpoint inhibiting therapy [154].
Only two patients in our cohort had MSI high tumors, one patient with BTC and one patient with CRC [101]. This small number can be expected for BTC, as the numbers of MSI high BTC range between 5% and about 10%, differing slightly between the localizations, and the total number of patients with BTC in our population was 19 [30]. Even though high MSI in primary colorectal cancer is relatively common, with an estimated frequency of 15%, it is less common in metastatic colorectal cancer [7, 88, 162]. An investigation by Koopman et al. found high MSI in 3.5% of samples from advanced CRC [87]. All patients with

CRC in our approach had metastatic disease at the time of presentation to the MTB and sequencing was performed on tissue obtained from metastatic site in 24 cases, and from recurrent disease in 4 cases. So, the low proportion of patients with MSI high tumor can be explained by the analyzed population.
Interestingly, patients with MSI high tumors comprised only 11.7% of all patients that showed high TMB. Inversely, all patients showing high MSI also showed high TMB. Patients with high MSI were among the patients with the highest TMB values of the whole cohort. This observation is in line with an investigation in a broad range of different tumor types, reporting that only 16% of patients with high TMB had tumors classified as MSI high, but in 97% high MSI, TMB values were of > 10 Var/MBp [89]. Of note, 3 of the patients of our cohort that achieved disease control due to therapy with checkpoint inhibitors were treated regardless of their MSI status.

Taken together, reliable, and generally valid biomarkers on benefit from immune checkpoint inhibition as well as on resistance are still an unmet need in the context of MTBs.

4.4 SOMATIC MUTATIONS AS TARTGETS

Next to high TMB and clinically relevant germline alterations NGS also revealed somatic mutations that were identified as molecular targets.
In the context of the MTB Tübingen, interdisciplinary experts discussed the actionability of somatic targets mainly based on the availability of targeted drugs and reports on successful implementation of the treatment due to the respective biomarkers.
The most frequent somatic targets found in our cohort involved the pathways of cell cycle regulation and mitogenic signaling. Next, treatment recommendations based on alterations in *CDKN2A* as well as genes of the FGF and ErbB family and their respective subsequent pathways will be exemplarily discussed. These targets have shown to be important in other reports, too [104, 122, 123]. Especially *PI3K* (or *PIK3CA*) is frequently found to be altered and identified as “actionable” in solid tumors [104, 106, 110, 120, 122, 123]. Of note, alterations in

PI3K, or *PIK3CA*, were not identified as targets in our approach, but are well-known to affect pathways downstream of both the FGFR and ErbB signaling [66, 74, 75].

4.4.1 *CDKN2A* and *CDK4/6*

A very frequently identified target in our cohort were *CDKN2A/B* deletions, found in 6 patients with PC, 2 patients with UGC, one patient with CRC and one patient with BTC, with additional *CDK6* amplification in 2 cases (Table 4) [101].

The high frequency of *CDKN2A/B* deletions in PC is common, with data of *CDKN2A* and *CDKN2B* alterations in general found in about 60 % of PC [187]. Alterations of *CDKN2A* and *CDKN2B* have also been reported for esophageal adenocarcinoma and EBV-associated gastric cancer, which is found to show *CDKN2A* promotor hypermethylation [15, 19], but are less common in BTC [188]. Due to deletion or deleterious mutations of *CDKN2A* cell cycle regulation is abolished by loss of p16(INK4a), a gene product of *CDKN2A* [189]. With loss of p16(INK4a), inhibition of CDK4/6 is lacking, and CDK4/6 activity is unregulated [189]. Also, aberrant activity of CDK4/6, due to overexpression or function gaining mutation, leads to unregulated progression through the cell cycle, uncontrolled cell division and proliferation [51]. It hence has a tumorigenic effect. Palbociclib is an inhibitor of CDK4/6, a role that physiologically is assumed by the CDK inhibitors, p16(INK4) and related proteins, encoded by *CDKN2A* [54, 190].

However, at the moment, phase 2 or 3 studies with CDK4/6 inhibitors are not in the center of personalized approaches. In pancreatic cancer it was shown that on patient derived xenografts or mouse models tumor responses to Palbociclib correlated positively with higher levels of Rb [189, 191]. However, *CDKN2A* deletion or loss of function of p16(INK4a) did not seem to be a reliable predictive biomarker [191]. Of note, patients with mutations in *KRAS*, *TP53*, or *CDKN2A* showed worse outcome compared with patients with wildtype *KRAS*, *TP53*, or *CDKN2A* in a phase 1b study that tested efficacy and safety of Palbociclib in combination with nab-paclitaxel in metastatic adenocarcinoma of the pancreas [192].

Apart from that, Palbociclib showed efficacy in the treatment of liposarcoma, a tumor type that in over 90% of cases harbors amplifications of CDK4 [193]. Loss of p16(INK4a) was found to be a good predictive marker for the efficacy of Palbociclib in preclinical studies on glioblastoma multiforme (GBM) xenograft [194], and, next to proficiency of Rb, on ovarian cancer cell lines [195]. Inconsistent data is given by a phase 1 study of patients with advanced head and neck squamous cell cancer who received combination therapy of Palbociclib and cetuximab [196]. A disease control rate of 98% was reported with 8 of 9 patients showing at least SD [196]. There were two patients that reached PR, and both had negative immunohistochemistry results of p16(INK4a) expression, while among the patients that reached SD there was an equal number of positive and negative immunohistochemistry results for p16(INK4a) expression [196].

Profound data on potent predictive biomarkers indicating molecular subtypes of pancreatic cancer benefiting from CDK4/6 inhibitors are scarce, though presence of Rb seems to be important [189, 191, 194]. Though treatment implementation with Palbociclib was recommended in all 9 cases with *CDKN2A/B* alteration in our cohort, only in 3 cases this recommendation could be followed. Of those 3 cases, 2 patients weren't available for follow up, and in 1 case only progressive disease could be reached as best performance status (Table 4, 5). In summary, although preclinical data suggest a promising role of *CDKN2A/B* as a target for CDK4/6 inhibition in PC, this has to be questioned. Currently, treatment approaches with Palbociclib as a monotherapy in PC only suggested in selected cases by the Tübingen MTB based on these data and an additional report [197].

4.4.2 Targeting of the FGF/R pathway

FGFR alterations are considered to be found in a subset (5-10%) of all cancer types, which makes them important potential targets across different entities [67]. In our cohort, for 6 patients with CAA *FGFR* alterations were identified as targets (Table 4).

In intrahepatic CCA, elevated rates of 10 – 30% of *FGFR* alterations have been observed [67]. Although CNVs are the most common *FGFR* alterations in general, *FGFR* fusions, for example, with the BICC family RNA-binding protein 1

(BICC1), seem to be quite frequent in CCA [68]. The reason for this frequency remains unknown [67].

5 of the 8 *FGFR* alterations found in the patients with CAA of our cohort were *FGFR2* fusion genes, 3 of them with *BICC1* (Table 4). However, there was no implementation of therapy in the cases of *FGFR-BICC1* fusion due to disease control under standard treatment or poor performance status.

By today, there is an approval for the selective *FGFR* inhibitor Pemigatinib in locally advanced or metastasized and previously treated CCA with *FGFR* fusion or rearrangement in the USA and the EU [29, 67]. Approval was given due to results of an open-label phase 2 study that showed disease control in 82% of all patients with *FGFR* fusions or rearrangements, with 2,8% showing complete and 32,7% showing partial response [198]. Of note, none of the other patients without *FGFR* fusion or rearrangement in this study showed an objective response [198].

In our cohort, the fusion gene *FGFR-AHCYL2* together with an *FGFR* alteration was a rationale for treatment with Lenvatinib for one patient (Table 4). This patient showed durable disease control with PR and a PFS of 9 months (Table 5). At the time of data cut-off this patient was still alive and OS at this time was 13.1 months (Table 5).

Alterations of *FGFR* are important targets because of their high prevalence across different entities. In our cohort they made up a high proportion of the identified targets in BTC. Since a substantial part of *FGFR* alterations are fusion genes, it is important to include assays that are able to detect these fusions. In the meantime, further FGFR-inhibitors have been approved by the FDA for the treatment of CCAs with a FGFR2-fusion [199-201].

4.4.3 Targeting of the ErbB pathway

Equally important in the field of mitogenic signaling is the ErbB family of growth factor receptors. However, not only in the context of pathogenesis the ErbB pathway plays a central role, but also in Precision Oncology, as Trastuzumab was one of the first established targeted drugs [13, 107].

2 of 3 patients of our cohort that received targeting of the ErbB signaling pathway achieved durable disease control (Table 5). In one patient with *KRAS* and *BRAF*

wildtype CRC (CRC22) we found an *ERBB2* amplification that was targeted with Trastuzumab plus Lapatinib (Table 4). *ERBB2* amplifications are found in a small subset (3%) of metastatic CRC, especially in tumors that do not exhibit mutations in *KRAS* or *BRAF* [74]. Efficacy of the combination of Trastuzumab plus Lapatinib has been reported for a cohort of metastatic CRC with overexpression of ErbB2 [202]. With Trastuzumab, an anti-ErbB2 antibody and Lapatinib, an ErbB1 and ErbB2 inhibitor, both ErbB1 and ErbB2 are targeted [202]. Trastuzumab in this context also can hinder the upregulation of ErbB3 that results from continuous treatment with Lapatinib [202]. This treatment approach is a good example for the capability of a combination therapy in Precision Oncology. It led to durable disease control with a PFS of 8.5 months for one patient (CRC22) of our cohort (Table 5).

One patient with PC (PC3) received ErbB1 and ErbB2 inhibition with Pertuzumab plus Erlotinib due to *NRG1* fusion (*TMEM66*:*NRG1*, *NRG1*:*CDH6*) and increased ErbB3 expression (Table 4). NRG1 fusions lead to increased PI3K-AKT signaling via ErbB3 activation, as well as the MAPK pathway and hence to proliferative, pro-tumorigenic effects [70, 203]. NRG1 fusions are rather rare in cancer (< 1%) [70, 72]. The increased expression of ErbB3 in this case has been observed in combination with NRG1 fusions in other patients before [203, 204]. This is possibly due to increased availability of the ligand and hence upregulation of the ErbB/PI3K/NFκ pathway [70, 203]. The targeting of ErbB1 (EGFR) and ErbB2 with Pertuzumab plus Erlotinib therefore has a molecular rationale. In our cohort, it led to a PR with a PFS of 4.6 months (Table 5). This patient (PC3) was also reported in another publication that was focused on NRG fusions [72].

All in all, alterations in the ErbB family genes provide molecular rationales that can be targeted successfully within the approach of Precision Oncology, in particular by extension of established treatment options and application of combination therapy.

4.5 OUTCOME ON MTB-RECOMMENDED TREATMENT OPTIONS

Treatment recommendations based on molecular biomarkers could be given for 41 (43%) of 96 patients with advanced GI tumors by the MTB, which implies that for 4 out of 10 patients there was an additional therapy option beyond established treatment lines [101].

The part of patients for which targetable alterations were found in early approaches of MTB implementation at other hospitals ranges around 39% to 65% of patients [104, 106, 120, 122]. However, these are numbers from investigations of unselected patient groups, that equally focused on gastrointestinal, gynecological, urogenital or CNS cancers [104, 106, 120, 122].

The present analysis focuses on the implementation of molecular guided treatment in GI cancer. Still, within this group, each entity is unique in its characteristics and requirements.

The highest rate of target identification in our cohort was within patients with BTC, with an additional treatment option for 73.7 %, i.e., for 14 out of 19 patients. The finding of a high number of targetable alterations has been described for BTC before [26, 27]. This is especially promising since treatment of advanced BTC with conventional agents is not highly successive [26, 27].

At the time of presentation to the MTB, for patients with advanced BTC who were not eligible to surgery due to metastasis or local extent there was only the combination of gemcitabine and cisplatin with a mean survival of 11.7 months [205]. Durable disease control could be achieved for 2 patients of our cohort of BTC by administration of the IDH1 inhibitor BAY 1436032 or Lenvatinib and OS even was 18 months and 13.1 months, respectively (Table 5).

As of late there is an FDA approval of the IDH1 inhibitor Ivosidenib for third line treatment of *IDH1* mutated CCA as well as an FDA and EMA approval of the FGFR inhibitor Pemigatinib for second line treatment of intrahepatic CCA that exhibits FGFR fusions or rearrangements [29, 198, 206, 207]. That means promising new therapeutic options for these subtypes of BTC. Furthermore, it underlines our observation that patients with BTC can notably benefit from the approach of Precision Oncology [101].

On the other side of the spectrum, no target could be identified for patients with NET of our cohort [101]. This could be due to the small cohort of patients with NET in our approach. However, a small number of targets in NET in general also is in line with other recent publications reporting that pancreatic neuroendocrine cancers were among the entities with the lowest fraction of patients with identified driver mutations [96].
In conclusion, GI cancers comprise a group of tumors that differ with regard to the applicability of the approach of Precision Oncology. However, in particular for rare tumors like NET Precision Oncology may be a chance to find treatment solutions [106, 110]. The inclusion of additional genes in existing sequencing panels as well as the implementation of further diagnostic may help to detect the important pathways in rare cancer types [96].

Apart from the identification of targets, the implementation of treatment as well as the assessment of a potential benefit are critical parameters for the evaluation of MTB work and success.
CRC was the most frequent tumor type in our cohort and of 13 patients with treatment recommendation 11 patients received the treatment (Table 4, 5). The disease control rate for patients with CRC available for follow-up was 50 %. 4 of these patients that reached disease control had a PFS of >4 months (Table 5). This is long compared to earlier studies on molecular profiling and administration of matched therapies in CRC, where the median time to treatment failure was 7.9 weeks [208].
Also, for patients with PC, HCC and UGC targets could be identified, and treatment recommendations were given by the MTB. However, of 5 patients with PC that were available for best response analysis, only one patient reached disease control with PR and a PFS of 4.8 months (Table 5). This underlines the general experience that PC is a hard-to-treat disease [39].
No patient with UGC achieved disease control due to the implemented treatment, while one patient with HCC showed a PFS of 8.4 months and an OS of 13.8 months on treatment with Nivolumab (Table 5).

Looking at this small numbers of patients that reached SD or PR under the recommended treatment one must note that the general number of patients with treatment initiation was very small in some entity groups. This pertains to patients with BTC, HCC or UGC in particular. Across all entities the main reason for not following treatment recommendation was poor performance status. A worse performance status impeded the initiation of therapy in 16 cases, including patients that had died before the treatment could be started (Fig. 9).
Furthermore, 6 patients were not available for follow-up and assessment of best response, which in 3 cases was due to rapid clinical deterioration after starting the treatment (Fig. 9, Table 4).
As depicted above, the analyzed cohort is a population of patients with advanced GI cancers at metastatic status in the majority of cases. Available standard treatment options often were exhausted at the time of MTB presentation. This makes it hard to provide additional treatment options in a sufficiently short time before the patients' performance status and the dynamic of the present malignant disease allows nothing but best supportive care. Our cohort's characteristics are typical for patient populations within the approach of Precision Oncology when compared to other reports [104, 110].
On the other hand, there were 6 patients whose tumors harbored targetable alterations but for who no treatment recommendation for molecularly matched therapies could be started because of sustained disease control under standard treatment at the time of MTB presentation [101]. Thus, presenting patients to the MTB at the right time remains challenging.

All patients of our cohort who showed an OS of at least 12 months under the MTB-recommended treatment had reached disease control with either SD or PR (Fig 10). So, with the idea of duration of disease control as a surrogate end point for the assessment of benefit from the administered treatment beyond best response, PFS and OS were compared between patients who reached PR or SD for at least 3 months and patients who reached PD [101].
It was shown that the median PFS of the patients who reached disease control (SD or PR) was significantly longer with 7.8 months (95% CI, 4.2 to 11.4 months)

versus 2.2 months (95% CI, 1.5 to 2.8 months; P < .0001) in patients with PD [101]. The same could be shown for the median OS with 18.0 months (95% CI, 10.4 to 25.6 months) in patients with disease control versus 3.8 months (95% CI, 2.3 to 5.4 months; P < .0001) in patients with PD [101].

Another way of assessing the success of therapy in pretreated cohorts is the comparison between the PFS on the current treatment (PFS2) with PFS on the previous treatment (PFS1). A PFS2/PFS1 ratio of ≥ 1.3 is assumed to show clinical benefit on the latter treatment [103, 209]. For 9 patients of our cohort no data could be obtained, due to lack of information on PF1 in 5 cases and due to drop out and thus no assessment of PFS2 in 4 cases. A ratio of ≥ 1.3 was achieved in 7 (43.8%) out of 16 patients available for this analysis (2 CRC, 2 PC, 2 BTC, 1 HCC).

Across the whole cohort, of 20 patients available for best response analysis, 45% (9 out of 20) reached either PR or SD (Table 5). This is in line with the rate of patients with a favorable PFS2/PFS1 ratio.

It is challenging to identify parameters that reflect benefit, and the achievement of disease control needs to be accompanied by the improvement of other relevant outcomes as well [101]. All in all, patients that achieved PR or SD seemed to benefit with regard to PFS and OS [101]. The median PFS of 7.8 months and OS of 18.0 months is a considerable success in a cohort of advanced cancers with progress on the last approved treatment option.

4.6 CONCLUSION

Parts of this work have been published previously by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]. For further information on authorship regarding distinct aspects of the work, please also consult the 'Erklärungen zum Eigenanteil' (declaration of own contribution).

4.6.1 Main results of the study

Over the last decades, the knowledge of the molecular pathogenesis of cancer has grown substantially and, together with the acquired technologies, has been integrated into clinical oncology [210, 211]. This is especially reflected by the increase of approvals for targeted therapies and the introduction of new therapeutic approaches [212].

With the start of the Molecular Tumor Board (MTB) at Tuebingen University in April 2016, a new quality and amount of clinical data on molecular profiles as well as on treatment outcomes was documented for all cancer patients that were discussed within this interdisciplinary board. Between April 2016 and February 2018, this included 96 patients with advanced gastrointestinal cancer (32 with CRC, 22 with PC, 19 with BTC, 11 with HCC, 9 with UGC and 3 with NET). 90% of the patients of our cohort had metastatic disease and the mean number of systemic anticancer pretreatments was 2.8 at the time of presentation to the MTB [101]. This is in line with the characteristics of patient populations in other reports of the implementation of Precision Oncology [104, 106]. However, within these cohorts the fraction of gastrointestinal tumors often only accounted for small numbers of patients. To our knowledge, our cohort presented at that point the largest reported approach of the implementation of Precision Oncology in this disease entity that was analyzed in such detail [101].

After performing comprehensive genetic profiling with panel analysis of 337 to 710 genes or whole exome sequencing in 91 patients (94,8%) and 5 patients (5,2%) respectively (Fig. 3), we found targetable alterations for 47 (49%) of the 96 presented patients (Table 4).

To our surprise there was an unexpected high number of secondary germline findings in our cohort [101]. Reasons for this could be the smaller sample size in our study as well as the relatively large panel sizes [101, 134]. By applying the ACMG classification system to our study, germline variants classified as pathogenic and likely pathogenic could be identified in as many as 16 patients (17%). In 3 cases of PC and 2 cases of CRC this supported treatment recommendations.

Apart from that, recommendations for immune checkpoint inhibition accounted for a substantial proportion of all treatment rationales. 8 patients were treated with checkpoint inhibitors due to high TMB, and the disease control rate (DCR) was 50% with a duration of at least 4.8 months [101]. Disease control with SD or PR was reached by 3 patients with CRC who received Pembrolizumab and one patient with HCC who received Nivolumab (Table 4, 5).

TMB was the main rationale for the recommendation of treatment with immune checkpoint inhibitors in our approach. Although TMB as a biomarker has limitations, correlation between high TMB and benefit from checkpoint inhibition has been reported frequently [153]. In the EU, though, admission of immune checkpoint inhibiting agents is in part linked to status of PD-L1 expression [8, 10]. However, high TMB was shown to outperform expression of PD-L1 in recent meta-analyses [182, 184].

A very frequently identified somatic target in our cohort were CDKN2A/B deletions, found in 6 patients with PC, 2 patients with UGC and one patient with BTC [101]. Although preclinical data suggest a promising role of CDKN2A/B as a target for CDK4/6 inhibition with Palbociclib in PC, this must be questioned. Currently, treatment approaches with Palbociclib as a monotherapy in PC are only suggested in selected cases by the Tübingen MTB, which is also supported by an additional report by Baghdadi et al. [197].

The highest rate of target identification in our cohort was within patients with BTC, with an additional treatment option for 73.7 %, i.e., for 14 out of 19 patients. Frequent targets were FGFR alterations, especially FGFR fusion genes. *FGFR-AHCYL2* together with an *FGFR* alteration was a rationale for treatment with

Lenvatinib for one patient with BTC (BTC14) (Table 4). This patient showed durable disease control with PR and a PFS of 9 months (Table 5). At the time of data cut-off this patient was still alive and OS at this time was 13.1 months (Table 5). Other reports underline our observation that patients with BTC can notably benefit from the approach of Precision Oncology [26, 27].

All in all, treatment recommendations based on molecular biomarkers could be given for 41 (43%) of 96 patients with advanced GI tumors by the MTB, which implies that for 4 out of 10 patients there was an additional therapy option beyond established treatment lines [101]. Across all entities the main reason for not following treatment recommendation was poor performance status. For the assessment of benefit from the implemented treatment beyond best response, PFS and OS were compared between patients who reached PR or SD for at least 3 months and patients who reached PD [101]. It was shown that the median PFS as well as the median OS of the patients who reached disease control (SD or PR) was significantly longer [101]. The median PFS and OS was 7.8 months, and 18.0 months, respectively [101]. All patients of our cohort who showed an OS of at least 12 months under the MTB-recommended treatment had reached disease control with either SD or PR (Fig 10). We considered this as a considerable success in a cohort of advanced cancers with progress on the last approved treatment option.

4.6.2 Limitations of the study

There are several limitations of the analysis though.

First and foremost, both the cohorts regarding the tumor type, and the cohorts regarding one specific target or recommended treatment, are still small, particularly when it comes to patients with therapy start. This does not permit to draw conclusion for bigger populations.

There is no compared matched cohort that was not presented to an MTB, or did receive the same treatment, but without showing the target. Also, there was no follow-up on the patients that were presented to the MTB but for who no targets could be identified. Therefore, a comparison of OS between patients who

received molecular matched therapy and those who did not, was not possible. In addition, because the patient's presentation to the MTB has not been performed in a randomized fashion, there clearly is a selection bias in the analysis of outcomes.

Because it was a retrospective analysis, some parameters could not be obtained. The assessment of the performance status of each patient at the time of MTB presentation or at the time of initiation of treatment was attempted but was not possible. Documented information was too inconsistent to estimate a valid value. Another shortcoming is that there was no assessment of quality of life during the course of presentation and implementation of treatment. If there had been, one could not only have assessed benefit in terms of improved PFS or OS, but also in terms of well-being, a very important aspect, in particular in the late life of cancer patients, as it often is in heavily pretreated cohorts. There was no complete documentation of discontinuation of treatment due to adverse events, though patients that discontinued treatment out of other reasons than progression of their disease often showed a poor performance status in general. Especially because one aim of Precision Oncology is not only to provide more effective but also safer treatment options, a more comprehensive assessment of both adverse events and quality of life would have been of further value.

4.6.3 Outlook

Although the size of the part of our cohort with implementation of treatment makes it difficult to statistically assess the impact of our approach, there are patients that clearly had a benefit regarding OS and PFS. The comparison of PFS and OS between patients with disease control and patients without disease control in the first follow up showed a significant difference. Also, some achievements of OS and PFS in patients were noteworthy. Nevertheless, it is not clear if the chosen surrogate of best response for at least 3 months is valid and first should be confirmed in a larger population of patients [101].

Apart from that there is a substantial number of patients that did not benefit from the administered treatment although there had been a molecular rationale.

The aim must be to find the right predictive markers to constantly enlarge the number of patients responding on the targeted treatment in the first place [101]. In the context of the MTB Tübingen, interdisciplinary experts discussed the actionability of somatic targets mainly based on the availability of targeted drugs (Table 2) and reports on successful implementation of the treatment due to the respective biomarkers to that time.

As a part of Precision Oncology, treatment is administered in a histology agnostic fashion and with off-label use. From early attempts we can learn that histologic classification can give advice on molecular characteristics of targeted tumors [213]. The classification of a tumor to a molecular subtype within the respective entity, on the other hand, takes into account important driver mutations, gene expression and the upregulation of pathways that lead to cancer development and progression [15, 214]. Thereby, a framework for treatment decisions is provided and heterogeneity within a tumor type is addressed [7, 214]. Further analysis of selected patient groups provided with novel therapies will help to validate the clinical utility of molecular subtypes.

One important issue concerning the diagnostic tests that were used for this study is a constantly further evolving field of new technologies and further improvements. A growing number of further techniques will constantly improve the knowledge of individual cancers which is thought to help addressing knowledge gaps. A constant improvement in complex diagnostic procedures should provide more rationales for the administration of combination therapies and thus additional prospects for therapeutic success. Knowledge on biomarkers for the prediction of response and resistance augments through the increasing amount of phase II or III studies and meta-analyses in the field.

Herein, the existence of MTBs is crucial to meet the challenge of interpretation of novel data and their implications for clinical practice. Within this approach, it must be secured that full documentation is accomplished for all patients that are presented to the MTB, notwithstanding whether or not treated with targeted agents. Then information on findings and treatment success as well as unsuccess can be further analyzed and shared. This includes documentation on

performance status, adverse events, as well as quality of life [101]. Thereby, the circumstances of treatment administration can be understood objectively and benefit beyond OS and PFS can be assessed. An answer to this need could be one-person trials, or N-of-1 trials, especially for the patients that receive off-label drugs and cannot be included in ongoing clinical trials [101, 215]. Equally, the evaluation of data generated by the increasing application of novel therapies within the clinical routine apart from MTBs will contribute to an improvement of targeted therapeutic concepts.

Taken together, an iterated evaluation of the whole process from patient presentation to assessment of benefit as well as the integration of new data in this process are important to constantly improve recommendations and increase the number of patients that benefit from targeted therapies.

All in all, this work shows that the application of novel sequencing procedures led to the identification of pathogenic and likely pathogenic germline variants and to additional individual treatment options beyond established therapy lines [101]. Patients with advanced GI cancer could benefit from the approach of Precision Oncology and implementation of the MTB at the University Hospital Tübingen. At the same time the integration of molecular profiling and targeted treatments into clinical oncology remains to be an ongoing process. Novel and complex diagnostics have to be implemented and MTB recommendations must be improved continuously to further enlarge the proportion of patients that benefit from targeted treatments.

5 SUMMARY

We evaluated 96 patients with GI tumors that were presented to the MTB Tübingen between April 2016 and February 2018 on different parameters including tumor entity, sequencing results, presence of germline mutations, MTB decision, treatment implementation and outcome. Parts of this work have been published previously by Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101]. The analysis shows that for 41 of the 96 patients a targeted treatment could be recommended and that a treatment implementation of the recommended therapy was performed for 25 patients. Patients that reached “stable disease” or “partial response” according to radiological criteria showed a longer median progression free survival as well as a longer median overall survival.

Detected targetable gene mutations were, among others, mutations in CDKN2A/B, CDK6, BRCA2 and FGFR2. For 10 patients, a high tumor mutational burden led to the treatment recommendation. Therapy recommendations included PD1 inhibition, CDK4/6 inhibition, PARP inhibition and inhibition of the FGFR pathway. Furthermore, the results of this study reveal “pathogenic” (class 5) and “likely pathogenic” (class 4) germline mutations in 16 patients, that partly were responsible for treatment recommendation.

Overall, an additional treatment option could be identified for nearly 43% of patients with advanced GI cancers. This observation is in line with the reported observations of other MTBs in Germany and around the world. Of note, those reports did not investigate patients with GI cancer separately from other solid tumors. Our investigation thereby constitutes the first detailed depiction of the implementation of MTB decisions in a collective with GI tumor diseases. Nevertheless, such a selected cohort also exhibits considerable heterogeneity, regarding diagnostic results as well as response to the respective therapeutic attempt.

Taken together, it could be shown that the concept of Precision Oncology is applicable on patients with advanced GI cancer and that in this cohort a control rate to MTB-guided therapy could be reached in 45% of treated patients.

6 ZUSAMMENFASSUNG

Wir analysierten den Verlauf von 96 Patient*innen mit gastrointestinalen Tumoren, die zwischen April 2016 und Februar 2018 im MTB des Universitätsklinikums Tübingen vorgestellt wurden. Hierbei wurden verschiedene Parameter wie Tumorentität, Sequenzierungsergebnis, das Vorliegen von Keimbahnmutationen, Therapieempfehlung, Therapieumsetzung und Outcome in der Analyse berücksichtigt. Teile dieser Arbeit sind im Vorfeld bereits erfolgreich publiziert worden und finden sich daher wieder bei Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101].

Die Auswertung zeigte, dass bei 41 der 96 Patienten eine zielgerichtete Therapie durch das MTB empfohlen werden konnte, bei 25 Patient*innen kam es zur Umsetzung der empfohlenen Therapie. Patient*innen, die eine „partielle Remission“ (PR) oder einen „stabilen Verlauf“ (SD) nach radiologischen Kriterien zeigten, hatten sowohl ein längeres progressionsfreies Überleben als auch ein längeres Gesamtüberleben im Vergleich zu Patient*innen, die einen Progress zeigten. Als Zielstrukturen zeigten sich Mutationen unter anderem in den Genen *CDKN2A/B*, *CDK6*, *BRCA2* und *FGFR2*, bei 10 Patient*innen führte eine hohe Mutationslast zur Therapieempfehlung. Empfohlene Therapien umfassten unter anderem Checkpoint-Inhibitoren, CDK4/6-Inhibitoren, PARP-Inhibitoren und Inhibitoren des FGFR Pathways.

Darüber hinaus offenbarte die Auswertung das Vorliegen von „pathogenen“ (Klasse 5) oder „wahrscheinlich pathogenen“ (Klasse 4) Keimbahnmutationen bei 16 Patient*innen, die teilweise zu Therapieempfehlungen führten.

Insgesamt konnte für ein Kollektiv mit fortgeschrittenen gastrointestinalen Tumorerkrankungen in knapp 43% der Fälle eine potenzielle zusätzliche Therapieoption gefunden werden. Diese Beobachtung stimmt in ihrer Größenordnung mit Beobachtungen anderer MTBs aus Deutschland und weltweit überein. Dabei muss beachtet werden, dass bisher gastrointestinale Tumorerkrankungen nicht gesondert von anderen soliden Tumorerkrankungen im Detail betrachtet worden waren. Unsere Untersuchung stellte demnach die

erste detaillierte Beschreibung der Umsetzung von MTB-Beschlüssen in einem Kollektiv mit gastrointestinalen Tumorerkrankungen dar.
Nichtsdestotrotz weist auch eine solche selektierte Kohorte eine beachtenswerte Heterogenität auf, sowohl was die Ergebnisse der Diagnostik als auch das Ansprechen auf die jeweiligen Therapieversuche betrifft.
Zusammenfassend konnte jedoch gezeigt werden, dass das Konzept der Präzisionsmedizin für Patient*innen mit fortgeschrittenen gastrointestinalen Tumorerkrankungen anwendbar ist, und in diesem Kollektiv bei 45% der therapierten Patient*innen eine Kontrolle der Tumorerkrankung (PR oder SD) erreicht werden konnte.

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8 ERKLÄRUNG ZUM EIGENANTEIL

Diese Arbeit wurde an der Medizinischen Klinik des Universitätsklinikums Tübingen unter Betreuung von Prof. Dr. med. Michael Bitzer durchgeführt.

Teile dieser Arbeit sind im Vorfeld bereits erfolgreich publiziert worden und finden sich daher wieder bei Bitzer, Ostermann, et al., *JCO Precision Oncology*; https://doi.org/10.1200/po.19.00359 (2020) [101].

Die Konzeption der Untersuchung und Fragestellung erfolgte in enger Zusammenarbeit mit Herrn Prof. Dr. med. Michael Bitzer sowie weiterhin mit Frau Dr. Janina Beha, stellvertretende Geschäftsführerin des Zentrums für Personalisierte Medizin (ZPM), Wissenschaftliche Koordinatorin der ZPM-Geschäftsstelle, Koordinatorin für Molekulare Diagnostik und Frau M. Sc. Kristina Ruhm, Koordinatorin des Molekularen Tumorboards (MTB).

Die Datenerhebung wurde von mir durchgeführt, im Fall von schwer zugänglichen Daten mit Unterstützung durch Herrn Prof. Dr. med. Bitzer, Frau Dr. Janina Beha und Frau M. Sc. Kristina Ruhm.

Die statistische Auswertung erfolgte nach Beratung durch Prof. Dr. Peter Martus bezüglich der Auftragung und Interpretation der Daten in einer Überlebenskurve nach der Kaplan-Meier-Methode durch mich.

Die Konzeption des Molekularen Tumor Boards (MTB) sowie der damit verbundenen elektronischen Informationsplattform erfolgte im Vorfeld der durch mich durchgeführten Untersuchung durch die Universitätsklinik Tübingen bzw. das Zentrum für Personalisierte Medizin und nicht durch mich. Die Beschreibung der Organisation des MTBs im Abschnitt 2.3 des Kapitels „Material and Methods“ basiert und bezieht sich daher auf Informationen, die durch die Organisatori*innen des MTBs im Rahmen der oben genannten Publikation

bereitgestellt wurden. Daher verweise ich in meiner Arbeit an entsprechenden Stellen auf die oben genannte Publikation.

Ebenso erfolgte die genetische Sequenzierung der Tumorproben wie im Abschnitt 2.4 des Kapitels „Material and Methods“ aufgeführt nicht durch mich, entsprechend basiert und bezieht sich die Beschreibung dessen auch hier auf Informationen, die durch die Mitarbeitenden des Instituts für Medizinische Genetik und Angewandte Genomik, Universität Tübingen, sowie der CeGaT GmbH and Praxis für Humangenetik, Tübingen im Rahmen der oben genannten Publikation bereitgestellt wurden. Daher verweise ich in meiner Arbeit an entsprechenden Stellen auf die oben genannte Publikation.

Ich versichere, das Manuskript selbstständig nach Anleitung durch Herrn Prof. Dr. med. Bitzer verfasst zu haben. Dies geschah zum Teil im Rahmen der gemeinsamen Arbeit an der oben genannten, gemeinsam erarbeiteten Publikation und betrifft vor allem Eckpunkte der Diskussion sowie die Kapitel 1.3.3 und 1.4 der Einleitung. Teile dieser Arbeit konnten in der zitierten Publikation bereits erfolgreich veröffentlicht werden. Grundlage für die Publikation waren die Arbeiten im Rahmen dieser Promotionsarbeit. Daher verweise ich in meiner Arbeit an entsprechenden Stellen auf die oben genannte Publikation.

Ich versichere, keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Berlin, den

Leonie Ostermann

9 LISTE DER PUBLIKATIONEN

Teile der vorliegenden Dissertationsschrift wurden bereits in den folgenden Publikationen veröffentlicht:
Parts of this work have been published previously in the following publications:

Michael Bitzer, Leonie Ostermann, Marius Horger, Saskia Biskup, Martin Schulze, Kristina Ruhm, Franz Hilke, Öznur Öner, Konstantin Nikolaou, Christopher Schroeder, Olaf Riess, Falko Fend, Daniel Zips, Martina Hinterleitner, Lars Zender, Ghazaleh Tabatabai, Janina Beha and Nisar P. Malek - Next-Generation Sequencing of Advanced GI Tumors Reveals Individual Treatment Options - JCO Precision Oncology - 2020 - no. 4 (2020) - S. 258-271

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