

Comprehensive Genomic and Transcriptomic Analysis for Guiding Therapeutic Decisions in Patients with Rare Cancers



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ABSTRACT

The clinical relevance of comprehensive molecular analysis in rare cancers is not established. We analyzed the molecular profiles and clinical outcomes of 1,310 patients (rare cancers, 75.5%) enrolled in a prospective observational study by the German Cancer Consortium that applies whole-genome/exome and RNA sequencing to inform the care of adults with incurable cancers. On the basis of 472 single and six composite biomarkers, a cross-institutional molecular tumor board provided evidence-based management recommendations, including diagnostic reevaluation, genetic counseling, and experimental treatment, in 88% of cases. Recommended therapies were administered in 362 of 1,138 patients (31.8%) and resulted in significantly improved overall response and disease control rates (23.9% and 55.3%) compared with previous therapies, translating into a progression-free survival ratio >1.3 in 35.7% of patients. These data demonstrate the benefit of molecular stratification in rare cancers and represent a resource that may promote clinical trial access and drug approvals in this underserved patient population.

SIGNIFICANCE: Rare cancers are difficult to treat; in particular, molecular pathogenesis-oriented medical therapies are often lacking. This study shows that whole-genome/exome and RNA sequencing enables molecularly informed treatments that lead to clinical benefit in a substantial proportion of patients with advanced rare cancers and paves the way for future clinical trials.

See related commentary by Eggermont et al., p. 2677.

INTRODUCTION

Comprehensive molecular profiling can be applied to guide targeted treatment in patients with cancer, a method commonly referred to as precision oncology (1), and retrospective analyses suggested that such biomarker-stratified

strategies are associated with clinical benefit (2–4). Recently, several prospective studies have also investigated the value of precision oncology approaches. Common to these studies is a clinical benefit rate associated with molecularly informed therapy decisions of about one third across tumor types (5–10). In addition, these trials demonstrated that

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the efficacy of certain molecularly informed therapies, such as those targeting BRAF^{V600} mutations (11–14) or NTRK rearrangements (15), is primarily determined by the presence of specific genomic alterations rather than by the histologic entity.

However, in many other cases, a patient's individual treatment response is highly dependent on the tumor type, that is, the context in which the biomarker occurs (12, 16). This circumstance makes it difficult to study the efficacy of many therapies in rare cancers, such as, for example, soft-tissue sarcomas [except gastrointestinal stromal tumor (GIST), for which five approved targeted agents exist], which prevents their use despite known actionable molecular alterations in these diseases. To overcome some of these limitations and accelerate the discovery of effective biomarker–drug combinations, including their tissue dependence, novel adaptive study designs have been implemented (8). Rapid accrual into such trials either relies on the capacity of single, high-volume cancer centers or requires multi-institutional cooperation (17). Another challenge that has stood in the way of implementing precision oncology approaches for rare cancers is that negative evidence for drug efficacy in unstratified clinical trials that used a “one-size-fits-all” strategy has underestimated the value of therapies and discouraged further drug development efforts. This problem is also exemplified by the early negative evaluation of olaparib in ovarian cancer (18). Thus, the efficacy of most approved targeted drugs remains unexplored in rare cancers (19–21).

The complexity and heterogeneity of solid tumors call for the comprehensive assessment of variables that influence the effectiveness of targeted therapies. In addition to the respective predictive biomarker(s), these variables include accompanying molecular changes, the histologic and possibly molecular tumor type, pharmacokinetics, and pharmacogenomic parameters. Improvements in the precision of targeted cancer therapies, primarily in the positive predictive value of biomarker–drug combinations, can be achieved by a detailed understanding of an individual patient's tumor. This insight has led to a growing number of precision oncology platforms adopting comprehensive methods to determine

the molecular profiles of solid tumors (5, 8, 9, 22). However, widespread access for patients with rare cancers to a clinical platform that uses broad and multilayered molecular characterization to inform therapeutic decisions and implement them in a coherent clinical workflow has not yet been achieved.

Another difficulty for molecularly stratified pan-cancer studies is the rigidity of current clinical trial endpoints. These were defined for large, uniformly conducted phase III studies but often fail in personalized oncology, especially in the analysis of retrospective data and small patient cohorts, which has hindered therapeutic progress in patients with rare cancers (8, 15). As an encouraging development, novel clinical endpoints such as the progression-free survival ratio (PFSr) (23) and new study designs using retrospective real-world data (24) have been proposed in the recent past to address this dilemma.

To investigate the clinical value of whole-genome/whole-exome sequencing (WGS/WES) and RNA sequencing (RNA-seq) in younger adults with advanced cancers across histologies and in patients with advanced rare cancers across age groups, the German Cancer Consortium (DKTK) has established MASTER (Molecularly Aided Stratification for Tumor Eradication Research), a multicenter, prospective observational study based on a common workflow for diagnostics, therapeutic decision-making, and structured follow-up. We present the molecular and clinical results for the first 1,310 patients enrolled, about three quarters of whom represent rare cancers, showing that comprehensive genomic and transcriptomic analysis provides diagnostic and therapeutic benefits for this prognostically unfavorable patient population.

RESULTS

Comprehensive Genomic and Transcriptomic Analysis of Patients with Rare Cancers

Between March 2012 and November 2018, we registered 2,340 patients for potential participation in the MASTER trial, which was designed for adults with advanced cancer across histologies who are younger than 51 and patients with rare tumors, including rare subtypes of more common entities, regardless of age. Until the data cutoff on November 21, 2018, we had analyzed—using a standardized workflow (Fig. 1A) for selection and consenting of patients, sample processing, molecular profiling, bioinformatic analysis, and technical validation of potentially actionable findings (25, 26)—1,484 patients who met the inclusion criteria, and discussed 1,310 (88.3%) in a multidisciplinary molecular tumor board (MTB; Fig. 1B). Frequent reasons for exclusion are shown in Fig. 1B. Relevant subgroups of patients considered in this study are summarized in Supplementary Table S1.

The 1,310 cases discussed in the MTB, which takes place twice a week as a video conference to enable external partners to participate, included 31 cases (2.4%) and two cases (0.2%) that were analyzed twice or three times, respectively, as well as 107 patients (8.2%) who died shortly after the molecular analysis. All patients had incurable disease, and 1,096 (83.7%) had received at least one prior systemic treatment (median, 3; range, 1–15). The median age of patients at the time their cases were discussed in the MTB was 45 years (range, 16–82; Table 1; Supplementary Fig. S1A), and their median

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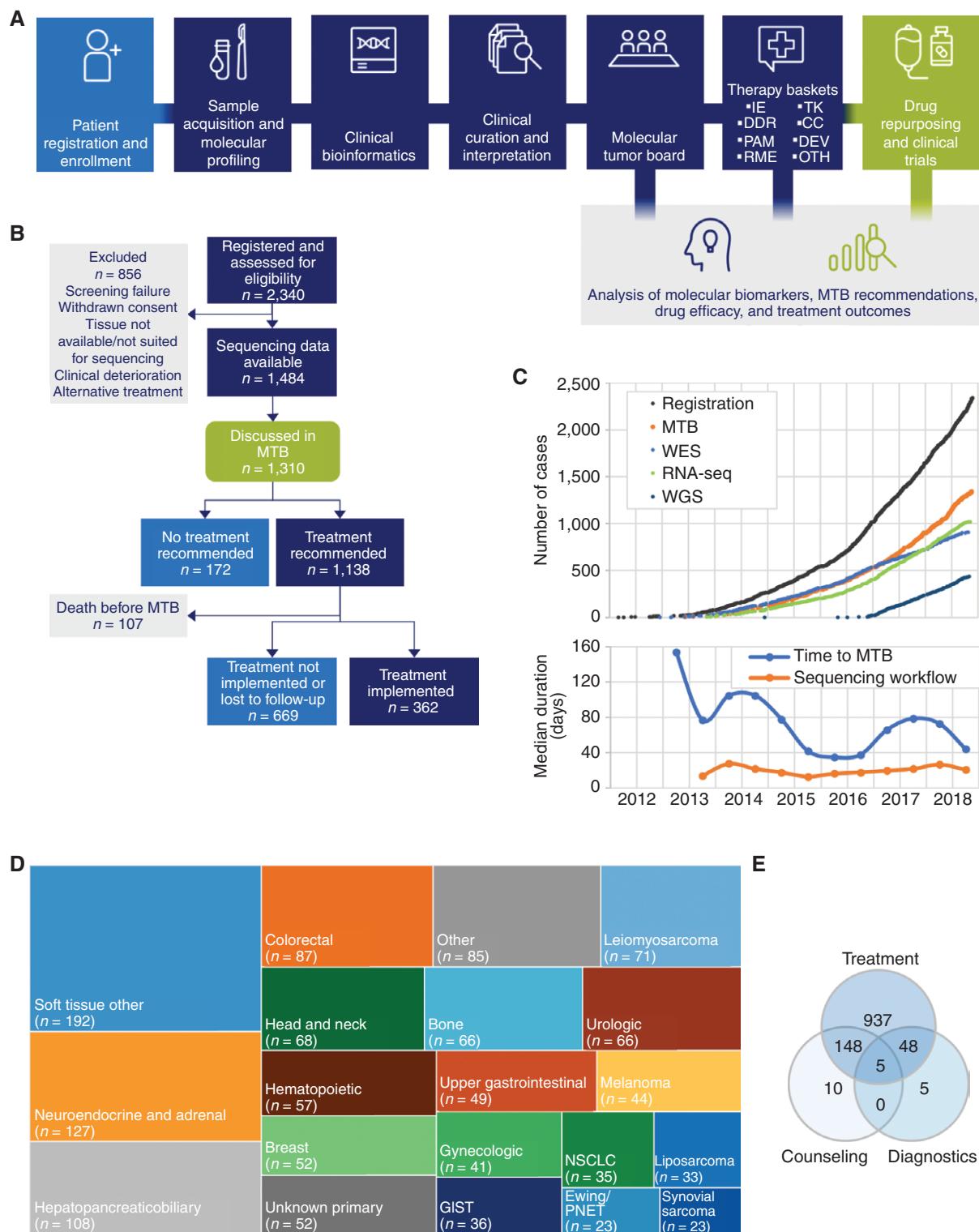


Figure 1. Workflow and patient accrual. **A**, Standardized workflow for patient registration and enrollment, sample acquisition and molecular profiling, bioinformatic analysis, molecular tumor board (MTB) discussion, assignment to treatment baskets (IE, immune evasion; DDR, DNA damage repair; PAM, PI3K-AKT-mTOR; RME, RAF-MEK-ERK; TK, tyrosine kinases; CC, cell cycle; DEV, developmental regulation; OTH, other), and clinical outcome analysis. **B**, Screening, recruitment, and analysis populations. **C**, Patient registrations, molecular analyses, MTB discussions (top; WES, whole-exome sequencing; WGS, whole-genome sequencing; RNA-seq, RNA sequencing), and turnaround times (bottom; blue, median time from sample receipt to MTB; orange, median duration of sample processing, sequencing, and bioinformatic analysis) over time. **D**, Patient subcohorts (GIST, gastrointestinal stromal tumor; NSCLC, non-small cell lung cancer; PNET, primitive neuroectodermal tumor). **E**, Number of patients with a recommendation for experimental therapy, diagnostic reevaluation, or genetic counseling.

Table 1. Characteristics of patients discussed in the molecular tumor board

Cohort	n	Median age (years)		Sex (n)		ICD-O-3 codes (n)	Rare cancer (%)	Median number of prior systemic therapies	Median OS (months) ^a
		F	M	F	M				
All	1,310	44.9	46.1	639	671	221	75.5	3	10.4 (9.2-11.6)
Bone	66	33.3	39.8	20	46	12	100	1	15.4 (11.3-19.1)
Breast	52	42.7	55.0	50	2	6	9.6	2	7.2 (4.9-15.8)
Colorectal	87	42.7	45.7	41	46	13	2.3	3	4.5 (3.4-7.3)
Ewing sarcoma/PNET	23	32.7	31.1	10	13	3	100	3	7.3 (3.7-NA)
GIST	36	46.6	49.1	11	25	1	100	2	22.8 (12.0-NA)
Gynecologic	42	49.4	-	41	0	19	85.7	3	7.3 (4.9-18.7)
Head and neck	68	46.8	49.0	25	43	16	100	1	12.1 (10.2-NA)
Hematopoietic	57	46.1	47.1	22	35	28	100	3	9.1 (5.0-NA)
Hepatopancreaticobiliary	108	45.2	46.7	54	54	18	57.4	1	9.7 (6.9-13.6)
Leiomyosarcoma	70	49.0	56.0	50	20	1	100	3	12.5 (9.2-18.5)
Liposarcoma	33	44.8	53.0	18	15	7	100	1	20.6 (11.4-NA)
Melanoma	44	45.8	41.5	21	23	10	31.8	3	7.7 (4.6-11.9)
Neuroendocrine/adrenal	127	42.2	49.7	59	68	14	100	3	10.3 (6.1-22.7)
NSCLC	35	44.7	47.7	23	12	6	17.1	2	11.6 (6.8-22.4)
Other	85	45.8	45.7	34	51	49	88.2	2	13.8 (9.7-20.8)
Soft tissue other	192	40.6	42.9	81	111	39	100	2	11.3 (9.1-14.0)
Synovial sarcoma	19	42.1	32.1	8	11	4	100	4	10.5 (3.7-NA)
Unknown primary	52	48.5	48.1	32	20	19	100	2	10.4 (5.9-15.4)
Upper gastrointestinal	49	39.2	43.8	20	29	9	32.7	2	6.4 (2.3-14.7)
Urologic	65	47.4	45.3	19	47	22	46.2	2	10.8 (5.0-23.2)

Abbreviations: ICD-O, International Classification of Diseases for Oncology; OS, overall survival; PNET, primitive neuroectodermal tumor; GIST, gastrointestinal stromal tumor; NSCLC, non-small cell lung cancer; NA, not applicable.

^aNumbers in parentheses are 95% confidence intervals.

overall survival from the time of the MTB was 10.4 months (659 deaths during follow-up; Table 1; Supplementary Fig. S1B), with a median follow-up for the entire cohort of 18.4 months.

The MASTER program originated from NCT Heidelberg in 2012 and was extended to all partner sites of the DKTK, representing 10 comprehensive cancer centers, and their respective catchment areas from March 2016. As a result, the frequency of patient registrations, molecular analyses, and MTB discussions increased steadily (Fig. 1C). In addition, the spectrum of molecular techniques evolved in that we initiated RNA-seq in all patients since November 2013 and WES has been replaced by WGS in a substantial proportion of patients since November 2016 (Fig. 1C). By implementing standardized and largely automated core processes, we achieved a stable turnaround time for sample processing, sequencing, and bioinformatic analysis, with a median of 21 days. In contrast, the other parts of the workflow, such as the initial histologic review of the tissue and the clinical evaluation of the molecular data, and thus the time from sample receipt to MTB, were subject to fluctuations. In particular, the overall turnaround

time increased with the program's expansion to all DKTK sites in 2016. However, this delay due to increased case volume and more complex logistics was overcome as the study progressed, resulting in a median overall turnaround time of 44 days at the end of the observation period in November 2018 (Fig. 1C).

In line with the conception of MASTER as a cross-entity study, the cohort included patients representing 81 ICD-10 topography and 221 ICD-O-3 morphology codes. Because of this diversity, we categorized the cases into larger subcohorts based on histologic and clinical characteristics (Fig. 1D). The most common diagnosis was soft-tissue sarcoma ($n = 351$; 26.8%), followed by neuroendocrine and adrenal tumors ($n = 127$; 9.7%) and hepatopancreaticobiliary tract cancers ($n = 108$; 8.2%). The proportion of rare cancers—defined by an incidence of less than six per 100,000 persons per year in the European population, including rare subtypes of more common entities (<https://www.rarecancerseurope.org>)—in the entire cohort was 75.5%, and two independent tumors were examined in one patient.

Identification of Clinically Actionable Genomic and Transcriptomic Biomarkers

Of the 1,310 patients discussed in the MTB, 1,153 (88.0%) received one or more molecularly informed recommendations (median, 3; range, 1–11) for clinical management, which were related to three areas: diagnostic reevaluation, genetic counseling, and experimental therapy (Fig. 1E). The rapid identification of clinically actionable genomic and transcriptomic biomarkers as the basis for MTB recommendations, as well as the visualization of the molecular data in an automatically generated MTB presentation, were achieved through a proprietary bioinformatics workflow for extracting clinically relevant information from WGS/WES and RNA-seq data. This workflow included (i) alignment, (ii) calling of single-nucleotide variants (SNV), small insertions and deletions (indels), somatic copy-number alterations (sCNA), structural variants (SV), and gene fusions, (iii) evaluation of gene expression, and (iv) detection of potentially actionable molecular changes from a curated list adapted from available precision oncology databases and continuously expanded throughout the study (Supplementary Table S2). To be able to include sCNAs and RNA expression, for which in most cases no therapeutically relevant cutoffs have been established, in decision-making by the MTB, these continuous variables were assigned categorical labels (gain or loss of DNA copies, high or low RNA expression) that allowed them to be considered or rejected as predictive parameters for a specific treatment.

In addition to evaluating alterations of single genes or genomic regions, we took advantage of the fact that WGS/WES and RNA-seq can capture composite biomarkers that provide information about sensitivity to homologous recombination deficiency (HRD)-directed therapies, such as PARP inhibition, or immune checkpoint blockade. Genomic measures of HRD included mutational signatures (Supplementary Fig. S2), in particular the single-base substitution signature 3 (SBS3), which is highly prevalent in HRD-driven cancers and strongly associated with *BRCA1* or *BRCA2* loss (27), and the HRD score based on loss of heterozygosity (LOH) and the numbers of large-scale state transitions (LST) and telomeric allelic imbalances (TAI; refs. 28–30). To predict benefit from CTLA4, PD-1, or PD-L1 blockade, we reported (i) tumor mutational burden (TMB) with a cutoff of 100 nonsynonymous SNVs and indels, which corresponded to the 79.5% percentile, (ii) microsatellite instability (MSI), (iii) aneuploidy, and (iv) viral integrations (31–34).

In the course of the study, we used a total of 472 genes and six composite biomarkers for therapeutic decisions, which were assigned to seven biomarker baskets based on the cellular pathways or processes involved [tyrosine kinases (TK), PI3K-AKT-mTOR (PAM), RAF-MEK-ERK (RME), cell cycle (CC), developmental regulation (DEV), DNA damage repair (DDR), and immune evasion (IE)]. Genes that were not associated with these pathways or processes were placed in a basket labeled “other” (OTH; Fig. 1A). We also determined for each biomarker whether it was associated with a loss of function (LOF) or a gain of function (GOF) regarding the affected cellular pathways or processes. Clinically actionable LOF genes included *CDKN2A/B*, *PTEN*, *NFI*, *STK11*, and genes

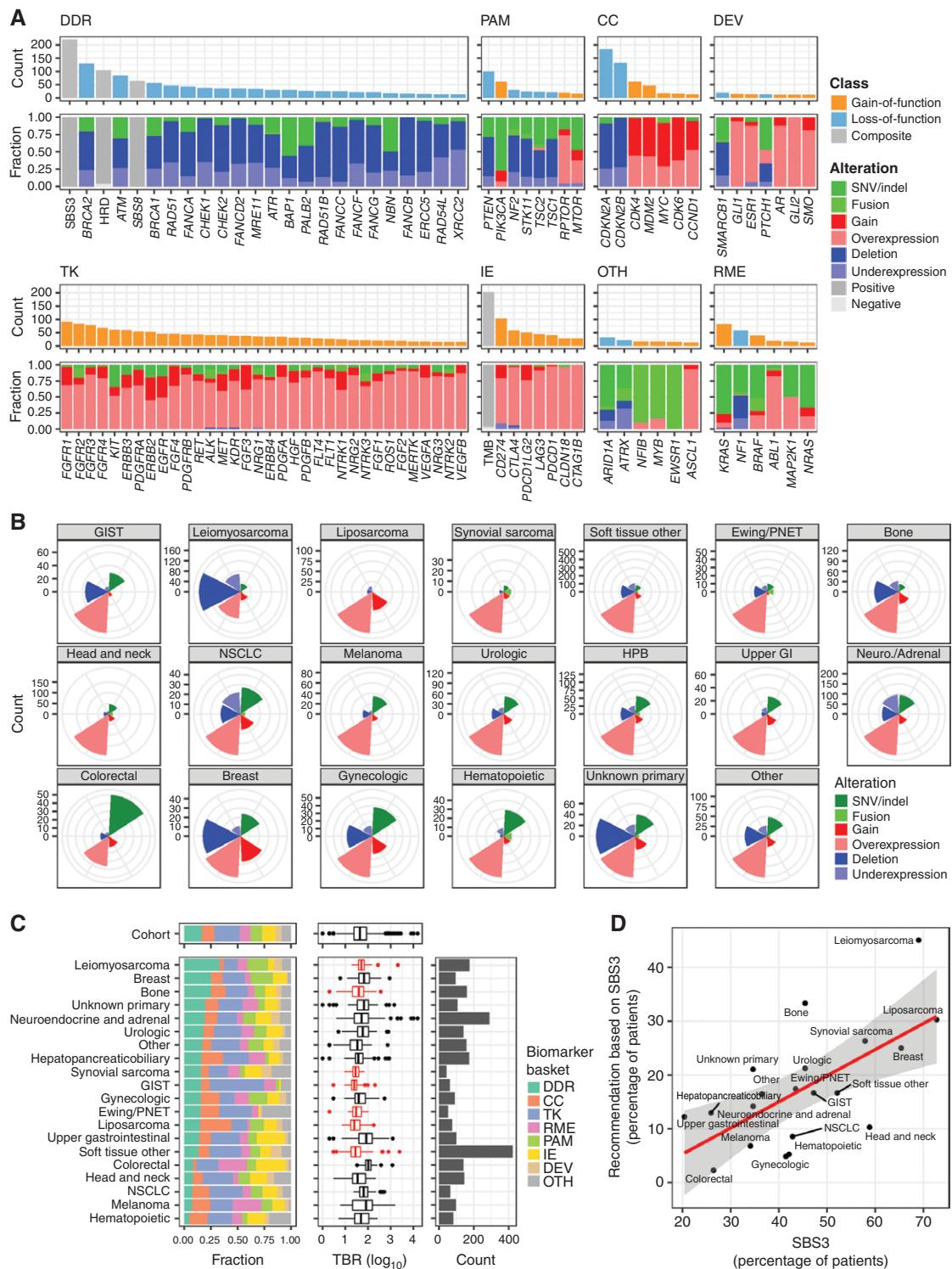
involved in homologous recombination DNA repair, such as *BRCA1*, *BRCA2*, *ATM*, *CHEK2*, and *RAD51*. Other tumor suppressor genes often affected by LOF mutations were only occasionally used for therapeutic decisions, such as *TP53* and *RB1* proficiency as a prerequisite for therapy with MDM2 or CDK4/6 inhibitors, respectively. The most frequent GOF biomarkers included genes encoding members of the FGFR, PDGFR, VEGFR, and ERBB receptor tyrosine kinase families and their respective ligands, components of the MAPK signaling pathway, and immune checkpoint molecules such as CD274 (PD-L1) and CTLA4 (Fig. 2A).

We also evaluated the clinically relevant information that comprehensive genomic and transcriptomic analysis can provide in addition to more focused molecular diagnostic approaches. To this end, we examined the types of alterations underlying the biomarkers identified in the 908 patients with available WGS/WES and RNA-seq data among the 1,138 cases that received a recommendation for experimental therapy (Fig. 1E). In these patients, the majority of biomarkers (47.1%) were based on RNA expression, followed by sCNAs (25.5%), SNVs/indels (13.2%), composite biomarkers (8.7%), and gene fusions (5.3%). In the 230 patients without RNA-seq data, sCNAs predominated (46.6%), followed by SNVs/indels (32.0%), composite biomarkers (16.6%), and gene fusions (3.7%; Supplementary Fig. S3). Because of the high frequency of expression-based biomarkers, we examined the added value of transcriptomic analysis in more detail. We observed that 698 of 908 patients (76.9%) with RNA-seq data had at least one biomarker that was based on gene expression and that the MTB recommendations for these patients considered more biomarkers (median, 2 vs. 1; $P < 0.0001$, Wilcoxon rank-sum test). In addition to the detection of known and previously unrecognized gene fusions (35–37), the data were used to verify intratumoral expression of specific SNVs and indels and to evaluate the transcriptional effects of gene amplifications and deletions.

Distribution of Clinically Actionable Biomarkers across Baskets and Tumor Entities

We next examined the distribution of clinically actionable biomarkers across the different baskets and histologic entities. With regard to the former, we observed that most biomarkers based on which treatments were recommended fell into the DDR (26.8%), TK (25.8%), and IE (10.7%) baskets (Fig. 2A). LOF biomarkers were enriched in the DDR and PAM baskets and concerned, for example, alterations of *BRCA* family members and *PTEN*, *NF2*, or *STK11*, respectively. GOF biomarkers mainly occurred in the TK and IE baskets, affecting, for example, various receptor tyrosine kinases and CD274 (PD-L1) or CTLA4, respectively (Fig. 2A). RNA-based biomarkers were found in the context of several baskets. In the TK category, the availability of RNA-seq data increased the number of treatment recommendations. In contrast, the consideration of gene expression in the PAM basket eliminated several possible recommendations, because many biomarkers in these categories were deletions (Fig. 2A) and, based on unimpaired gene expression, sCNAs that were unlikely to affect gene function could be identified (Supplementary Fig. S4A).

With regard to the occurrence of clinically actionable biomarkers in the different patient subcohorts, we observed an



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Figure 2. Biomarkers used for recommendations by the molecular tumor board. **A**, Incidence of the 100 most frequent biomarkers and relative proportions of associated biomarker classes and alteration types, sorted by basket (DDR, DNA damage repair; PAM, PI3K-AKT-mTOR; CC, cell cycle; DEV, developmental regulation; TK, tyrosine kinases; IE, immune evasion; OTH, other; RME, RAF-MEK-ERK; NFE, NF1B). **B**, Relative contribution of different alteration types to the biomarkers detected in different patient subcohorts (GIST, gastrointestinal stromal tumor; PNET, primitive neuroectodermal tumor; NSCLC, non-small cell lung cancer; HPB, hepatopancreaticobiliary; GI, gastrointestinal; Neuro., neuroendocrine); SNV, single-nucleotide variant; indel, small insertion and deletion. **C**, Biomarkers in the different patient subcohorts [left, according to basket; middle, TMB (box plots, median and the first and third quartiles; whiskers, 1.5-fold of the interquartile range; points, outliers; sarcoma subcohorts indicated in red); right, total number of cases]. **D**, Smoothed linear model of the relationship between the detection of the single-base substitution signature 3 (SBS3) and its use for treatment recommendations by the molecular tumor board (shaded area, 95% confidence interval).

uneven distribution. For example, there was an enrichment of molecular drug targets from the CC basket in well-differentiated/dedifferentiated liposarcomas, which are characterized by amplification of *CDK4* and *MDM2* (Fig. 2B and C). In contrast, MTB decisions did not lead to the enrichment of established actionable biomarkers in other histologic entities, such as GIST (activating SNVs or indels in *KIT*) and non-small cell lung cancer (NSCLC; *ALK* or *ROS1* fusions), which reflected that patients with these diseases were only included in the MASTER trial if they had previously received standard therapy following current guidelines (Fig. 2B). Deletions and reduced gene expression as predictive biomarkers were particularly enriched in patients with breast cancer, leiomyosarcoma, bone sarcoma, and carcinoma of unknown primary site (CUP; Fig. 2B). This distribution correlated with the predominance of biomarkers from the DDR basket in these entities (Fig. 2C), particularly indicators of HRD such as inactivation of the *BRCA1* and *BRCA2* tumor suppressor genes, which were also detectable in other subcohorts not typically associated with DDR alterations, for example, in patients with neuroendocrine neoplasms (NEN) or GIST (Fig. 2B and C). As supporting evidence for HRD, other parameters were routinely determined, such as the SBS3, whose prevalence in the individual subcohorts correlated with the frequency of MTB recommendations based on this biomarker (Fig. 2D), indicating its possible clinical utility. Composite biomarkers linked to the IE basket were detected in all subcohorts (Fig. 2C). For example, we identified subgroups of cases in several entities, such as NEN, adrenal tumors, and leiomyosarcoma, that had a high TMB—the most common molecular feature in this category—Independent of MSI and were, therefore, candidates for therapy with immune checkpoint inhibitors (Fig. 2C). Elevated RNA expression was found in all subcohorts as predictive molecular alteration (Fig. 2B), especially RNA-based biomarkers from the IE and TK baskets. In specific subcohorts, the availability of RNA-seq data was associated with MTB recommendations from a larger number of treatment baskets (Supplementary Fig. S4B). For example, in patients with synovial sarcoma or leiomyosarcoma, treatments from the IE basket were only recommended on the basis of RNA data.

Importantly, our broad diagnostic approach allowed us to identify clinically actionable biomarkers in addition to molecular alterations with established diagnostic or therapeutic relevance. For example, in patients with GIST or NSCLC and acquired resistance to tyrosine kinase inhibitors, we detected biomarkers from the TK basket that were not among the primary molecular drivers for these diseases and comprised, for example, alterations of FGFR and MET signaling, respectively (Fig. 2C). We also addressed and reported entity-independent biomarkers such as KRAS^{G12D} mutations, which cannot be targeted directly but often prompted the recommendation of combination therapies aimed at other pathway components, for example, MEK1/2, and possible synthetic lethal interactions to counteract innate or acquired resistance mechanisms.

Recommendations by the Molecular Tumor Board

As noted above, 1,153 of 1,310 patients (88.0%) discussed in the MTB received at least one molecularly informed recommendation for clinical management (Fig. 1E). In 58 of 1,310 cases (4.4%), the molecular findings prompted us to recom-

mend histologic and clinical reevaluation (Supplementary Table S3); this was particularly common in patients with CUP ($n = 21$) and soft-tissue sarcoma ($n = 27$; Fig. 3A). In 156 of 1,075 patients (14.2%), we identified pathogenic or likely pathogenic germline variants based on a preselected panel of autosomal dominant or recessive cancer predisposition genes (Supplementary Table S4), which we integrated into the routine workflow in August 2015. These variants triggered recommendations for independent validation and genetic counseling (Fig. 3B) and were also considered for therapeutic decisions, such as in the case of inactivating *BRCA1/2* mutations or other changes associated with HRD (Supplementary Table S4). In seven additional patients, genetic counseling was recommended on the basis of clinical information, for example, early age of disease onset and entity. Overall, the molecular rationale for treatment recommendations included germline variants as relevant biomarkers in 116 of 1,310 patients (8.9%; Supplementary Table S5).

In 1,138 of 1,310 patients (86.9%) discussed in the MTB, the data provided a rationale for experimental therapy beyond current guidelines. Treatment recommendations were assigned to the same eight baskets as the individual biomarkers, but based on the mechanism of action of the recommended drug(s) rather than the functional consequences of the respective genetic alterations (Figs. 2C and 3C). Differences in the categorization of biomarkers and treatment recommendations reflected the nonunique relationship between biomarkers and possible therapeutic agents. For example, molecular changes that were assigned to a specific biomarker basket due to the primary function of the affected gene (e.g., an inactivating *NF1* mutation categorized into the RME basket) could inform the selection of drugs from several treatment baskets (e.g., of a MEK inhibitor from the RME basket or an mTOR inhibitor from the PAM basket). While biomarkers and treatment recommendations from the CC ($R^2 = 0.96$), DDR ($R^2 = 0.98$), IE ($R^2 = 0.98$), PAM ($R^2 = 0.96$), RME ($R^2 = 0.95$), and TK ($R^2 = 0.99$) baskets were highly correlated, we observed less congruence for the DEV ($R^2 = 0.84$) and OTH ($R^2 = 0.81$) baskets. This discrepancy reflected the lack of access to therapies matched to biomarkers from the DEV basket, such as NOTCH or WNT signaling inhibitors, and the heterogeneity of the OTH basket, which included recommendations for antihormonal therapies and targeted agents, for example, IDH1 and EZH2 inhibitors, as well as suggestions for conventional chemotherapy, which could not be assigned to a distinct treatment basket, but could be selected according to biomarkers (Supplementary Fig. S5).

To weight the decisions of the MTB and facilitate clinical implementation, we took two steps. First, each recommendation was assigned a molecular evidence level according to a scheme for assessing and classifying predictive biomarker-drug relationships developed in the NCT/DKTK MASTER network (Supplementary Table S6; ref. 38). We observed that recommendations based on clinical data collected in the same histologic entity (NCT/DKTK levels m1A–C) could be made in 200 of 1,138 patients (17.6%) and that only 8.2% of all recommendations fell into this group, reflecting the lack of molecularly stratified clinical trials in patients with rare cancers (Fig. 3C). In contrast, 46.5% of recommendations were based on corollary evidence obtained in other entities

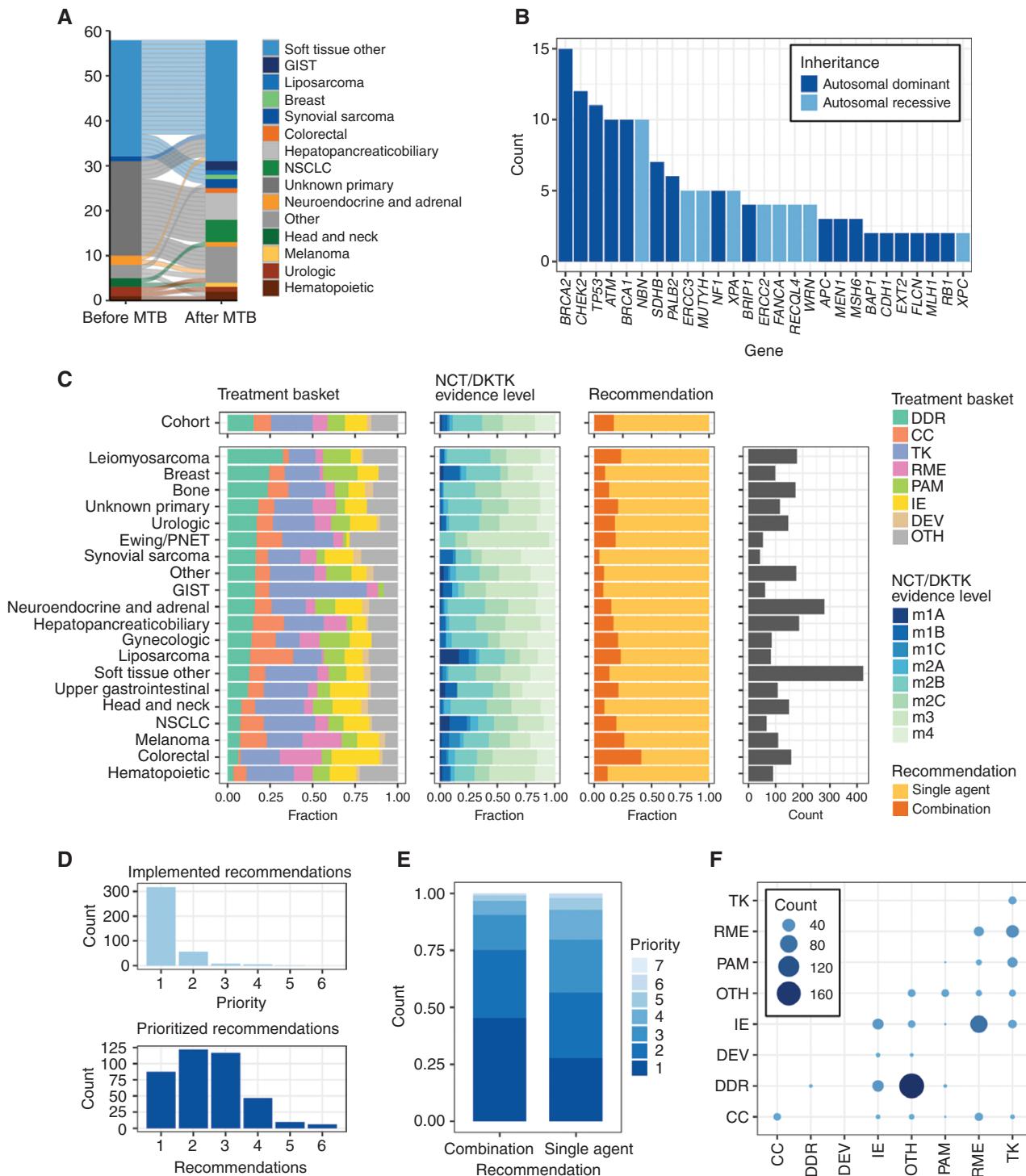


Figure 3. Recommendations by the MTB. **A**, Patients with a recommendation for diagnostic reevaluation based on genomic and transcriptomic analysis (left, original subcohort; right, subcohort suggested by the MTB). GIST, gastrointestinal stromal tumor; NSCLC, non-small cell lung cancer. **B**, Cancer predisposition genes with pathogenic germline variants in at least two patients. **C**, Treatment recommendations in the different patient subcohorts (left, according to basket; second from left, NCT/DKTK evidence levels; second from right, combination vs. single-agent therapy; right, total number of cases). **D**, Implementation of treatments recommended by the MTB according to priority level (top, priority levels of implemented recommendations; bottom, total number of recommendations per case in patients who received molecularly guided therapy). **E**, Priority levels of combination versus single-agent therapies recommended by the MTB. **F**, Number of combination therapies recommended by the MTB according to the corresponding baskets (TK, tyrosine kinases; RME, RAF-MEK-ERK; PAM, PI3K-AKT-mTOR; OTH, other; IE, immune evasion; DEV, developmental regulation; DDR, DNA damage repair; CC, cell cycle).

(NCT/DKTK levels m2A–C), 28.2% were based on preclinical data (NCT/DKTK level m3), and 17.1% were informed by theoretical considerations alone (NCT/DKTK level m4; Fig. 3C). We also retrospectively applied the European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets (ESCAT; ref. 39). We found that 4.6% and 3.7% of recommendations fell into ESCAT tiers I and II, which correspond to NCT/DKTK levels m1A–C (Supplementary Fig. S6A and S6B), 25.8% into ESCAT tier III (defined by clinical benefit demonstrated in other tumor types or for similar molecular targets), 30.8% into ESCAT tier IV (defined by preclinical and *in silico* evidence), 0.7% into ESCAT tier V (defined by evidence supporting co-targeting approaches), and 17.7% into ESCAT tier X, which corresponds to NCT/DKTK level m4. The remaining 16.6% of recommendations could not be assigned an ESCAT tier. Second, we prioritized treatment recommendations based on the integrated consideration of evidence levels, the clinical efficacy of biomarker–drug combinations, relevant patient characteristics (e.g., performance status and response to prior treatments), and drug availability. Of the 1,138 patients whose molecular profiles provided information to guide clinical management, 892 (78.4%) received alternative treatment recommendations. The value of prioritization for clinical decision-making was evident from the fact that among the 362 patients who received experimental therapy, the highest-ranked recommendations were implemented in 83.7% of cases (Fig. 3D).

We also noticed that the median number of treatment recommendations per patient increased over time from one (range, 1–4) in 2014 to three (range, 1–9) in 2018 (Supplementary Fig. S7A). This development related in particular to combination therapies, which may be based on one or more biomarkers. During the entire observation period, most recommendations were based on single agents (82.8%); however, the proportion of patients for whom we recommended at least one combination therapy increased from 5.0% in 2014 to 53.9% in 2018, and we found that combination therapies were frequently prioritized over single agents by the MTB (Fig. 3E; Supplementary Fig. S7B). Combinations were recommended in all subcohorts, especially in patients with colorectal cancer, melanoma, liposarcoma, and leiomyosarcoma (Fig. 3C). The consideration of combination therapies mostly resulted from evidence for the lack of efficacy of single agents in specific histologic contexts, for example, BRAF inhibition in BRAF^{V600}-mutated colorectal cancer (12), and increased with the availability of combinations within molecularly stratified clinical trials, for example, olaparib and trabectedin within the TOP-ART study of the NCT/DKTK MASTER network (ClinicalTrials.gov Identifier NCT03127215). Because of these factors, we most often recommended DDR-directed treatment in conjunction with chemotherapy, followed by approaches assigned to the RME and IE baskets and the TK and RME baskets (Fig. 3F).

Outcome of Molecularly Informed Therapies

As described in the previous section, recommended therapies were administered (within clinical trials, under compassionate use protocols, or, for the most part, off-label) in 362 of 1,138 patients (31.8%; Fig. 4A). Reasons for nonimplementation of MTB decisions included, for example, worsening of a patient's general condition, death before treatment could be given, and

lack of access to or reimbursement of the recommended drug(s). To determine the clinical benefit associated with recommended therapies, the MASTER program involves collecting all medical health records for 24 months from the MTB as part of a structured follow-up. Using the data cutoff on November 21, 2018, we here analyzed 251 patients concerning response to molecularly informed treatment [complete response (CR), partial response (PR), stable disease (SD) for at least eight weeks, mixed response (MR), or progressive disease (PD)] and 300 patients concerning the progression-free survival (PFS) times associated with molecularly informed treatment and the last systemic therapy before molecular analysis (Fig. 4A).

Among 181 patients evaluable for a best response comparison (Fig. 4A; Supplementary Fig. S8A), the overall response rate (ORR; defined as the proportion of patients with CR or PR) and disease control rate (DCR; defined as the proportion of patients with CR, PR, or SD for at least eight weeks) associated with molecularly informed treatment improved from 16.3% to 23.9% and from 46.3% to 55.3%, respectively, compared with the last systemic therapy before comprehensive genomic and transcriptomic analysis (Fig. 4B). Recommendations that resulted in clinically relevant disease control or an MR as an indicator of biological efficacy were distributed across all evidence levels (Fig. 4B). Among the different subcohorts, the ORR was highest in patients with NSCLC, synovial sarcoma, gynecologic cancers, hepatopancreaticobiliary cancers, and CUP (Fig. 4C). To assess whether inhibition of specific signaling pathways or cellular processes was particularly effective across entities, we compared the different treatment baskets. We observed that drugs from seven of the eight treatment baskets elicited objective responses, with the ORR ranging from 29.2% and 27.7% in the IE and OTH baskets, respectively, to 0% in the DEV basket (Fig. 4C).

A common endpoint in precision oncology trials is an intrapatient PFSr, defined as the PFS interval associated with molecularly informed therapy (PFS2) divided by the PFS interval associated with the last prior systemic therapy (PFS1), >1.3 or, in some studies, >1.33 or >1.5 (5–7, 23). Of 300 patients evaluable for this outcome measure (Supplementary Fig. S8B), 107 (35.7%) had a PFSr >1.3 (Fig. 4D). To better represent real-world assessments by physicians, we recently proposed a modified PFSr (mPFSr) that applies clinically relevant PFS1 and PFS2 cutoffs but does not lead to a significant change in the overall proportion of patients who are considered to benefit from molecularly informed therapy (40). Accordingly, there were 115 of 300 patients (38.3%) with an mPFSr >1.3 (Supplementary Fig. S9A). We also examined the relationship between PFSr values and the NCT/DKTK evidence levels or ESCAT tiers assigned to treatments (Fig. 4E and F; Supplementary Fig. S9B and S9C). While the proportion of therapies associated with a PFSr >1.3 was highest for MTB recommendations that were based on NCT/DKTK evidence levels m1A–C (55%–72%) or ESCAT tiers I–II (25%–83%), a clinically meaningful proportion of PFSr values >1.3 also resulted from treatments associated with NCT/DKTK evidence levels m2A–C (21%–36%) and ESCAT tiers IIIA–B (34%–38%), as well as from recommendations based on preclinical data or biological rationale (NCT/DKTK evidence levels m3–4; 34%–36%).

The cohort of 300 patients with PFSr data also enabled us to determine in which histologic entities molecularly informed

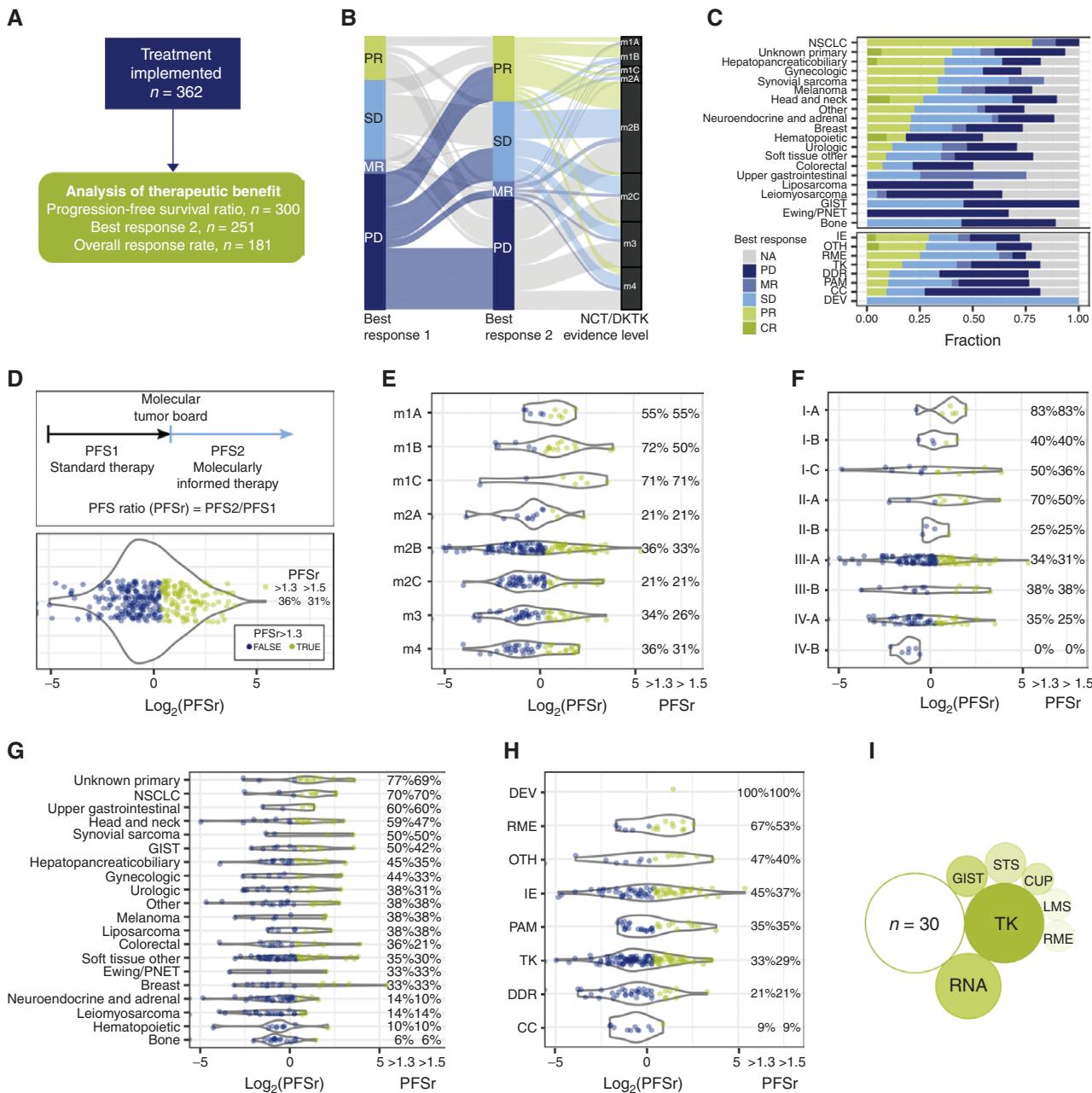


Figure 4. Outcome of molecularly informed therapies. **A**, Patients available for analysis of clinical benefit from molecularly informed treatment. **B**, Best response associated with the last systemic therapy before genomic and transcriptomic analysis (BR1) versus best response associated with molecularly informed therapy (BR2) and NCT/DKTK evidence levels assigned to the treatment recommendation underlying BR2. PR, partial response; SD, stable disease; MR, mixed response; PD, progressive disease. **C**, Distribution of BR2 in the different patient subcohorts (top) and treatment baskets (bottom), ranked by overall response rate. **D**, Distribution of PFSr values in the NCT/DKTK MASTER cohort. PFS1, progression-free survival interval associated with the last systemic therapy before genomic and transcriptomic analysis; PFS2, progression-free survival interval associated with molecularly informed therapy. Green circles, patients with a PFSr > 1.3 . **E**, Distribution of PFSr values in the NCT/DKTK MASTER cohort according to NCT/DKTK evidence levels. Green circles, patients with a PFSr > 1.3 . **F**, Distribution of PFSr values in the NCT/DKTK MASTER cohort according to European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets tiers. Green circles, patients with a PFSr > 1.3 . **G**, Distribution of PFSr values in the NCT/DKTK MASTER cohort according to patient subcohort. Green circles, patients with a PFSr > 1.3 . **H**, Distribution of PFSr values in the NCT/DKTK MASTER cohort according to treatment basket. Green circles, patients with a PFSr > 1.3 . **I**, Representation of factors, weighted by size and color of the respective circle, associated with exceptional clinical benefit (PFSr > 1.3 or CR/PR as BR2 after PD as BR1, achieved by implementing treatment recommendations based on NCT/DKTK evidence levels m3–4). TK, tyrosine kinase inhibitor treatment; RNA, RNA-based biomarker; GIST, gastrointestinal stromal tumor; STS, soft-tissue sarcoma; CUP, carcinoma of unknown primary site; LMS, leiomyosarcoma; RME, RAF-MEK-ERK basket.

therapies were particularly effective. In 16 of the 20 subcohorts defined on the basis of histologic and clinical characteristics (Fig. 1D), more than one third of the patients achieved a PFS_r >1.3. Most successful implementations were seen in patients with carcinomas of the upper gastrointestinal tract, CUP, NSCLC, and hepatopancreaticobiliary cancers. In the large and heterogeneous soft-tissue sarcoma subcohort, 35% of patients had a PFS_r >1.3, and specific subtypes with sufficient case numbers for a separate evaluation, such as synovial sarcoma and GIST, were among the entities associated with the most significant therapeutic benefit. In contrast, genome- or transcriptome-directed therapies for patients with bone sarcomas were mostly ineffective (Fig. 4G; Supplementary Fig. S9D). When measuring the PFS_r across treatment baskets, we noticed that the highest rates of therapeutic success were associated with drugs targeting the RAF-MEK-ERK or PI3K-AKT-mTOR pathways, immune checkpoint inhibitors, and a group of diverse compounds assigned to the OTH basket, which included androgen receptor antagonists, EZH2 inhibitors, and IDH1 inhibitors (two patients each). Similar to the response analysis, drugs from the DDR and CC baskets were associated with the lowest probability of a PFS_r >1.3 (Fig. 4H; Supplementary Fig. S9E). Exploratory analysis suggested that a PFS_r >1.3 was associated with significantly improved overall survival ($P = 0.00027$, log-rank test), supporting its use as a surrogate outcome measure (Supplementary Fig. S10).

Finally, we scrutinized the 30 patients in whom a response or PFS_r >1.3 was achieved by implementing treatment recommendations that were based on NCT/DKTK evidence levels m3–4. These favorable cases occurred in various entities, including GIST, soft-tissue sarcoma, and CUP, and were associated with RNA-based biomarkers and mainly tyrosine kinase inhibitor treatments (Fig. 4I; Supplementary Table S7).

DISCUSSION

Our evaluation of MASTER, an ongoing multicenter observational study by the DKTK, demonstrates the feasibility and clinical utility of a structured precision oncology workflow in patients with rare cancers, a population underrepresented in previous studies. An important ambition of MASTER is the systematic application of comprehensive molecular profiling based on WGS/WES and RNA-seq to improve the clinical care of patients with a spectrum of diseases whose pathogenesis is often incompletely understood and for whom few treatment options are available. One challenge is to provide such diagnostics with sufficiently high throughput and within a clinically relevant timeframe. We experienced that a semiautomated core workflow for sample processing, WGS/WES and RNA-seq, and bioinformatic analysis can be completed in three weeks, whereas time-consuming manual steps such as histologic evaluation, biological curation, and clinical interpretation require continuous optimization. In the field of rare cancers, the situation is further complicated by the fact that cross-institutional cooperation is required to recruit sufficient patient numbers, while at the same time, a uniform and standardized workflow for molecular analysis must be ensured. We have addressed this by dividing the screening and selection of patients among

11 comprehensive cancer centers that are members of the DKTK and combining this decentralized element with a shared, centrally coordinated workflow for tissue processing, molecular profiling, and bioinformatics. This dual strategy enables patients throughout Germany to access the MASTER program and integrates all levels of cancer patient care, from university hospitals to oncologists in private practice, with more than 100 partners to date; however, the decentralized approach is inevitably associated with a sizable dropout rate during the screening process. We anticipate that the importance of regional, national, or even international precision oncology networks will increase further to provide access to molecularly informed therapies for the largest possible proportion of patients with cancer. Recent examples are the Drug Rediscovery protocol in the Netherlands (8) and the precision oncology workflow established within the Cancer Core Europe consortium (41).

The systematic application of comprehensive molecular analyses has the potential to substantially broaden the spectrum of molecular alterations that can be used to guide clinical management in patients with rare cancers. Particularly promising in this respect are the use of RNA-seq and the detection of composite biomarkers. Current precision oncology practice is primarily based on the analysis of DNA-sequencing data. Routine assessment of gene expression profiles is the logical next step, and recent efforts have led to the development of an RNA-based score to match individual patients with targeted therapies (6). Our data indicate four particularly informative aspects of RNA-seq analyses, that is, (i) the detection of gene fusions, (ii) the verification of intratumoral expression of SNVs and indels, (iii) the evaluation of transcriptional effects of gene amplifications and deletions, and (iv) the diagnostic classification of unclear disease patterns. A challenge for any RNA analysis is that for most rare cancers, it is impossible to obtain matched normal controls to take into account the tissue background because the respective cellular origin is unknown. To circumvent this issue, we applied a rank-based pan-cancer methodology; however, the increasing use of RNA-seq and other emerging technologies in a clinical setting will undoubtedly require additional strategies and standardization. We are confident that cross-institutional collaboration in larger consortia will be valuable in this respect as well. Furthermore, the clinical potential of RNA analysis is far from exhausted. Promising applications include estimating targetable protein and pathway activities and related approaches that might have even more power for predicting cancer cell vulnerabilities than genomics (42–46). The importance of composite biomarkers can be illustrated by the molecular correlates of HRD. These are of clinical interest because they indicate sensitivity to PARP inhibitors. However, some studies suggest that more patients than those with inactivating mutations in “classical” genes such as *BRCA1* and *BRCA2* benefit from these drugs and that the concept of “BRCAness” as a therapeutic liability might extend beyond breast, ovarian, and prostate cancers (47). Therefore, we applied a WGS/WES-based multicomponent biomarker to identify the genomic imprints of HRD in rare cancers, the clinical value of which is currently being investigated in a histology-independent clinical trial (ClinicalTrials.gov Identifier NCT03127215). It is also apparent that predicting the response to immune

checkpoint inhibitors requires considering numerous parameters beyond PD-L1 expression and TMB, whose value as singular markers across entities has been challenging to determine. In addition, the number of putative predictors of response and resistance to these agents is rapidly increasing (48, 49); thus, the development of robust biomarkers within registry and, most importantly, interventional clinical trials will benefit from sufficiently broad diagnostic approaches such as the one employed in NCT/DKTK MASTER, which have been shown to be well-suited for early adoption of investigational biomarkers such as TMB and MSI.

Our experience shows that the generation and bioinformatic processing of comprehensive genomic and transcriptomic data in patients with rare cancers can be standardized and semiautomated. We have also succeeded in reducing the complexity of WGS/WES and RNA-seq data by specific filtering strategies and available knowledge databases. However, the clinical application of heterogeneous molecular information requires the unique expertise of physicians trained in molecular biology. Their task is to evaluate genetic alterations concerning their functional relevance and assign them to individualized therapeutic decisions, which consider the respective clinical constellation and must be consented to in a multidisciplinary MTB. To support decision-making by the MTB, several guidelines and classifications of available evidence have been developed in recent years (38, 39, 50). However, an assessment of their clinical relevance in large patient populations has been lacking. Our study includes the application of two schemes for classifying predictive biomarker-drug relationships. It thus provides, for the first time, an insight into the real-world distribution of evidence levels in a large cohort of patients with rare cancers. It is essential to note that knowledge of specific biomarkers' actionability and the complement of molecularly targeted drugs are rapidly evolving. Therefore, our evaluation of evidence levels and MTB recommendations should not be considered a self-contained dataset. Instead, revisiting the resource presented here in the future is warranted to capture the actionable genome and transcriptome of rare cancers as completely as possible.

Our cohort of patients with advanced rare cancers was ideally suited to investigate a precision oncology approach in a prospective observational setting, as these patients have few therapeutic options and a dismal outcome. We found that comprehensive genomic and transcriptomic analysis enabled clinical management recommendations at multiple levels in patients who had exhausted all standard therapies, including those based on established molecular biomarkers. Of particular importance, we observed a meaningful clinical benefit, defined as a PFS_r or mPFS_r >1.3, in approximately one third of the patients in whom the efficacy of molecularly informed treatment could be assessed, accounting for 8.2% of the total MTB population. This is comparable to the results of other recently reported pan-cancer precision oncology studies (6–8). Thus, our study provides the first evidence for the clinical benefit of a precision oncology approach in a large and diverse cohort of rare cancers. A limitation of these results, inherent in all observational or registry studies, is that patient outcome was measured retrospectively based on available medical records, and that response to experimental therapy was not assessed in a standardized way, for example, according to

Response Evaluation Criteria In Solid Tumors, in the majority of cases. We addressed this issue by implementing regular follow-up intervals and structuring outcome assessments based on the consensus of a curator team of oncologists and medical geneticists. Another drawback is the possible selection bias of an observational trial that results from preferentially including and treating patients with prognostically favorable characteristics. In addition, the exploratory analyses presented were retrospectively designed and performed and thus not powered to examine biomarker/treatment baskets or patient subcohorts for significant differences in clinical outcomes.

While we consider our findings in a high-risk patient population with an unmet medical need encouraging, further steps must be taken to increase the potential benefit of molecularly informed approaches to managing rare cancers. First, comprehensive molecular analyses should be considered earlier in the course of the disease. This can address the diagnostic uncertainty associated with many rare cancers, provide early genetic counseling to families affected by pathogenic germline alterations, and minimize the attrition of patients qualifying for experimental therapy. Second, the rational combination of targeted drugs based on individual molecular profiles will be a necessary next step to increase their clinical efficacy. An increasing number of clinical trials pursue this strategy, although only a few use comprehensive molecular profiling for therapy stratification (7). We show that a multidisciplinary precision oncology workflow based on WGS/WES and RNA-seq can yield combinatorial strategies to treat advanced rare cancers and generate evidence on the efficacy or futility of such combinations (35), thus informing future clinical trial design. Finally, the most important measure to improve clinical translation is to investigate the predictive value of complex molecular information in controlled clinical trials that allow most, if not all, patients with rare cancers access to individualized therapies. For this reason, we are working to link the MASTER platform to a growing portfolio of multicenter basket protocols that also apply composite biomarkers (ClinicalTrials.gov Identifier NCT03127215) and combination therapies (NCT04551521).

Our intermediate-term goal is to expand the MASTER program, which belongs to a new class of clinical protocols that promise to close the evidence gap in precision medicine (24), to other patient characterization methods, for example, epigenomic, proteomic, and functional profiling and pharmacogenomic analyses. In the long term, we will extend our stratification approach to modalities beyond medical therapy to increase the precision of any cancer-specific intervention based on understanding individual tumors' distinct properties. Finally, in addition to informing choices regarding targeted therapy, the data generated through broad molecular profiling frequently provide starting points for exploratory research projects, particularly investigations into the functional and mechanistic consequences of individual molecular lesions or combinations thereof.

METHODS

Study Design and Patient Population

NCT/DKTK MASTER is a prospective, continuously recruiting, multicenter observational study for biology-driven stratification of adults with advanced cancer across histologies who are younger

than 51 and patients with rare tumors, including rare subtypes of more common entities, regardless of age (25). In keeping with the therapeutic intent of the study, patients must have exhausted curative treatment options, be in good general condition (Eastern Cooperative Oncology Group performance status of 0 or 1), and provide written informed consent for banking of tumor and control tissue, molecular analysis, and the collection of clinical data under a protocol (S-206/2011) approved by the Ethics Committee of the Medical Faculty of Heidelberg University. The study was conducted in accordance with the Declaration of Helsinki.

Genomic and Transcriptomic Analysis

The processing of tumor and control specimens and technical details of the WGS/WES and RNA-seq analyses are described in the Supplementary Methods.

Bioinformatic Analyses

Alignment of WGS/WES data was performed with BWA, first with BWA aln and from 2017 with BWA mem. Calling of SNVs was carried out with an in-house pipeline based on SAMtools mpile-up and bcftools, indel calling with a pipeline based on Platypus. Structural variants were detected with a pipeline based on CREST; from 2017, SVs in WGS data were detected using the in-house tool SOPHIA (<https://bitbucket.org/utopprak/sophia/src/master>). Somatic copy number alterations in WGS data were detected with ACEseq (<https://aceseq.readthedocs.io/en/latest>); sCNAs in WES data were initially analyzed by an in-house pipeline using VarScan2 and from 2017 with CNVkit. To quantify genomic instability, LOH (28) and the numbers of LSTs (29) and TAIIs (30) were computed using the output of the pipelines for sCNA detection in WGS and WES data. Microsatellite instability was assessed with MSISensor. Supervised analysis, that is, fitting of mutational signatures, was performed with YAPSA (<http://bioconductor.org/packages/3.12/bioc/html/YAPSA.html>). Alignment of RNA-seq data was performed with STAR. Gene expression was quantified using a custom script based on coverageBed from the BEDtools package. Gene fusions were identified from RNA-seq data using Arriba (<https://github.com/suhrig/arriba>). Further details on the pipelines used, including version numbers and parameter choices, and the integration and visualization of the different data layers are described in the Supplementary Methods.

Clinical Evaluation of Molecular Alterations and Assessment of Outcome Parameters

Evaluations of biomarkers' clinical actionability and assignments of molecularly informed therapies were performed in semiweekly, multicenter MTB conferences, which included at least one clinician familiar with the individual case, a bioinformatician, a molecular oncologist, a medical geneticist, a pathologist, and a tumor biologist. All MTB decisions were centrally documented, curated, and evaluated retrospectively by ten specialists and fellows in medical oncology or medical genetics (C.E. Heilig, C. Heining, P. Horak, D. Hanf, A. Jahn, S. Kreutzfeldt, A. Mock, L. Möhrmann, L. Ruhnke, and V. Teleanu) according to a prespecified set of rules. Structured follow-ups were performed 3, 6, 12, 18, and 24 months from the MTB. The clinical benefit associated with molecularly informed therapies implemented until the data cutoff on November 21, 2018, was assessed by the above team of curators based on real-world clinical and radiology reports using a prespecified set of rules. Complicated and ambiguous cases were discussed in a weekly conference to reach a consensus regarding the validity and evaluability of the underlying data and reevaluate clinical outcome if necessary. Further details on the guidelines for clinical evaluation of molecular alterations and assessment of outcome parameters are described in the Supplementary Methods.

Data Availability

Sequencing data have been deposited in the European Genome-phenome Archive (<https://www.ebi.ac.uk/ega/datasets>) under accession EGAS00001004813.

Authors' Disclosures

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REFERENCES

- Subbiah V, Kurzrock R. Challenging standard-of-care paradigms in the precision oncology era. *Trends Cancer* 2018;4:101–9.
- Jardim DL, Schwaederle M, Wei C, Lee JJ, Hong DS, Eggermont AM, et al. Impact of a biomarker-based strategy on oncology drug development: a meta-analysis of clinical trials leading to FDA approval. *J Natl Cancer Inst* 2015;107:djv253.
- Schwaederle M, Zhao M, Lee JJ, Eggermont AM, Schilsky RL, Mendelsohn J, et al. Impact of precision medicine in diverse cancers: a meta-analysis of phase II clinical trials. *J Clin Oncol* 2015;33:3817–25.
- Schwaederle M, Zhao M, Lee JJ, Lazar V, Leyland-Jones B, Schilsky RL, et al. Association of biomarker-based treatment strategies with response rates and progression-free survival in refractory malignant neoplasms: a meta-analysis. *JAMA Oncol* 2016;2:1452–9.
- Massard C, Michiels S, Ferte C, Le Deley MC, Lacroix L, Hollebecque A, et al. High-throughput genomics and clinical outcome in hard-to-treat advanced cancers: results of the MOSCATO 01 Trial. *Cancer Discov* 2017;7:586–95.
- Rodon J, Soria JC, Berger R, Miller WH, Rubin E, Kugel A, et al. Genomic and transcriptomic profiling expands precision cancer medicine: the WINTHER trial. *Nat Med* 2019;25:751–8.
- Sicklick JK, Kato S, Okamura R, Schwaederle M, Hahn ME, Williams CB, et al. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. *Nat Med* 2019;25:744–50.
- van der Velden DL, Hoes LR, van der Wijngaart H, van Berge Henegouwen JM, van Werkhoven E, Roepman P, et al. The Drug Rediscovery protocol facilitates the expanded use of existing anticancer drugs. *Nature* 2019;574:127–31.
- Tredan O, Wang Q, Pissaloux D, Cassier P, de la Fouchardiere A, Fayette J, et al. Molecular screening program to select molecular-based recommended therapies for metastatic cancer patients: analysis from the ProfiLER trial. *Ann Oncol* 2019;30:757–65.
- Belin L, Kamal M, Mauborgne C, Plancher C, Mulot F, Delord JP, et al. Randomized phase II trial comparing molecularly targeted therapy based on tumor molecular profiling versus conventional therapy in patients with refractory cancer: cross-over analysis from the SHIVA trial. *Ann Oncol* 2017;28:590–6.
- Diamond EL, Subbiah V, Lockhart AC, Blay JY, Puzanov I, Chau I, et al. Vemurafenib for BRAF V600-mutant erdheim-chester disease and langerhans cell histiocytosis: analysis of data from the histology-independent, phase 2, open-label VE-BASKET study. *JAMA Oncol* 2018;4:384–8.

12. Hyman DM, Puzanov I, Subbiah V, Faris JE, Chau I, Blay JY, et al. Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. *N Engl J Med* 2015;373:726–36.
13. Kopetz S, Grothey A, Yaeger R, Van Cutsem E, Desai J, Yoshino T, et al. Encorafenib, binimetinib, and cetuximab in BRAF V600E-mutated colorectal cancer. *N Engl J Med* 2019;381:1632–43.
14. Subbiah V, Puzanov I, Blay JY, Chau I, Lockhart AC, Raje NS, et al. Pan-Cancer efficacy of vemurafenib in BRAF (V600)-mutant non-melanoma cancers. *Cancer Discov* 2020;10:657–63.
15. Drilon A, Siena S, Ou SI, Patel M, Ahn MJ, Lee J, et al. Safety and antitumor activity of the multitargeted pan-TRK, ROS1, and ALK inhibitor entrectinib: combined results from two phase I trials (ALKA-372-001 and STARTRK-1). *Cancer Discov* 2017;7:400–9.
16. Hyman DM, Piha-Paul SA, Won H, Rodon J, Saura C, Shapiro GI, et al. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. *Nature* 2018;554:189–94.
17. Flaherty KT, Gray R, Chen A, Li S, Patton D, Hamilton SR, et al. The molecular analysis for therapy choice (NCI-MATCH) trial: lessons for genomic trial design. *J Natl Cancer Inst* 2020;112:1021–9.
18. Ledermann JA. PARP inhibitors in ovarian cancer. *Ann Oncol* 2016; 27:i40–i4.
19. DeSantis CE, Kramer JL, Jemal A. The burden of rare cancers in the United States. *CA Cancer J Clin* 2017;67:261–72.
20. Gatta G, van der Zwan JM, Casali PG, Siesling S, Dei Tos AP, Kunkler I, et al. Rare cancers are not so rare: the rare cancer burden in Europe. *Eur J Cancer* 2011;47:2493–511.
21. Munoz J, Kurzrock R. Targeted therapy in rare cancers—adopting the orphans. *Nat Rev Clin Oncol* 2012;9:631–42.
22. Robinson DR, Wu YM, Lomigro RJ, Vats P, Cobain E, Everett J, et al. Integrative clinical genomics of metastatic cancer. *Nature* 2017; 548:297–303.
23. Von Hoff DD, Stephenson JJ Jr, Rosen P, Loesch DM, Borad MJ, Anthony S, et al. Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. *J Clin Oncol* 2010;28:4877–83.
24. Dickson D, Johnson J, Bergan R, Owens R, Subbiah V, Kurzrock R. The master observational trial: a new class of master protocol to advance precision medicine. *Cell* 2020;180:9–14.
25. Horak P, Klink B, Heining C, Groschel S, Hutter B, Frohlich M, et al. Precision oncology based on omics data: the NCT Heidelberg experience. *Int J Cancer* 2017;141:877–86.
26. Lier A, Penzel R, Heining C, Horak P, Frohlich M, Uhrig S, et al. Validating comprehensive next-generation sequencing results for precision oncology: the NCT/DKTK molecularly aided stratification for tumor eradication research experience. *JCO Precis Oncol* 2018; 2:1–13.
27. Davies H, Glodzik D, Morganella S, Yates LR, Staaf J, Zou X, et al. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. *Nat Med* 2017;23:517–25.
28. Abkovich V, Timms KM, Hennessy BT, Potter J, Carey MS, Meyer LA, et al. Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. *Br J Cancer* 2012;107:1776–82.
29. Popova T, Manie E, Rieunier G, Caux-Moncoutier V, Tirapu C, Dubois T, et al. Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. *Cancer Res* 2012;72:5454–62.
30. Birkbak NJ, Wang ZC, Kim JY, Eklund AC, Li Q, Tian R, et al. Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. *Cancer Discov* 2012;2:366–75.
31. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet* 2019;51:202–6.
32. Naumann RW, Hollebecque A, Meyer T, Devlin MJ, Oaken A, Kerger J, et al. Safety and efficacy of nivolumab monotherapy in recurrent or metastatic cervical, vaginal, or vulvar carcinoma: results from the Phase I/II CheckMate 358 Trial. *J Clin Oncol* 2019;37:2825–34.
33. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 2015;372:2509–20.
34. Davoli T, Uno H, Wooten EC, Elledge SJ. Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. *Science* 2017;355:eaaf8399.
35. Heining C, Horak P, Uhrig S, Codo PL, Klink B, Hutter B, et al. NRGR1 fusions in KRAS wild-type pancreatic cancer. *Cancer Discov* 2018;8:1087–95.
36. Haas BJ, Dobin A, Li B, Stransky N, Pochet N, Regev A. Accuracy assessment of fusion transcript detection via read-mapping and de novo fusion transcript assembly-based methods. *Genome Biol* 2019; 20:213.
37. Weinberg F, Griffin R, Frohlich M, Heining C, Braun S, Spohr C, et al. Identification and characterization of a BRAF fusion oncoprotein with retained autoinhibitory domains. *Oncogene* 2020;39:814–32.
38. Leichsenring J, Horak P, Kreutzfeldt S, Heining C, Christopoulos P, Volkmar AL, et al. Variant classification in precision oncology. *Int J Cancer* 2019;145:2996–3010.
39. Mateo J, Chakravarty D, Dienstmann R, Jezdic S, Gonzalez-Perez A, Lopez-Bigas N, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). *Ann Oncol* 2018;29: 1895–902.
40. Mock A, Heilig CE, Kreutzfeldt S, Huebschmann D, Heining C, Schrock E, et al. Community-driven development of a modified progression-free survival ratio for precision oncology. *ESMO Open* 2019;4:e000583.
41. Tamborero D, Dienstmann R, Rachid MH, Boekel J, Baird R, Brana I, et al. Support systems to guide clinical decision-making in precision oncology: The Cancer Core Europe Molecular Tumor Board Portal. *Nat Med* 2020;26:992–4.
42. Alvarez MJ, Shen Y, Giorgi FM, Lachmann A, Ding BB, Ye BH, et al. Functional characterization of somatic mutations in cancer using network-based inference of protein activity. *Nat Genet* 2016;48: 838–47.
43. Alvarez MJ, Subramaniam PS, Tang LH, Grunn A, Aburi M, Rieckhof G, et al. A precision oncology approach to the pharmacological targeting of mechanistic dependencies in neuroendocrine tumors. *Nat Genet* 2018;50:979–89.
44. Schubert M, Klinger B, Klunemann M, Sieber A, Uhlig F, Sauer S, et al. Perturbation-response genes reveal signaling footprints in cancer gene expression. *Nat Commun* 2018;9:20.
45. Dempster JM, Krill-Burger JM, McFarland JM, Warren A, Boehm JS, Vazquez F, et al. Gene expression has more power for predicting *in vitro* cancer cell vulnerabilities than genomics. *bioRxiv* 2020.
46. Paull EO, Aytes A, Jones SJ, Subramaniam PS, Giorgi FM, Douglass EF, et al. A modular master regulator landscape controls cancer transcriptional identity. *Cell* 2021;184:334–51.
47. Pilie PG, Gay CM, Byers LA, O'Connor MJ, Yap TA. PARP inhibitors: extending benefit beyond BRCA-mutant cancers. *Clin Cancer Res* 2019;25:3759–71.
48. Conway JR, Kofman E, Mo SS, Elmarakeby H, Van Allen E. Genomics of response to immune checkpoint therapies for cancer: implications for precision medicine. *Genome Med* 2018;10:93.
49. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science* 2018;359:1350–5.
50. Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn* 2017;19:4–23.