Next-Generation Sequencing of Advanced GI Tumors Reveals Individual Treatment Options

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PURPOSE Precision oncology connects highly complex diagnostic procedures with patient histories to identify individualized treatment options in interdisciplinary molecular tumor boards (MTBs). Detailed data on MTB-guided treatments and outcome with a focus on advanced GI cancers have not been reported yet.

PATIENTS AND METHODS Next-generation sequencing of tumor and normal tissue pairs was performed between April 2016 and February 2018. After identification of relevant molecular alterations, available clinical studies or in-label, off-label, or matched experimental treatment options were recommended. Follow-up data and a response assessment that was based on radiologic imaging were recorded.

RESULTS Ninety-six patients were presented to the MTB of Tuebingen University Hospital. Sixteen (17%) showed “pathogenic” or “likely pathogenic” germline variants. Recommendations on the basis of molecular alterations or tumor mutational burden were given for 41 patients (43%). Twenty-five received the suggested drug, and 20 were evaluable for best response assessment. Three patients (15%) reached a partial response (PR), and 6 (30%), stable disease (SD), whereas 11 (55%) had tumor progression (progressive disease). Patients with SD for ≥3 months or PR compared with progressive disease showed both a statistically significant longer median PFS (7.8 months [95% CI, 4.2 to 11.4 months] v 2.2 months [95% CI, 1.5 to 2.8 months], P < .0001) and median OS (18.0 months [95% CI, 10.4 to 25.6 months] v 3.8 months [95% CI, 2.3 to 5.4 months], P < .0001).

CONCLUSION Next-generation sequencing diagnostics of advanced GI cancers identified a substantial number of pathogenic or likely pathogenic germline variants and unique individual treatment options. Patients with PR or SD in the course of MTB-recommended treatments seemed to benefit with respect to PFS and OS.

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INTRODUCTION The challenge of precision oncology is to connect highly complex diagnostic procedures with individual patient histories to identify optimal treatments. The complexity of this approach resulted in the implementation of interdisciplinary molecular tumor boards (MTBs) at academic centers. Detailed data on MTB-guided treatment suggestions, and specifically the outcomes of patients, have only been reported for nonselected patient groups, including for a variety of different tumors. However, each tumor entity, including GI cancers, harbors unique features that will have to be considered to identify the patients who will benefit the most from such an approach. Recent reports highlight the experiences of MTBs in everyday practice. A series from the MD Anderson Cancer Center that examined hot-spot regions in 11 to 50 genes for 2,000 cancer tissues found actionable mutations for 39% of patients. Only 11% of these selected patients could be enrolled in genotype-matched clinical trials. A report from the Mayo Clinic identified actionable mutations in 65% of 141 patients, but only 29 (21%) were subsequently treated in clinical trials or with targeted Food and Drug Administration (FDA)-approved drugs, with an objective response or stable disease (SD) for ≥4 months in 10 patients. Freiburg University reported 104 patients with recommendations that were based on diagnostic tests that ranged from an 8-gene panel to whole-exome sequencing (WES). Thirty-three patients received a recommended treatment; 11 had an objective response, and 8 additional patients reached SD at ≥10 weeks. Eight of 9 patients in that cohort with solid tumors and partial response (PR) received checkpoint inhibitors.
CONTEXT

Key Objectives
Academic molecular tumor boards (MTBs) bridge the gap between a growing complexity in diagnostic procedures and clinicians at the bedside. This retrospective study investigated the course of patients with advanced gastrointestinal (GI) cancers that have been considered for personalized treatment options, including the outcome receiving MTB-guided treatment.

Knowledge Generated
Next-generation sequencing (NGS) revealed a relevant number of germline variants, suggesting genetic counseling. More than one quarter of patients who did not have further established therapeutic options could be treated according to MTB recommendation. Responding patients with prolonged disease stabilization seemed to derive a meaningful survival benefit from cancer genome sequencing and matched treatments.

Relevance
Patients with GI cancers can benefit from currently available NGS sequencing procedures. In perspective, a continued improvement of MTB recommendations and the inclusion of additional complex diagnostic procedures might substantively enhance treatment opportunities in everyday clinical practice for these patients in the near future.

The sequencing of matched tumor and normal tissue is an important procedure for the precise identification of potentially actionable genes.\textsuperscript{5,6} This inevitably leads to the detection of germline alterations. A recent investigation in 10,389 patients across 33 cancer types detected pathogenic or likely pathogenic germline variants in 8% of all patients with cancer and in 8.8% of patients with GI tumors.\textsuperscript{7} The frequency of germline mutations varied greatly across cancer types in general but also across different GI cancers, ranging from 2.2% for cholangiocarcinoma to 14.1% for pancreatic cancer (PC).\textsuperscript{7}

To our knowledge, our experience with GI tumors in an academic MTB is to date the largest reported series of this group with detailed information of comprehensive sequencing data, subsequent board recommendations, and documented outcome. MTB recommendations included approved drugs for the disease, clinical studies, approved drugs against the driver mutations in different indications (off-label use), and matched experimental treatments.

PATIENTS AND METHODS

Patients and MTB Organization
All 96 patients with GI tumors at the University Hospital Tuebingen referred to the MTB between April 2016 and February 2018 were included in this retrospective, open-label, histology-agnostic analysis, which was reviewed and approved by the local ethics committee (511/2018BO). Before patients consented to next-generation sequencing (NGS) of their tumor tissue, they were informed by a specialist in clinical genetics. If relevant germline alterations were detected, genetic counseling by a clinical geneticist was offered. The MTB consists of an interdisciplinary team coordinated by the Tuebingen Center for Personalized Medicine and includes experts in clinical and translational oncology, pathology, bioinformatics, molecular biology, radiology, and human genetics. An electronic Web-based platform (MTB platform) was established to introduce patients to the MTB team with all necessary information to prepare and follow up the weekly face-to-face meetings and subsequently document treatment outcomes (Data Supplement). Best response assessment was based on radiologic imaging in line with RECIST 1.1, for checkpoint inhibitor therapy, iRECIST, or for hepatocellular carcinomas (HCC), mRECIST criteria.\textsuperscript{8-10}

Genetic Tumor/Normal Characterization
Tumor and normal tissues were genetically characterized either by NGS panel sequencing of full coding sequences or by WES (more information in Data Supplement). After the identification of relevant molecular alterations in the MTB, available clinical studies or in-label, off-label, or matched experimental treatments were recommended. 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 Tuebingen.

Statistical Analysis
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. Of the 25 patients who were treated according to MTB recommendations, 5 could not be included in this analysis because they did not have any follow-up imaging for response monitoring.
RESULTS

Diagnostic Procedure and Clinical Work-Up

Ninety-six patients received NGS of advanced GI tumors and were presented to the MTB. This cohort included 32 colorectal cancer (CRC), 22 PC, 19 biliary tract cancer (BTC), 11 HCC, 9 upper GI tract cancer (UGC), and 3 neuroendocrine tumors (NET; Fig 1A). The mean number of systemic anticancer pretreatments was 2.8 (Fig 1B). Ninety-one patients received a gene panel analysis that examined between 337 and 710 genes, and 5 patients had WES (Fig 1C). Genes represented in the different panels are summarized in the Data Supplement, together with the total size and the average and median coverage of the 649 and 710 gene panels.

Germline Variants in GI Cancers

Germline findings were ranked according to a 5-tiered classification.11,12 Sixteen patients (17%) had germline variants classified as “pathogenic” or “likely pathogenic” (Figs 1D-1F), and in 1 patient with gastric cancer, 2 likely pathogenic variants were detected (PALB2 and FANCM). Five patients (5%) had pathogenic or likely pathogenic germline variants in one of the BRCA2, MSH6, or SDHB genes. These alterations belong to a list of secondary findings that should be reported because of the possibility for interventions to significantly reduce morbidity and mortality.13 In addition, 3 patients showed germline variants in pharmacologically relevant genes, 2 in DPYD and 1 in G6PD (Data Supplement) without showing unusual adverse reactions during chemotherapy. Seventeen additional patients had a variant of unknown significance (Data Supplement), of which the CHEK2 variant p.Ile157Thr was found in 3 different patients. This variant has been reported with classifications ranging from variant of unknown significance to pathogenic.14

A germline alteration was directly responsible for MTB recommendations for 5 patients (5%): BRCA2 for poly(ADP-ribose)-polymerase (PARP) inhibition (3 PC), CHEK2 combined with 2 somatic ATM truncations for ATR-inhibition and carboplatin (1 CRC), and MSH6 and therefore a resulting high tumor mutational burden (TMB) for pembrolizumab (1 CRC). Regarding patient histories, one had a microsatellite instability (MSI)—high CRC; the 3 patients with BRCA mutations had 1 first-degree relative with breast,
FIG 2. 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
pharyngeal, or bladder cancer; and the patient with a CHEK2 mutation had a first-degree relative with ovarian cancer. Taken together, for 19 out of 96 patients (20%), germline variants classified as pathogenic, likely pathogenic, or pharmacogenomic could be identified (Fig 2A).

**TMB**

TMB is currently regarded as relevant in predicting therapeutic success with checkpoint inhibitors beyond tumors with mismatch repair deficiency. This has been shown for patients with CRC and UGC, but is less clear for hepatobiliary cancer and PC. The median TMB ranged from 6.3 Var/Mbp for CRC to 2.3 Var/Mbp for BTC (Fig 3A). The 3 NET showed low TMB between 0.5 and 1.1 Var/Mbp. For MTB discussions, a high TMB was important for therapy decisions regarding checkpoint inhibitors, defined as ≥ 10 mutations per megabase. For tumors known to have a low TMB in general, those with values between 7.5 and 10 Var/Mb were considered TMB-high. Checkpoint inhibition on the basis of high TMB was recommended for 10 patients (4 CRC, 3 BTC, 1 HCC, 1 gastric cancer, 1 PC; Table 1). Eight of these 10 patients were also tested for MSI, but only 2 patients were found to have a MSI-high tumor.

**Molecular Target Identification by the MTB**

At least 1 potential molecular target was identified in 47 of 96 patients (49%; Figs 2B and 3B). The frequency of target identification varied among tumor types (Fig 3C, Table 2), with 74% for BTC, 56% for upper GI cancers, 50% for PC, 44% for CRC, and 27% for HCC. In 3 cases of NET, no target could be identified. The most frequently altered genes with single-nucleotide variants or copy number variants were CDKN2A/B, BRCA2, IDH1, ERBB2, MYC, FGF3, FGF4, FLT3, FGFR4, CDK6, BRAF, and ATM (Table 2; Data Supplement). Three different fusion genes were detected exclusively with FGFR2 in BTC. Of 10

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**FIG 3.** Tumor mutational burden and target identification per tumor type. (A) Tumor mutational burden was calculated for 88 patients. Two patients had 3, and 3 patients had 2, different calculations of separate tissue samples over the observation period, which are included in the analysis. The median is shown for each tumor type. (B) Frequency of potential target identification by the molecular tumor board (MTB), patients with target identification but no treatment, and patients with MTB-recommended treatment initiation. (C) Frequency of target identification per tumor type. BTC, biliary tract cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; NET, neuroendocrine tumor; PC, pancreatic cancer; UGC, upper GI tract cancer; w/o, without.

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**TABLE 1.** Mutational burden per tumor type.

| Tumor Type | TMB (Var/Mbp) | No. (%) |
|------------|---------------|---------|
| BTC        | 10.0          | 14 (74) |
| CRC        | 0.5-1.1       | 5 (56)  |
| UGC        | 7.5-10        | 11 (50) |
| HCC        | 14 (44)       | 14 (44) |
| PC         | 2.3-4.0       | 3 (27)  |

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**TABLE 2.** Molecular target identification per tumor type.

| Tumor Type | Target Identification | No. (%) |
|------------|-----------------------|---------|
| BTC        | 14 (74)               |         |
| CRC        | 5 (56)                |         |
| UGC        | 11 (50)               |         |
| PC         | 14 (44)               |         |
| HCC        | 3 (27)                |         |

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| Diagnosis and Patient No. | No. of Pretreatments | Board Recommendation | Treatment | Duration (days) | Drug Availability | Best Response | PFS (days)b | Overall Survival (days)b | Remarks |
|--------------------------|----------------------|-----------------------|-----------|----------------|------------------|---------------|-------------|------------------------|---------|
| CRC1                     | 3                    | BET inhibition caused by BRD4 mutation | BET-inhibitor molibresib | 35 | Individualized exp treatment | PD | 29 | 103 |                  |
| CRC2                     | 2                    | PD-1 inhibition caused by MLH1 mutation and high TMB (10.4 Var/Mbp) | Pembrolizumab | 113 | Off-label | PD | 113 | 127 | After 3 cycles, already suspected pseudoprogression |
| CRC6                     | 5                    | FGFR pathway inhibition caused by FGF3,4,19 amplification (approx. 5x) | Regorafenib | 65 | Approved | PD | 66 | 103 |                  |
| CRC9                     | 6                    | PD-1 inhibition caused by high TMB (8.1 Var/Mbp) | Pembrolizumab | 516 | Off-label | SD | 257 | 549c | Individual peptide vaccination under continued treatment with pembrolizumab after progression |
| CRC11                    | 6                    | Indirect Myc inhibition caused by Myc amplification (approx. 3x) | Aurora-A-kinase inhibitor MLN-8237 | 41 | Individualized exp treatment | PD | 41 | 68 |                  |
| CRC17                    | 6                    | ERBB2 inhibition caused by ERBB2 amplification (> 10x) | Trastuzumab plus lapatinib | 90 | Off-label | no F/U | no F/U | 158 | No response monitoring available |
| CRC20                    | 5                    | ATR inhibition caused by mutations in ATM, CHEK2 (germline), and TP53BP1 | ATR inhibitor AZD6738 plus carboplatin | 197 | Individualized exp treatment | SD | 112 | 276 |                  |
| CRC22                    | 3                    | ERBB2 inhibition caused by ERBB2 amplification (> 10x) | Trastuzumab plus lapatinib (FOLFIRI) | 258 | Off-label | SD | 258 | 537c | 55% decrease in CEA value, lowest result on day 129; at day 146, lapatinib was stopped and FOLFIRI added to trastuzumab |
| CRC23                    | 6                    | PD-1 inhibition caused by high TMB (8.1 Var/Mbp) | Pembrolizumab | 175 | Off-label | PR | 175 | 319 | PD caused by progression and new lesions |
| CRC24                    | 3                    | ATR inhibition caused by mutations in ATM | ATR inhibitor AZD6738 plus carboplatin | 89 | Individualized exp treatment | PD | 84 | 305 |                  |
| CRC25                    | 2                    | PD-1 inhibition caused by MSH6 germline mutation and high TMB (118 Var/Mbp) | Pembrolizumab | 238 | Off-label | SD | 238 | 486c | 55% decrease in CEA value, lowest result on day 129; at day 146, lapatinib was stopped and FOLFIRI added to trastuzumab |

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### TABLE 1. Patients Treated According to MTB Recommendation (Continued)

| Diagnosis and Patient No. | No. of Pretreatments | Board Recommendation | Treatment | Duration (days) | Drug Availability | Best Response | PFS (days) | Overall Survival (days) | Remarks |
|---------------------------|----------------------|----------------------|-----------|----------------|------------------|---------------|------------|------------------------|---------|
| PC                        |                      |                      |           |                |                  |               |            |                        |         |
| PC3                       | 3                    | Inhibition of ERBB1 and ERBB2 caused by 2 NRG1 fusion genes and increased ERBB3 and NRG3 expression | Pertuzumab plus erlotinib | 148 | Off-label | PR | 146 | 284 | KRAS wildtype PC, CA19-9 at treatment start 1,260 kU/L, normalization after 66 days |
| PC8                       | 5                    | PD-1 inhibition caused by high TMB (16.2 Var/Mbp) | Pembrolizumab | 71 | Off-label | PD | 69 | 148 | |
| PC9                       | 5                    | PARP inhibition caused by BRCA2 mutation (germline) | Olaparib | 70 | Off-label | no F/U | no F/U | 84 | No response monitoring performed |
| PC10                      | 2                    | PARP inhibition caused by BRCA2 mutation (germline) | Olaparib | 43 | Off-label | PD | 42 | 46 | |
| PC12                      | 3                    | CDK4/6 inhibition caused by CDKN2A/B deletion | Palbociclib | 51 | Off-label | PD | 51 | 59 | |
| PC18                      | 1                    | PARP inhibition caused by BRCA2 mutation (germline) | Olaparib | 134 | Off-label | PD | 86 | 186 | |
| PC19                      | 3                    | CDK4/6 inhibition caused by CDKN2A/B deletion | Palbociclib | 20 | Off-label | no F/U | 37 | 36 | Only short therapy interval, no decrease of elevated CA19-9 or CEA levels observed, no response monitoring performed |
| BTC                       |                      |                      |           |                |                  |               |            |                        |         |
| BTC6                      | 2                    | PD-1 inhibition caused by high TMB (7.6 Var/Mbp) | Pembrolizumab | 89 | Off-label | PD | 85 | 116 | |
| BTC9                      | 1                    | IDH1 inhibition caused by IDH1 mutation | IDH1 inhibitor BAY 1436032 | 88 | Clinical study | SD | NA | 547 | SD for at least 110 days, start of gemcitabine and cisplatin before progression according to RECIST |
| BTC14                     | 3                    | FGFR pathway inhibition caused by FGFR2 fusion protein | Lenvatinib | 275 | Off-label | PR | 275 | 398c | |
| BTC18                     | 3                    | FGFR pathway inhibition caused by FGFR1 (4x) and FGFR4 (5x) amplifications | Ponatinib | 10 | Off-label | no F/U | no F/U | 21 | No response monitoring performed |
| UGC                       |                      |                      |           |                |                  |               |            |                        |         |
| UGC3                      | 2                    | PD-1 and CTLA4 inhibition caused by high TMB (15 Var/Mbp) | Nivolumab plus ipilimumab | 63 | Off-label | PD | 61 | 305 | Hyperprogression, disease control after checkpoint inhibition by ramucirumab and paclitaxel |
| UGC6                      | 3                    | CDK4/6 inhibition caused by CDKN2A/B deletion | Palbociclib | 4 | Off-label | no F/U | no F/U | 4 | Rapid clinical deterioration, no sufficient dosing possible, no response monitoring performed |

(Continued on following page)
| Diagnosis and Patient No. | No. of Pretreatments | Board Recommendation | Treatment | Duration (days) | Drug Availability\(^a\) | Best Response | PFS (days)\(^b\) | Overall Survival (days)\(^b\) | Remarks |
|--------------------------|---------------------|----------------------|-----------|-----------------|--------------------------|--------------|----------------|-----------------------------|---------|
| HCC                      | 1                   | PD-1 inhibition caused by high TMB (119 Var/exome) | Nivolumab | 217             | Off-label               | SD           | 254           | 420                         |         |

\(^a\)Drug availability: approved, off-label, clinical study, individualized exp treatment.

\(^b\)PFS and overall survival calculated from day of initiation of MTB-recommended treatment.

\(^c\)Patient alive at data cutoff.

NOTE. Data cutoff March 31, 2019.

Abbreviations: BET, bromodomain and extra-terminal motif protein; BTC, biliary tract cancer; CEA, carcinoembryonic antigen; CRC, colorectal cancer; exp, experimental; FOLFIRI, chemotherapy with folinic acid, 5-fluorouracil, irinotecan; F/U, follow-up; HCC, hepatocellular carcinoma; MTB, molecular tumor board; NA, not available; PARP, poly(ADP-ribose)-polymerase; PC, pancreatic cancer; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; TMB, tumor mutational burden; UGC, upper GI tract cancer.
CDKN2A/B alterations, 6 were detected in PC, 2 in UGC, 1 in BTC, and 1 in CRC. Three out of 4 BRCA2 alterations were detected in PC, and 1 in BTC (Table 2).

Outcome and Clinical Course of Patients

The course of all patients presented to the MTB is shown in Fig 2B. Six patients did not receive a recommendation because of complete tumor resection in 2 patients or sustained disease control during ongoing chemotherapy in 4 patients. Targets in these 6 patients would have been FGFR2 fusions, IDH1 mutation, CDKN2A/B deletion, or high TMB (Data Supplement). MTB recommendations for matched treatments were given for 41 patients (43%). Twenty-five patients with a mean of 3.4 previous anti-cancer treatments received the suggested medication (61% of patients with MTB recommendation; 26% of the whole cohort). The other 16 patients could not be treated because of poor performance status. The alterations in this subgroup without treatment included CDKN2A/B deletions, BRAF mutations, BRCA deletions, FLT1/3 amplification, FGFR2 fusion, IDH mutation, and high TMB (Data Supplement).

Treatment was initiated in 11 patients with CRC (34% of patients with CRC), 7 patients with PC (32% of patients with PC), 4 patients with BTC (21% of patients with BTC), 2 patients with UGC (22% of patients with UGC), and 1 patient with HCC (9% of patients with HCC). Detailed information for all treated patients is listed in Table 1, and the exact molecular alteration that led to the recommendation is included in the Data Supplement. The applied drugs were either used in-label, used off-label, obtained via a clinical study, or supplied for a matched experimental treatment (Table 1, Drug Availability column). Twenty patients were treated and evaluable for best response analysis. Three patients reached PR (15%) and 6, SD (30%), and 11 had tumor progression regardless of treatment (PD, 55%). The disease control rate was 45%. Patients who showed PD as best response were treated with checkpoint inhibition (high TMB), olaparib (BRCA2 germline variant), BRD4 inhibition (BRD4 mutation), regorafenib (FGFR alteration), aurora A kinase inhibition (Myc amplification), ATR-inhibitor with carboplatin (ATM mutation), or palbociclib (CDKN2A/B deletion; Table 1).

### Table 2. Target Identification per Tumor Type

| Diagnosis  | Patients Tested (No.) | Molecular Target Frequency |
|------------|-----------------------|---------------------------|
| CRC        | 32                    |                           |
| ATM        | 2                     |                           |
| ERBB2      | 2                     |                           |
| BRAF       | 1                     |                           |
| BRD4       | 1                     |                           |
| CDKN2A/B   | 1                     |                           |
| CHEK2      | 1                     |                           |
| FGFR3/4/19<sup>a</sup> | 1                |                           |
| FLT1/3     | 1                     |                           |
| MLH1       | 1                     |                           |
| MSH6       | 1                     |                           |
| MYC        | 1                     |                           |
| TMB        | 4                     |                           |
| BTC        | 19                    |                           |
| FGFR2-BICC1 | 3                    |                           |
| IDH1       | 2                     |                           |
| BRAF       | 1                     |                           |
| BRCA1      | 1                     |                           |
| BRCA2      | 1                     |                           |
| CDKN2A     | 1                     |                           |
| FGFR1      | 1                     |                           |
| FGFR2<sup>b</sup> | 1               |                           |
| FGFR4      | 1                     |                           |
| FGFR2-PRKCQ | 1                   |                           |
| FGFR2-AHCYL2 | 1                  |                           |
| TMB        | 3                     |                           |
| PC         | 22                    |                           |
| CDKN2A/B   | 6                     |                           |
| BRCA2      | 3                     |                           |
| CDK6       | 1                     |                           |
| ERBB3      | 1                     |                           |
| NRG3       | 1                     |                           |
| NRG1-CDH6  | 1                     |                           |
| TMEM66-NRG1 | 1                   |                           |
| TMB        | 1                     |                           |
| Upper GI tract | 9                   |                           |
| CDKN2A/B   | 2                     |                           |
| CDK6       | 1                     |                           |
| FGFR3/4    | 1                     |                           |
| IDH1       | 1                     |                           |
| TMB        | 1                     |                           |
| HCC        | 11                    |                           |
| IDH1       | 1                     |                           |

(Continued in next column)

Abbreviations: BTC, biliary tract cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; PC, pancreatic cancer; TMB, tumor mutational burden.

<sup>a</sup>FGF3/FGF4/FGF19 cluster amplification.

<sup>b</sup>Coincident FGFR2 mutation in tumor with FGFR2_AHCYL2 fusion gene.
Patients who reached either SD or PR were treated with PD-1 blocking antibodies (high TMB), ATR inhibitor with carboplatin (simultaneous mutations in ATM, CHEK2, and TP53BP1), trastuzumab with lapatinib (ERBB2 amplification), pertuzumab with erlotinib (NRG1 fusion, high-expression ERBB3 and NRG3), IDH1 inhibitor BAY 1436032 (IDH1 mutation), or lenvatinib (FGFR2 fusion gene; Table 1). For each treated patient, PFS and OS are shown in Figs 4A and 4B, respectively. The median PFS for all treated and evaluable patients was 2.8 months (range, 1.0-9.0 months) and the median OS of all treated patients was 5.2 months (range, 0.1 months to not reached).

Of note, all patients who survived > 1 year after treatment initiation reached either SD or PR (Data Supplement). Therefore, as a surrogate end point to estimate whether patients might benefit from the MTB-recommended treatment, the achievement of SD for at least 3 months or even PR as best response was compared with PD in a Kaplan-Meier estimation for PFS and OS. Patients who reached SD or PR showed both a statistically significant longer median PFS of 7.8 months (95% CI, 4.2 to 11.4 months) versus 2.2 months (95% CI, 1.5 to 2.8 months; \( P < .0001 \)) and median OS of 18.0 months (95% CI, 10.4 to 25.6 months) versus 3.8 months (95% CI, 2.3 to 5.4 months; \( P < .0001 \); Figs 4C and 4D).

**DISCUSSION**

Comprehensive NGS panel or WES of GI tumors and matched normal tissue samples was performed for 96 patients. In several studies, the inclusion of germline findings enabled a more comprehensive characterization of tumor biology, potential resistances, or even treatment opportunities. In this study, germline variants classified as pathogenic, likely pathogenic, or pharmacogenomic could be identified in 19 patients (20%). These numbers are higher than in a recent investigation that reported pathogenic or likely pathogenic germline predisposition variants in 8.8% of GI tumors, and could be mainly a result of the smaller sample size in our study. The importance of germline variants for genetic counseling usually has to be regarded in the context of associated tumor types. Whether this also holds true for therapeutic implications is unclear and is currently under discussion. For example, for BRCA mutations, the tumor lineage indeed seems to determine the therapeutic benefit of PARP inhibition. Conversely, emerging data suggest that patients with germline mutations that are not typically associated with a diagnosed cancer might nevertheless benefit from drugs that target this alteration. Germline variant reporting and the inclusion of experts in clinical genetics, should therefore be a prerequisite for MTBs.

TMB is regarded as relevant in predicting therapeutic outcome with checkpoint inhibitors. The minimum size of tumor panels to reliably calculate TMB was suggested to include at least 300 genes, or more precisely, 1.5 Mb of the target region, which is met in our approach. Of 8 patients treated with checkpoint inhibitors, 4 reached PR or SD with a duration of at least 4.8 months. One patient with CRC and high TMB (118 Var/Mb) who showed progression under PD-1 inhibition even responded after the addition of CTLA4 inhibition on progression. In the 4 patients who did not show any response to checkpoint inhibition, the molecular analysis did not reveal one of the currently discussed mechanisms of resistance. Of note, only a minority of patients with high TMB also had MSI-high tumors. This observation is in line with an investigation in a broad range of different tumor types, showing that only 16% with high TMB were classified as MSI high. Another study in 6,004 patients with CRC identified 465 patients with high TMB; however, only 65% of these could be classified as MSI high. These observations suggest that a reliable method for TMB estimation should be used if patients with GI tumors are evaluated for personalized treatment options.

Of 96 patients with advanced GI tumors, MTB treatment recommendations were given for 41 (43%), which means an additional therapy option beyond established treatment lines for 4 out of 10 patients. The identification of parameters that reflect the benefit to patients is challenging. Best response analysis revealed disease control in 45% of evaluable patients. However, the achievement of a PR or SD is meaningful only if other relevant outcomes are improved as well. We performed an exploratory analysis of PFS and OS for the patients who received MTB-recommended treatment. The difference in OS of > 14 months in our cohort, compared with patients with PD as best response (Fig 4D), is remarkable; however, it has to be interpreted with caution and should be confirmed in larger patient populations with GI or other cancers. If this observation could be confirmed, 1 goal in the constant improvement of MTB recommendations should be to gradually increase the percentage of patients who reach disease control at least 3 months in advanced cancers.

Early cohorts with molecular-matched therapies have been reported for CRC and BTC. In the first study, 68 out of 254 patients with advanced CRC received selected matched therapies to KRAS/BRAF/PIK3CA mutations, PTEN, or phosphorylated MET expression. PR or SD of ≥ 16 weeks were seen for only 11 patients, and median time-to-treatment failure was 7.9 weeks. In our cohort with an extended molecular profiling technique, actionable alterations in CRC were identified for 44%, and 4 out of 10 evaluable patients showed either PR or SD of ≥ 16 weeks. A subgroup with BTC from the MOSCATO-01 trial reported molecular targets in 23 of 34 patients, with 18 receiving matched therapies (53%). The overall response rate and PFS of ≥ 6 months in that study were 33% and 37%, respectively. In line with this observation, we identified targets in 74% of patients with BTC, which further emphasizes that BTC might be the most promising GI cancer subgroup for MTB-guided therapies.
To constantly improve the quality of future recommendations, several issues must be considered. First, the diagnostic era of characterizing tumors has just begun with panel or exome sequencing. Several techniques, such as transcriptome and epigenome analysis or whole-genome and single-cell sequencing, are expected to improve tumor characterization up front. Such a constant evolution will require more specialists in these methods to join academic MTBs, which means a new era of interdisciplinary patient care to bridge the gap between a growing complexity in diagnostic procedures and clinicians at the bedside. Second, recent data suggest that the administration of customized multidrug regimens that target multiple identified molecular alterations or a sequential treatment of standard chemotherapy followed by matched therapies could further improve therapeutic

**FIG 4.** Outcomes of patients treated according to molecular tumor board (MTB) recommendations. (A) Progression-free survival (PFS), defined as the time from start of an MTB-recommended treatment to radiographic progression or death, and (B) overall survival (OS), defined as the time from start of an MTB-recommended treatment to death as a result of any cause, in days. Shown are individual patient identifiers and treatments. (*) Patients who were treated according to identified germline alterations. Kaplan-Meier analysis of (C) PFS and (D) OS in evaluable patients according to best response. The estimation compared patients who reached a partial response (PR) or stable disease (SD) with patients with progressive disease (PD). Tick marks indicate censored data. BTC, biliary tract cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; PC, pancreatic cancer; UGC, upper GI cancer.
success. Recent examples in CRC are PD-L1 and CTLA-4 inhibition in MSI-high cancers\cite{41} or the combination of a BRAF inhibitor, MEK inhibitor, and anti–epidermal growth factor receptor antibody in BRAF V600E mutated cancers.\cite{42}

In conclusion, GI tumors are an important group in the field of personalized medicine. We were able to show that integrating NGS into everyday practice allowed us to identify an unexpectedly high number of germline variants and unique treatment options. Patients with advanced GI cancer who reach disease control with a matched treatment seem to benefit substantially with respect to PFS and OS. The inclusion of additional complex diagnostic procedures and the integration of individualized tumor characterization at an earlier disease stage might substantively enhance the treatment opportunities in everyday clinical practice for these patients in the near future.

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