The Pediatric Precision Oncology INFORM Registry: Clinical Outcome and Benefit for Patients with Very High-Evidence Targets

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ABSTRACT   INFORM is a prospective, multinational registry gathering clinical and molecular data of relapsed, progressive, or high-risk pediatric patients with cancer. This report describes long-term follow-up of 519 patients in whom molecular alterations were evaluated according to a predefined seven-scale target prioritization algorithm. Mean turnaround time from sample receipt to report was 25.4 days. The highest target priority level was observed in 42 patients (8.1%). Of these, 20 patients received matched targeted treatment with a median progression-free survival of 204 days [95% confidence interval (CI), 99–not applicable], compared with 117 days (95% CI, 106–143; $P = 0.011$) in all other patients. The respective molecular targets were shown to be predictive for matched treatment response and not prognostic surrogates for improved outcome. Hereditary cancer predisposition syndromes were identified in 7.5% of patients, half of which were newly identified through the study. Integrated molecular analyses resulted in a change or refinement of diagnoses in 8.2% of cases.

SIGNIFICANCE: The pediatric precision oncology INFORM registry prospectively tested a target prioritization algorithm in a real-world, multinational setting and identified subgroups of patients benefiting from matched targeted treatment with improved progression-free survival, refinement of diagnosis, and identification of hereditary cancer predisposition syndromes.

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

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INTRODUCTION

Children and adolescents with relapsed, progressive, or refractory high-risk malignant disease have a particularly poor prognosis. Survival rates of less than 20% following recurrence (1–12) suggest an urgent need for innovative treatment strategies. Based on the potential therapeutic options discovered in several large-scale pediatric sequencing projects (13, 14), a number of comprehensive pediatric precision oncology programs such as pediatric MATCH (15), MOSCATO-01 (16), the ZERO Childhood Cancer Program (17), and the INFORM (INdividualized Therapy FOr Relapsed Malignancies in Childhood) pilot study (18) have been established over recent years, as reviewed by Forrest and colleagues (19) and Mody and colleagues (20). These programs have identified a spectrum of molecularly actionable variants ranging from overexpression of targets to copy-number alterations, gene fusions, point mutations, etc. However, the significance and long-term clinical benefit of such comprehensive and tumor type–agnostic precision oncology studies in a real-world clinical setting are largely unknown for pediatric oncology.

The INFORM registry applies comprehensive molecular profiling in order to provide information on actionable gene variants, which may be used for subsequent clinical trial enrollment or experimental treatment approaches (compassionate or off-label use). One aim of the INFORM registry, and the focus of this report, is to prospectively investigate a predefined (18) seven-scale molecular target prioritization algorithm ranging from "very high" to "very low," based on the type of alteration and its disease-specific relevance, for its predictive power in a tumor type–agnostic approach. Importantly, the algorithm relies on functional molecular biological relevance of the target rather than evidence for clinical activity of a particular compound. The very high-priority targets are directly actionable genetic alterations with a proven link to tumorigenesis in the specific cancer type. High- and moderate-priority targets are those with a genetic alteration in a known cancer driver or activating an oncogenic pathway, with evidence either in the malignancy of interest or from other tumor types. Intermediate targets include genetic hits that sensitize to a given drug (e.g., activate a pathway) with evidence from tumors other than that being analyzed or highly overexpressed oncogenes that are known to drive the specific malignant disease. Borderline and low-priority targets are those that involve expression changes in oncogenic pathways or otherwise lead to drug sensitivity, with evidence either in the cancer type of interest or from other tumor types. Very low priority covers those with only circumstantial evidence of links to actionable drug targets.

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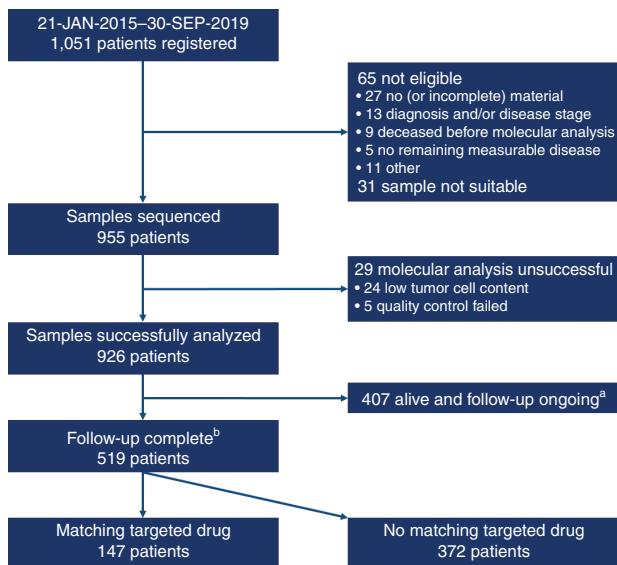
Figure 1. Patient disposition. [a]Registered after October 1, 2017, and still alive and with ongoing follow-up (because regular follow-up of 2 years not complete) at the data cutoff. [b]At least 2 years of regular follow-up completed, lost to follow-up, or deceased. This includes patients registered after October 1, 2017, who were lost to follow-up or deceased.

The registry routinely documents clinical follow-up, allowing evaluation of clinical benefit for this pediatric patient group with an urgent medical need.

At the time of the data cutoff, 1,051 patients had been registered in INFORM, of whom 519 patients had completed their clinical follow-up and were included in this analysis. This report describes prospective testing of a target prioritization algorithm and long-term clinical follow-up data of this large multinational and comprehensively molecularly profiled pediatric cohort in a real-world clinical setting.

RESULTS

Patients and Baseline Characteristics

Between January 21, 2015, and September 30, 2019, 1,051 patients were registered. Of these, the inclusion criteria were not fulfilled (most frequent reason: no or incomplete samples received, $n = 27$) for 65 patients, samples were not suitable for molecular analysis for 31 patients, and the molecular analysis was unsuccessful in 29 patients. Patient disposition is shown in Fig. 1. Of the remaining 926 patients, whole-exome sequencing (WES), low-coverage whole-genome sequencing (lcWGS), and DNA methylation analysis were successfully performed in almost all patients, with RNA sequencing successful for 84.3% of patients. No differences in profiling success rates were observed between national (German) or international sites (Supplementary Table S1). In total, 519 patients had completed their clinical follow-up of either at least 2 years or shorter in case of death or loss to follow-up and were subsequently included in this analysis. Patients with ongoing clinical follow-up shorter than 2 years at the time of the data cutoff (September 30, 2019; $n = 407$) were not included in this analysis. Patients were enrolled in a total of 72 centers in

Table 1. Patient baseline demographic and disease characteristics

Characteristics (at enrollment)	Received matching targeted drug	Did not receive matching targeted drug
N	147	372
Median age (range), y	13 (0–36)	12 (1–40)
Sex, n (%)		
Female	58 (39.5)	164 (44.1)
Male	89 (60.5)	208 (55.9)
Lansky/Karnovsky performance status, n (%)		
50	4 (2.7)	15 (4.0)
60	2 (1.4)	18 (4.8)
70	19 (12.9)	44 (11.8)
80	26 (17.7)	89 (23.9)
90	46 (31.3)	92 (24.7)
100	34 (23.1)	61 (16.4)
Missing	16 (10.9)	53 (14.2)
Disease status, n (%)		
Primary diagnosis	11 (7.5)	39 (10.5)
Refractory to first-line therapy	8 (5.4)	18 (4.8)
Relapse 1	71 (48.3)	198 (53.2)
Relapse 2	33 (22.4)	69 (18.5)
Relapse ≥3	23 (15.7)	44 (11.8)
Unknown	1 (0.7)	4 (1.1)
Diagnosis, n (%)		
Hematologic malignancy	**8 (5.4)**	**37 (9.9)**
Acute lymphoblastic leukemia	3 (2.0)	20 (5.4)
Acute myeloid leukemia	3 (2.0)	12 (3.2)
Non-Hodgkin lymphoma	1 (0.7)	5 (1.3)
Other	1 (0.7)	0
Solid tumor	**101 (68.7)**	**234 (62.9)**
Desmoplastic small round cell tumor	2 (1.4)	6 (1.6)
Ewing sarcoma	14 (9.5)	51 (13.7)
Malignant rhabdoid tumor	1 (0.7)	9 (2.4)
Neuroblastoma	21 (14.3)	51 (13.7)
Osteosarcoma	17 (11.6)	23 (6.2)
Rhabdomyosarcoma	15 (10.2)	49 (13.2)
Other soft tissue sarcoma	18 (12.2)	20 (5.4)
Other	13 (8.8)	25 (6.7)
CNS tumor	**38 (25.9)**	**101 (27.2)**
Atypical teratoid rhabdoid tumor	3 (2.0)	5 (1.3)
Ependymoma	7 (4.8)	20 (5.4)
High-grade glioma (including DIPG)	20 (13.6)	46 (12.4)
Medulloblastoma	5 (3.4)	18 (4.8)
Other	3 (2.0)	12 (3.2)
Metastatic disease (% of nonhematologic malignancies)	99 (71.2)	216 (64.5)
Subjected to molecular analysis at multiple time points (e.g., sequential relapses), n (%)	17 (11.6)	28 (7.5)

Abbreviation: DIPG, diffuse intrinsic pontine glioma.

the following eight countries: Austria (*n* = 5), Finland (*n* = 5), Germany (*n* = 396), Greece (*n* = 3), Poland (*n* = 2), Sweden (*n* = 36), Switzerland (*n* = 13), and the Netherlands (*n* = 59). Baseline characteristics are provided in Table 1. A total of 14 different pediatric malignant diseases were included in the study, including a group of rare diseases ("other"), resulting in a distribution of 64.5% non–central nervous system (CNS) solid tumors, 26.8% CNS tumors, and 8.7% hematologic malignancies (for details, see Table 1 and Supplementary

Table S2). To allow young adults who relapsed after a primary diagnosis during the pediatric age range (e.g., as frequently encountered with sarcomas), patients could be enrolled up to an age of 40 years, provided that they had had their primary pediatric-type diagnosis below the age of 21 years. One hundred and two patients (19.7%) were age ≥18 years at the time of inclusion. Fifty patients (9.6%) with newly diagnosed very high-risk malignancies for which no curative treatment exists [e.g., high-grade glioma (HGG)] were enrolled, whereas the

Table 2. Priority-level distribution (highest priority level per patient)

Priority level	Highest priority level for which patients received matched targeted drug, n (%)	Highest priority level per patient (all patients), n (%)
Very high	20 (13.6)	42 (8.1)
High	31 (21.1)	78 (15.0)
Moderate	24 (16.3)	105 (20.2)
Intermediate	33 (22.4)	124 (23.9)
Borderline	26 (17.7)	76 (14.6)
Low	6 (4.1)	12 (2.3)
Very low	2 (1.4)	5 (1.0)
Not applicable	5 (3.4)	4 (0.8)
No target	0	73 (14.1)
Total	147 (100)	519 (100)

others were enrolled upon refractory disease or relapse. Most patients with solid/CNS tumors (66.5%) were metastatic at enrollment.

Target Identification and Distribution

After enrollment and sample receipt, the mean turnaround time to target reporting within the context of a molecular tumor board was 25.4 days. Identified targets were classified by an interdisciplinary review according to a seven-scale prioritization algorithm ranging from "very high" to "very low," as reported previously (18). In 446 of the 519 patients (85.9%), at least one actionable target was identified, of which 225 (43%) were genetically altered targets (priority levels very high, high, and moderate; Table 2). The distribution of the highest priority level target per patient (multiple actionable alterations could be reported per patient) was: very high, 8.1%; high, 15.0%; moderate, 20.2%; intermediate, 23.9%; borderline, 14.6%; low, 2.3%; very low, 1.0%; and no actionable target available, 14.1% (actionable target not matching one of the priority levels, 0.8%). An overview of all reported actionable targets is provided in Supplementary Table S3. The most frequent very high priority level targets identified were *ALK* mutations, *BRAF* mutations, and *NTRK* fusions (Supplementary Table S4). The distribution of the highest priority level target per patient over the different diagnoses is depicted in Fig. 2. Considering disease groups with relevant case numbers, it was observed that the highest priority level (very high) occurred mostly in neuroblastoma and HGG. Priority levels high, moderate, and intermediate were more dominant in rhabdomyosarcoma and osteosarcoma. Ependymoma, Ewing sarcoma, other soft-tissue sarcomas, and other rare solid tumors tended to have a high proportion of targets of the lowest priority levels only (borderline, low, very low, and no target).

Treatment and Clinical Outcome

There was a trend to apply matching targeted drugs more frequently in patients with higher priority level targets than in patients with lower priority level targets (Table 2). Treatments matching one of the identified potential targets were applied in 147 patients (33.0% of the 446 patients with a potentially actionable target). The targeted agents applied were mostly small-molecule drugs, as well as immunotherapy (targeted antibodies including immune checkpoint inhibitors) in some instances (Supplementary Table S5a). The median duration of targeted treatment was 92 days. Seventeen patients were enrolled into clinical trials (3.8% of 446 patients with a potential actionable target and 11.6% of the 147 patients who received matching targeted drugs, with one patient enrolled in two trials). The following matching targeted drugs were applied in a trial setting: ceritinib ($n = 3$), crizotinib ($n = 1$), dabrafenib ($n = 1$), larotrectinib ($n = 5$), olaparib ($n = 2$), pazopanib ($n = 1$), ribociclib ($n = 3$), and tazemetostat ($n = 2$). All other patients received matching targeted drugs off-label or through compassionate use programs. Further details on drug treatments matching identified targets are provided in Supplementary Table S5a. During the study period, potent selective NTRK inhibitors became available (21–23). Outcome of patients with an *NTRK* fusion who received an NTRK inhibitor (e.g., larotrectinib) and patients with an *NTRK* fusion who received an alternative treatment (e.g., because NTRK inhibitors were not available then) is summarized in Supplementary Table S5b.

In the time from registration to completed sample receipt, 6 patients died and 5 had disease progression and were excluded from the progression-free survival (PFS) analyses (resulting in 508 evaluable patients). For overall survival (OS) analysis, the 6 patients who died before completion of sample receipt were excluded (resulting in 513 evaluable patients). Median PFS and OS for the whole cohort were 118 [95% confidence interval (CI), 106–145] and 290 (95% CI, 257–343) days, respectively (survival data for the individual patients can be found in Supplementary Table S2 and data on the disease level are provided in Supplementary Table S6 and Supplementary Fig. S1A–S1F). PFS and OS of all patients who received a matching targeted drug compared with all patients who did not receive matching targeted drugs did not show any significant differences (Fig. 3A and B). Of the 42 patients in whom a very high priority level target was identified, 20 received treatment with a matching targeted drug [all had at least evaluable disease for response evaluation (almost all metastatic)]. This resulted in a median PFS of 204 [95% CI, 99–not applicable (NA)] and OS of 354 days (95% CI, 165–NA), compared with
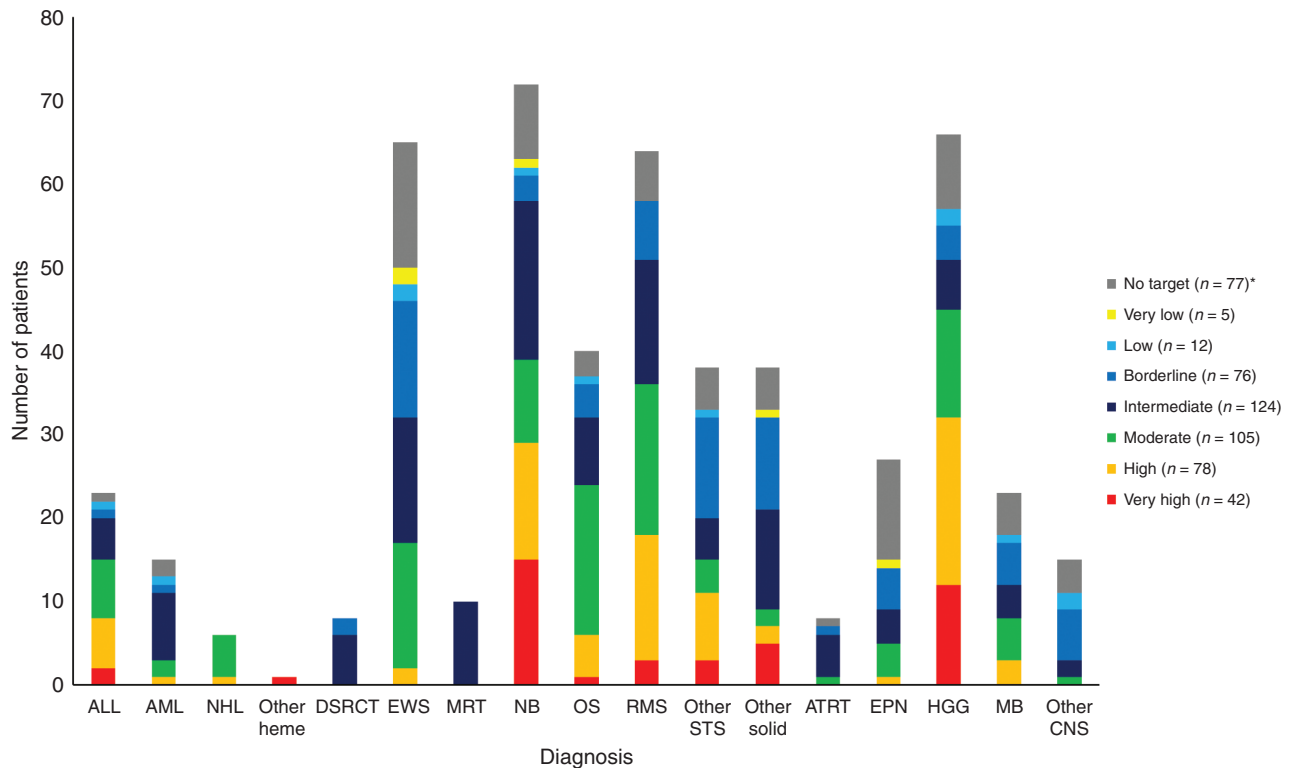
Figure 2. Distribution of the highest priority level target per patient over diagnoses. For each patient, only the highest priority level target is included. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATRT, atypical teratoid rhabdoid tumor; DSRCT, desmoplastic round cell tumor; EPN, ependymoma; EWS, Ewing sarcoma; HGG, high-grade glioma (including diffuse-intrinsic pontine glioma); MB, medulloblastoma; MRT, malignant rhabdoid tumor; NB, neuroblastoma; NHL, non-Hodgkin lymphoma; OS, osteosarcoma; other heme, other hematologic malignancies; RMS, rhabdomyosarcoma; STS, soft-tissue sarcoma. *, Including four patients with a target not matching one of the priority levels.

a PFS of 117 (95% CI, 106–143; $P = 0.011$) and OS of 290 days (95% CI, 256–343; $P = 0.32$) in all other patients (Fig. 3C and D). At baseline, of the 20 patients with a very high priority level target who received matching targeted treatment, 85% had a Lansky/Karnovsky performance status between 80 and 100, 10% were enrolled at primary diagnosis, 5% were enrolled refractory to first-line therapy, and 75% were enrolled at first or second relapse, whereas this was the case in 66.3%, 9.6%, 5%, and 71.3%, respectively, in the other 499 patients. Five of 20 patients (25%) with a very high priority level target who received matching targeted treatment also received other concomitant treatments, whereas 18 of 22 patients (81.8%) with a very high priority level target who did not receive matching targeted treatment received other conventional treatments (Supplementary Table S4). Comparison of patients with a very high priority level target who received matching targeted drugs and those who did not showed that patients who did not receive matching targeted drugs had comparable PFS to all other patients (Fig. 3E and F). Patients who received targeted drugs matching to other priority level targets did not show a significant PFS or OS improvement (Fig. 3G and H). Of the 372 patients who did not receive matched targeted treatment, 298 patients (80.1%) received other conventional medical systemic treatments, mostly chemotherapy. For the patients with an actionable target who were not treated

with a matched targeted drug ($n = 299$), the following reasons were reported: disease stabilized by other conventional therapy ($n = 86$), poor performance status due to progression of underlying malignant disease ($n = 71$), patient/guardians refused ($n = 25$), patient deceased before finalization of the molecular analysis and molecular tumor board ($n = 22$), poor performance status due to toxicity from previous treatment or treatments ($n = 13$), drug not available ($n = 13$), formulation not available ($n = 11$), insurance company declined cost coverage ($n = 6$), and various other reasons ($n = 133$; $n = 20$ patients with no information available; multiple reasons could be indicated). A comparable distribution was observed for patients with very high priority level targets who did not receive matched targeted treatment.

During the study period, new evidence for certain treatments or relevance of specific alterations emerged that changed the priority levels for some molecular alterations (the algorithm itself did not change). A retrospective analysis using priority levels according to current scientific and medical knowledge did show a change of priority level for 21.6% of the reported alterations that did not show significantly different outcomes per prioritization level. Also, new potential biomarkers were reported even if they could not be confidently ranked by the algorithm (yet). For example, a somatic BRCAness signature was reported as a potential biomarker
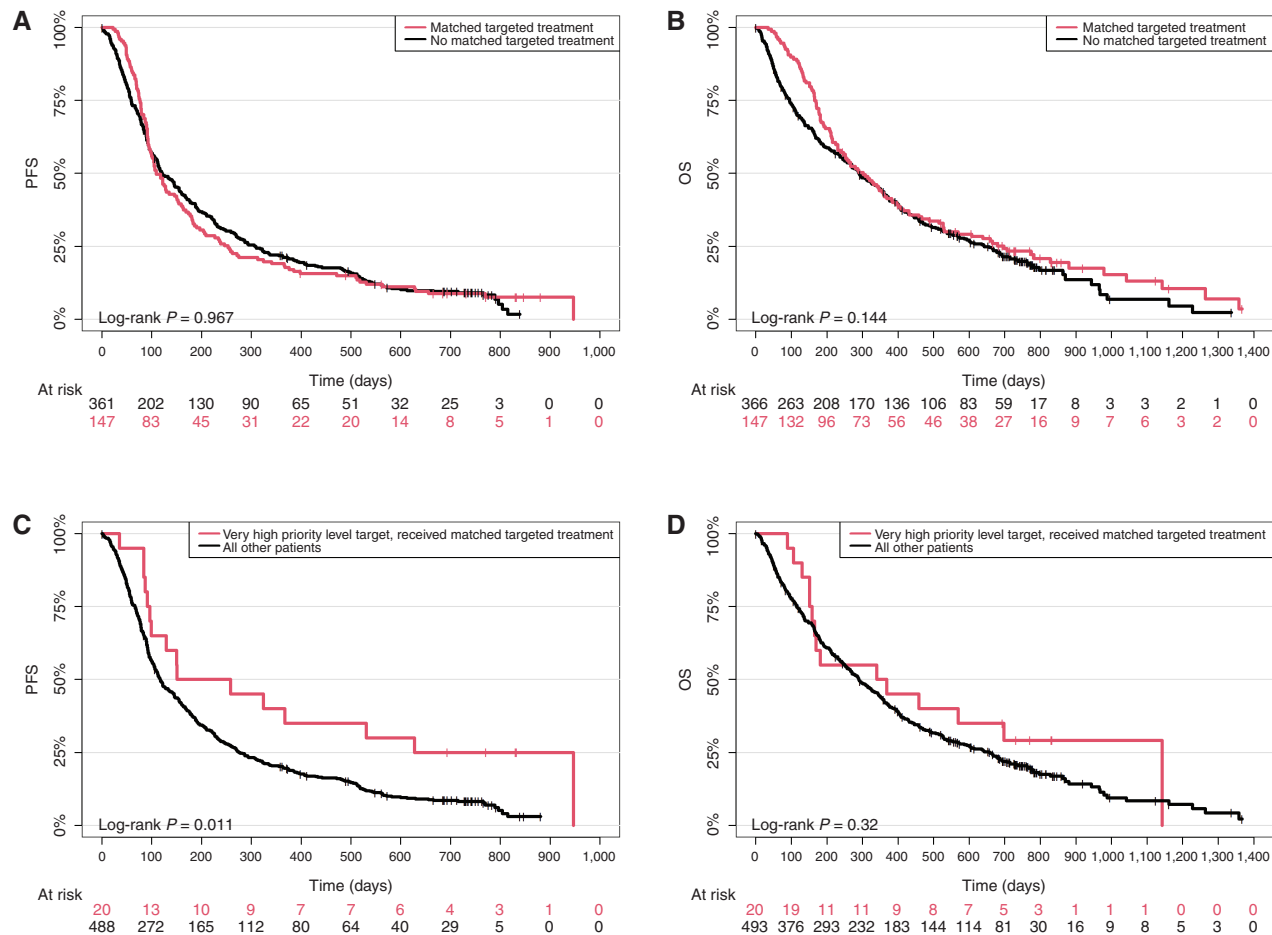
Figure 3. Survival analyses. **A,** PFS of patients separated by application of matching targeted drug versus all other patients ($P = 0.967$). **B,** OS of patients separated by application of matching targeted drug versus all other patients ($P = 0.144$). **C,** PFS of patients separated by application of matching targeted drug in very high priority level patients versus all other patients ($P = 0.011$). **D,** OS of patients separated by application of matching targeted drug in very high priority level patients versus all other patients ($P = 0.32$). (*continued on following page*)

for PARP inhibition on the basis of preclinical data (24), whereas according to current knowledge a BRCAness signature in the absence of true *BRCA* family mutation does not represent a biomarker for PARP inhibition and accordingly is not reported anymore.

Diagnosis Refinement

DNA methylation analysis and RNA sequencing for gene fusion detection allowed for reevaluation of tumor diagnosis in specific diseases. For CNS tumor and sarcoma samples, previously described methylation-based classifier scores were applied (25, 26), and furthermore, cancer type–defining fusions (e.g., *PAX3/7–FOXO1* in rhabdomyosarcoma or *EWSR1* fusions in Ewing sarcomas) were used for comparison between diagnosis at enrollment (mostly based on histology) and the molecularly informed diagnosis. For a total of 257 patients, a statement on molecular confirmation of diagnosis could be made. Of these, diagnosis was confirmed for 240 patients (93.4%). Molecular results suggested a change in diagnosis in 17 patients [6.6%; Ewing sarcoma ($n = 7$), medulloblastoma ($n = 4$), primitive neuroectodermal tumor ($n = 2$),

desmoplastic small round cell tumor ($n = 1$), osteosarcoma ($n = 1$), ependymoma ($n = 1$), and HGG ($n = 1$); Table 3]. For the Ewing sarcoma cases, no typical *EWSR1* fusion could be identified based on RNA-sequencing analysis, and the DNA methylation analysis classifier score did not reveal a high score for Ewing sarcoma (26). Thus, these tumors were not considered classic Ewing sarcoma and were classified as sarcoma according to the respective methylation classifier score and/or detected alternative alteration (e.g., in *BCOR* or *CIC*). Notably, four cases registered as recurrent medulloblastoma were indicated as HGG ($n = 3$) or CNS sarcoma ($n = 1$) by DNA methylation (Table 3). In addition, for four patients with CNS tumors, diagnosis could be further refined (e.g., assignment to specific subgroups within a tumor type).

Cancer Predisposition

Knowledge about a hereditary cancer predisposition syndrome (CPS) may be critical for the affected patient as it may influence treatment decisions and inform surveillance strategies for the patient and potentially affected relatives. During the informed consent process, patients and/or legal
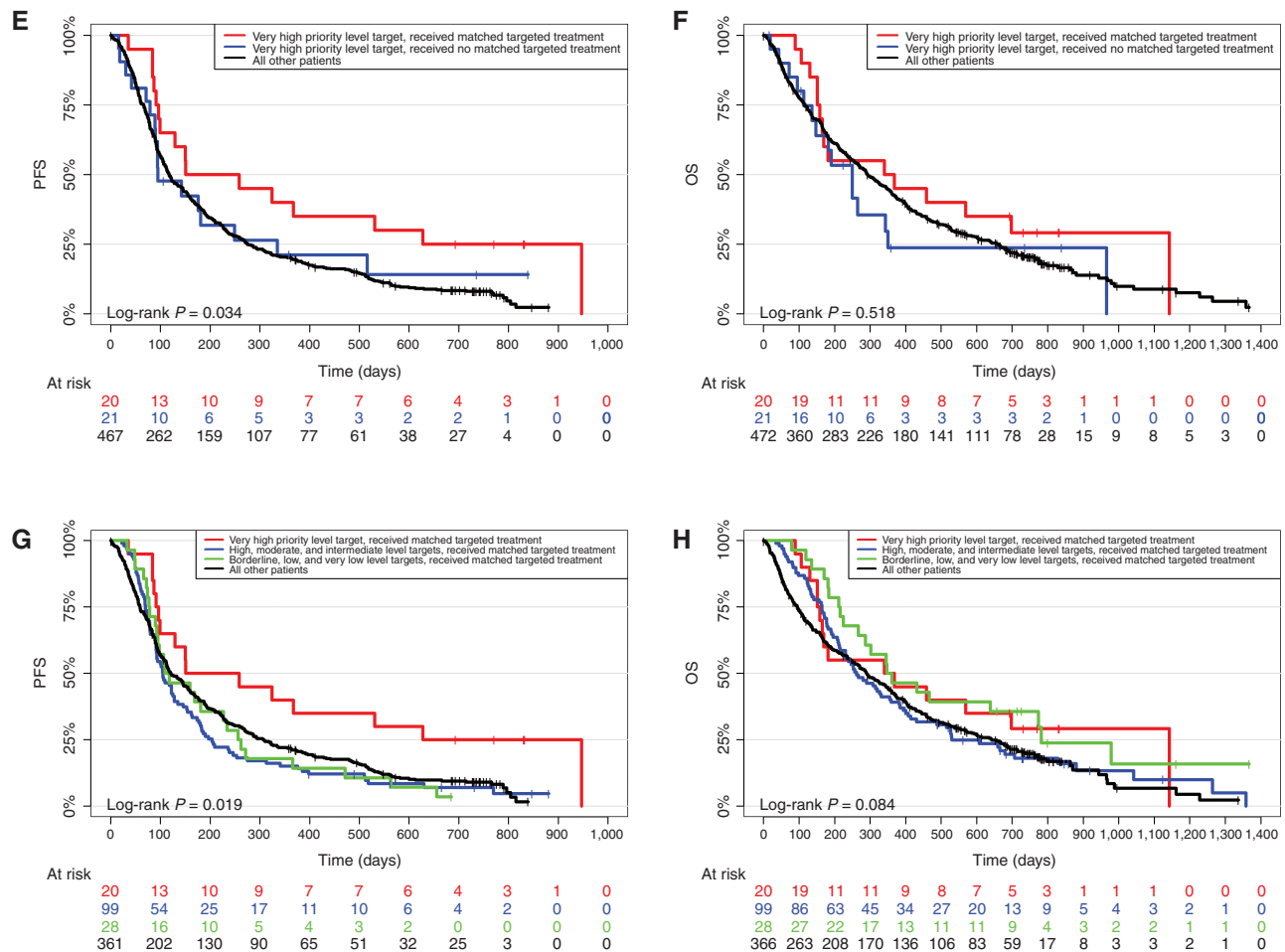
Figure 3. *(Continued)* **E,** PFS of patients separated by application of matching targeted drug for very high priority level patients versus very high priority level patients who did not receive matching target drugs versus all other patients (*P* = 0.034). **F,** OS of patients separated by application of matching targeted drug for very high priority level patients versus very high priority level patients who did not receive matching target drugs versus all other patients (*P* = 0.518). **G,** PFS of patients separated by application of matching targeted drug for very high priority level patients versus high, moderate, and intermediate level patients versus borderline, low, and very low level patients versus all other patients who did not receive matching target drugs. **H,** OS of patients separated by application of matching targeted drug for very high priority level patients versus high, moderate, and intermediate level patients versus borderline, low, and very low level patients versus all other patients who did not receive matching target drugs.

guardians were informed by the treating physician about the potential benefits and adverse effects that knowledge about a genetic cancer predisposition may entail. Patients and/or legal guardians consented to receive any relevant results in 94.2% of cases. We identified pathogenic or likely pathogenic (P/LP) variants in 39 patients (7.5%; Supplementary Table S7a). In 20 of 39 patients diagnosed with a CPS (51.3%), the cancer predisposition was first identified through our systematic screening of constitutional DNA and had not previously been clinically identified (Supplementary Table S7a). In our cohort, constitutional P/LP variants were most frequently identified in *TP53* (Li-Fraumeni syndrome; 7/39; 17.9%) in patients with osteosarcoma (*n* = 4), HGG (*n* = 1), undifferentiated sarcoma (*n* = 1), and medulloblastoma (*n* = 1). Four patients were diagnosed with constitutional mismatch repair deficiency caused by biallelic P/LP variants in the mismatch repair genes *PMS2* (HGG, *n* = 2) or *MSH6* (HGG, *n* = 1; adenocarcinoma, *n* = 1). Constitutional P/LP variants in

CHEK2 (Ewing sarcoma, osteosarcoma, and yolk sac tumor) and in *SMARCB1* (ATRT) were found in three patients each. Two patients each carried constitutional P/LP variants in *ALK, SDHB,* and *NF1*. Biallelic constitutional P/LP variants in *BRCA2* (Fanconi anemia) were also found in two patients (medulloblastoma with homozygous and HGG with compound heterozygous variants). Six patients carried heterozygous P/LP variants in DNA repair genes typically associated with adult-onset cancer (*ATM*, *BRCA1*, *BRCA2*, *PALB2*, *SLX4*), for which a driving role in the assessed pediatric tumors was not clear. Alterations in other genes were found in single patients only (Supplementary Table S7a).

DISCUSSION

Data from our INFORM registry demonstrate that comprehensive molecular profiling in a real-world multinational setting is feasible and beneficial. A turnaround time of less

Table 3. Molecular diagnosis change or refinement

ID	Histologic diagnosis at registration	Diagnosis molecularly confirmed	Molecular classification (subgroup)	Conclusive method(s)
INF_R_081	Desmoplastic small round cell tumor	No	Sarcoma (not further classifiable)	RNA-seq/DNA methylation
INF_R_037	Ewing sarcoma	No	Sarcoma (not further classifiable)	RNA-seq/DNA methylation
INF_R_075	Ewing sarcoma	No	Sarcoma (not further classifiable)	RNA-seq/DNA methylation
INF_R_101	Ewing sarcoma	No	Sarcoma (not further classifiable)	RNA-seq/DNA methylation
INF_R_262	Ewing sarcoma	No	Melanoma	RNA-seq/DNA methylation
INF_R_119	Ewing sarcoma	No	Sarcoma with *BCOR* alteration	RNA-seq/DNA methylation
INF_R_228	Ewing sarcoma	No	Sarcoma with *CIC* alteration	RNA-seq/DNA methylation
INF_R_557	Ewing sarcoma	No	Sarcoma with *CIC* alteration	RNA-seq/DNA methylation
INF_R_463	Osteosarcoma	No	Malignant peripheral nerve sheath tumor	DNA methylation
INF_R_263	Ependymoma	No	CNS high-grade neuroepithelial tumor with *BCOR* alteration	DNA methylation
INF_R_003	High-grade glioma	No	CNS high-grade neuroepithelial tumor with *BCOR* alteration	DNA methylation
INF_R_131	Medulloblastoma	No	Sarcoma (not further classifiable)	Histology/DNA methylation
INF_R_013	Medulloblastoma	No	High-grade glioma (not further classifiable)	DNA methylation
INF_R_223	Medulloblastoma	No	High-grade glioma (not further classifiable)	DNA methylation
INF_R_118	Medulloblastoma	No	High-grade glioma of receptor tyrosine kinase subgroup	DNA methylation
INF_R_178	Primitive neuroecto-dermal tumor	No	Pineoblastoma	DNA methylation
INF_R_496	Primitive neuroecto-dermal tumor	No	Pineoblastoma	DNA methylation
INF_R_057	High-grade glioma	Yes (refinement)	Infantile hemispheric glioma	DNA methylation
INF_R_292	High-grade glioma	Yes (refinement)	Infantile hemispheric glioma	DNA methylation
INF_R_707	High-grade glioma	Yes (refinement)	Pleomorphic xanthoastrocytoma	DNA methylation
INF_R_709	Oligoastrocytoma WHO grade III	Yes (refinement)	K27M-mutated high-grade glioma	DNA methylation

NOTE: Patients for whom molecular results suggested a change or refinement in diagnosis.

Abbreviations: RNA-seq, RNA sequencing; WHO, World Health Organization.

than 4 weeks is a clinically relevant window for these high-risk patients and particularly notable given the tasks carried out within this period: sample logistics, quality control, WES, lcWGS, RNA sequencing, DNA methylation analysis, bioinformatics processing, curation of target priorities, and conduction of the molecular tumor board. The weekly online interdisciplinary molecular tumor board in which targets and potential matching available approved treatments and matching open trials were discussed with the treating pediatric oncologist has developed into a well-recognized forum where expertise is shared and education is offered to its participants. Importantly, no differences in sequencing success rate were observed between national and international submissions. With a broad variety of pediatric-type hematologic malignancies, solid tumors, and CNS tumors represented, INFORM is truly agnostic to disease type. Notably, hematologic malignancies were somewhat underrepresented because of the availability of effective second- and third-line treatments and studies. The collection of long-term clinical follow-up data allowed for the initial identification of subgroups that may benefit from matched targeted treatment: patients with a very high priority level target who received matching targeted treatment had a significantly longer PFS compared with all other patients. In addition, molecular analyses added important diagnostic specifications and identified previously unknown hereditary cancer predisposition in a considerable number of patients.

In 43% of patients, a genetically altered target was identified (priority levels very high, high, and moderate), which is lower compared with other pediatric molecular profiling platforms [e.g., MOSCATO-01 (60.9%) and ZERO (71.4%); refs. 16, 17]. This discrepancy can be explained by a different definition of actionable altered genes (e.g., somatic *TP53* mutations were not considered actionable in INFORM). Importantly, "actionable" refers to a detected molecular alteration or affected pathway that theoretically would be targetable by an approved drug or an investigational agent and does not take into account the availability of a respective drug or

trial, particularly in regard to the pediatric population. This may explain the discrepancy between the number of actionable targets identified and the number of patients treated accordingly in INFORM. Furthermore, the methods used for ranking of actionable alterations (e.g., differentiation between genetic and nongenetic alterations) differ between the platforms. The ZERO program applies five levels (tiers; ref. 27) of supporting evidence based on the level of clinical or preclinical evidence in the respective disease (17), whereas the INFORM algorithm applies seven levels of functional molecular biological relevance (18). These differences in prioritization should be taken into account when comparing the clinical outcomes of both platforms. It will be very interesting to compare PFS and OS of both platforms according to both ranking systems at the time the ZERO program publishes long-term follow-up, as well (currently only response data are available; ref. 17). When comparing classification systems used by adult oncology platforms such as MASTER, which applies a similar molecular diagnostic platform as INFORM, one notices that most systems rely on clinical evidence (28). For example, the highest level used in MASTER (NCT/DKTK level m1A) is based on data from a prospective study or a meta-analysis in the same tumor type (29). In MASTER, 17.6% of patients received treatment recommendations of NCT/DKTK levels m1A to m1C (clinical data collected in the same histologic entity), which at this moment is an unobtainable standard in pediatrics because, unfortunately, such clinical evidence is only rarely available in this population (justifying the more functional biological approach used by INFORM).

The reported survival data of the population under study are in line with previous reports of pediatric patients in a phase I/II setting (3, 30), which underlines the enormous medical need in this population. We observed a doubling of PFS for patients with a very high priority level target who received matching targeted treatment compared with all other patients. The baseline performance status of the patients with a very high priority level target who received matching targeted treatment was slightly better compared with all other patients, and in this group, neuroblastoma and HGG were overrepresented. The disease status at enrollment was comparable in both groups, with 80.1% of all patients who were not treated with a matched drug receiving conventional oncologic treatments (mostly chemotherapy), which illustrates that most patients were at least in a sufficient clinical state to be able to receive further therapy. By design, an immortal time bias is observed for patients receiving matching targeted treatment because progression or death in this group could not occur before start of targeted therapy. Poor performance status due to progression of underlying malignant disease was one of the most frequent reasons for not applying matched targeted agents. Importantly, comparison of patients with a very high priority level target who received matching targeted drugs and those who did not showed that patients who did not receive matching targeted drugs had comparable PFS to all other patients and thus confirmed that the respective molecular aberrations are likely predictive for response to a targeted drug and not prognostic for improved outcome. At the start of the registry, a follow-up period of only 2 years

was planned (because it was not expected that a relevant number of patients would be alive after 2 years). This could be the reason for not detecting a significant OS difference between target priority levels, in addition to sample size and nonstandardized administration of other local and systemic therapies.

Inherent to its design as a registry with its noninterventional regulatory status, there are a number of obvious limitations to this study. A registry does not define the treatment of choice (e.g., the use of different ALK inhibitors for the same alteration in the same disease) or exclude other treatments in addition to matching targeted treatment. However, because other treatments are applied in all patient categories, this is likely not of relevance for the observed difference in PFS. Furthermore, the fraction of patients with a very high priority level target who received matching targeted treatment as well as other concomitant treatments was low, and it seems unlikely that the improved PFS can be explained by concomitant treatment. It could, however, dilute weaker signals of lower priority level targets, for example. Furthermore, eligibility criteria are less strict than in clinical trials. In addition, response evaluations are not defined with regard to timing or method (in contrast to clinical trials) and therefore not considered an appropriate outcome measure. Still, due to the high *a priori* chance of relapse in this high-risk population, "progressive disease" does not depend much on the response evaluation method applied. Therefore, its use for PFS determination is considered feasible. Data quality (consistency, accuracy, and completeness) of registries such as INFORM is hampered by the lack of on-site monitoring, audits, and inspections (31), which are especially important for items such as medication information and safety data. Therefore, extensive central monitoring was applied in INFORM to reduce errors and incompleteness as much as possible. Finally, with regard to time-dependent endpoints, a registry such as INFORM has a different baseline definition (completed sample submission and full registration) independent of treatment (or several treatments) compared with a clinical trial. However, despite the limitations of a registry, INFORM has the major advantage that it collects clinical follow-up of all patients regardless of molecular alterations and treatments under real-world clinical conditions, in contrast to clinical trials, which are mostly confined to a defined population with a malignancy harboring a certain biomarker and a respective drug combination.

Unfortunately, compared with adult oncology with numerous new innovative trials, biomarkers, and drugs, pediatric oncology is still lagging behind in access to new compounds (32), which is clearly exemplified by the low number of patients in the INFORM registry enrolled in clinical trials. Optimally, molecular diagnostics should be offered to patients at an earlier stage during their disease course and not only at a stage when precision oncology is essentially "the last hope." Notably, in INFORM, 32.6% of patients received molecular diagnostics only at second or later relapse. A protocol amendment allowing for enrollment in an earlier disease stage is currently under discussion within the steering committee. Furthermore, despite the availability of a genetically altered target (priority levels

very high, high, and moderate), 62% of patients did not receive a targeted agent at all. Surprisingly, 52% patients with a very high priority level target did not receive targeted treatment. Rational combination treatment regimens are important because pathway redundancy, tumor evolution, and molecular cross-talk make it unlikely that targeted monotherapies will result in durable responses, especially in advanced late-stage cancers (20). The clinical outcome data support the requirement of biomarker-driven, cross-entity phase I/II combination trials, as articulated by the INFORM pilot study and others (18, 32). As such, the INFORM consortium has initiated the INFORM2 series of multinational biomarker-driven seamless phase I/II combination trials (33). In addition, other groups have also started large pediatric trial initiatives, such as the European AcSé-ESMART study (clinicaltrials.gov identifier: NCT02813135) and pediatric MATCH in the United States (15). The first INFORM2 trial (INFORM2 NivEnt, clinicaltrials.gov identifier: NCT03838042) is currently recruiting, and further INFORM2 trials are in preparation. In parallel, the INFORM registry will create a continuously growing database for future personalized clinical trials.

In addition to the identification of actionable alterations, the application of the previously described DNA methylation–based classifier for CNS tumors and sarcomas (25, 26) as well as the detection of disease-specific fusions by RNA sequencing contributed to the reevaluation of tumor diagnoses. In a considerable number of cases, changes of the registration diagnosis or refinements were suggested based on molecular results. This is very important for further treatment decisions independent of particular targets.

Our data demonstrate that a significant fraction of pediatric malignancies develop on the background of a CPS, which is often not clinically evident, although some predispositions (e.g., Li-Fraumeni syndrome) may require adaptation of treatment (e.g., for medulloblastoma; clinicaltrials.gov identifier: NCT02066220). Because INFORM primarily enrolls patients with high-risk tumors, certain CPSs are underrepresented (e.g., *NF1* associated with low-grade glioma or *RB1* associated with retinoblastoma). In INFORM, we also identified a number of heterozygous alterations in genes involved in DNA double-strand repair and more prominently known to predispose to adult-onset cancers, such as hereditary breast and ovarian cancer (*ATM*, *BRCA1*, *BRCA2*, *PALB2*, or *SLX4*). In two of these cases, analysis revealed somatic loss of heterozygosity in the tumor, directly supporting a potential contribution of the event in constitutional DNA to tumor initiation or evolution. Still, some of these constitutional variants potentially are not directly related to the tumor and may represent incidental findings, although this requires a more detailed prospective investigation. Comparably stringent criteria and varying composition of predefined lists containing known cancer predisposition genes may partly explain different rates of reported constitutional variant findings between our study and, for example, the ZERO program (17). To be reported, P/LP variants affecting autosomal recessive genes required a clear association with the respective malignancy or a second hit. In addition, we did not report on findings in clear adult-onset cancer predisposition genes (e.g., *HOXB13* or *ATR*).

In conclusion, the INFORM registry has demonstrated that comprehensive and entity-agnostic pediatric precision oncology in a real-world, multinational setting is feasible. The prioritization algorithm identifies subgroups of pediatric patients with relapsed, progressive, or high-risk cancer benefiting from matched targeted treatment with improved PFS. For this population with an enormous medical need and particularly poor prognosis, this can be considered a hopeful start. Furthermore, molecular characterization of the tumors allows for refinement of diagnosis in some patients and identification of underlying CPS. This achievement has been acknowledged by the German health insurance companies, and negotiations to include INFORM diagnostics in their insurance coverage are ongoing. Despite the steadily decreasing costs of next-generation sequencing, this would be an important sustainability strategy with regard to the still-increasing number of samples submitted. To support such financing models in Germany and other participating countries in the future, a health economic impact analysis is planned as an objective in the still-ongoing registry. In most patients, only lower priority level molecular alterations could be identified, and these could not be shown to be associated with improved outcome when treated accordingly. Next to the methodologic limitations of a registry, an important reason for that can be that the relation between target and targeted drug is not always sufficiently tested. A systematic preclinical *in vivo* evaluation of targeted compounds in pediatric disease models is warranted [e.g., through ITCC-P4 (https://www.itccp4.eu)]. By doing that, we will learn more about predictive biomarkers, and the priority algorithm can be adapted accordingly. In addition, we propose that next-level precision oncology programs should encompass further layers of molecular and functional data (e.g., gene signatures, liquid biopsy methodologies, single-cell sequencing technologies, proteomics, drug sensitivity profiling), include patients at an earlier time point, and involve combination therapy strategies to hopefully increase the proportion of patients who may experience a clinical benefit.

METHODS

Study Design, Eligibility, and Patients

The INFORM registry is a prospective, noninterventional, multicenter, multinational, and feasibility registry collecting clinical, functional, and molecular data. In a weekly online tumor board, potential matching drugs and trials were discussed by an expert panel and the treating pediatric oncologist. The treating oncologist was involved in a direct discussion with the expert panel allowing for education and practical support, including a discussion on matching open clinical trials and available approved drugs. The use of target information for clinical decision-making was the responsibility of the treating pediatric oncologist. After a pilot phase (18), the registry opened January 21, 2015. A clinical follow-up of 2 years for all patients was planned (longer follow-up allowed). All patients who had finished their clinical follow-up, either at least 2 years or shorter in case of earlier study participation termination (e.g., if the patient died or was lost to follow-up), on September 30, 2019, were included in this report. Patients registered after October 1, 2017, and still alive and with ongoing follow-up less than 2 years were not included in this report. For patients with samples

not suitable for molecular analysis and/or unsuccessful molecular analysis, no further clinical follow-up was performed. The registry is still ongoing.

Eligible patients had clinically suspected refractory/relapsed/progressive malignant disease and received a biopsy (solid and CNS tumors) or bone marrow aspiration (e.g., leukemia) as part of their standard-of-care treatment at their local pediatric oncology center. Eligible pediatric diagnoses included high-risk acute lymphoblastic leukemia, acute lymphoblastic leukemia after stem cell transplantation, acute myeloid leukemia, non-Hodgkin lymphoma, desmoplastic small round cell tumor, Ewing sarcoma, malignant rhabdoid tumor, neuroblastoma, osteosarcoma, rhabdomyosarcoma, other soft-tissue sarcoma, ependymoma, HGG (including diffuse-intrinsic pontine glioma), medulloblastoma, and other pediatric cancers, including rare tumors such as nephroblastoma, hepatoblastoma, retinoblastoma, malignant endocrine tumors, germ cell tumors, and others. Patients with a primary diagnosis of HGG (including diffuse-intrinsic pontine glioma), soft-tissue sarcoma, embryonal tumor with multilayered rosettes, rare tumors, and exceptional other cases, for which no curative treatment is established, could already be enrolled at primary diagnosis. To allow young adults who relapsed after having had their primary diagnosis at a pediatric age (e.g., sarcoma), patients could be enrolled up to an age of 40 years, provided that they had their primary pediatric-type diagnosis before the age of 21 years. Patients should have measurable disease, and no established curative treatment options should be available. A life expectancy >3 months and sufficient general condition (Lansky ≥50 or Karnofsky ≥50) were requested.

The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients or their legally acceptable representative, or both (if possible), provided written informed consent. Approvals for the study protocol (and any modifications thereof) were obtained from independent ethics committees and the institutional review board at each participating center. The study was registered with the German Clinical Trial Register, number DRKS00007623.

Outcomes

The primary objectives of the INFORM registry were (a) to establish logistics for personalized treatment [on an (inter)national scale], (b) to initiate a comprehensive database providing information about individual druggable targets with parallel documentation of clinical follow-up, and (c) to investigate a predefined molecular target prioritization algorithm, based on the type of alteration and its disease-specific relevance, as described previously (18), for its prognostic and predictive power in a tumor-type agnostic approach. Secondary objectives included further evaluation of clinical benefit (PFS and OS), potential diagnostic refinements by molecular methods such as DNA methylation analysis, and identification of possible hereditary predisposition syndromes.

Procedures

Patients were recruited in Austria, Finland, Germany, Greece, Poland, Sweden, Switzerland, and The Netherlands. Before enrollment, the Society for Pediatric Oncology and Hematology in Germany (GPOH) study group of the respective cancer type or the respective national coordinator for other countries was consulted by the treating center to confirm eligibility. Written informed consent was obtained by the local treating physician, and the patient was registered in a globally accessible web portal (MARVIN, XClinical) in a pseudonymized fashion. Fresh-frozen tumor material of the current refractory/relapsed/progressive disease as well as matching nonmalignant material (EDTA blood, saliva) was submitted. After centralized sequencing/molecular analysis, raw data were subjected to bioinformatics processing and biological filtering, resulting in a list of prioritized actionable targets. The definition of "actionable" refers to a detected molecular alteration or affected pathway that theoretically would be targetable by an approved drug or an investigational agent in any phase of clinical development, either directly or indirectly in the affected pathway (16). Alterations included copy-number alterations, single-nucleotide variants, indels, gene fusions, outlier expression of individual genes, and expression of fusion transcripts. In a weekly online molecular tumor board, an expert panel consisting of molecular biologists, pediatric oncologists including the GPOH coordinator of the respective disease (or the respective national coordinator), and pharmacologists discussed, reviewed, and prioritized the identified targets together with the treating pediatric oncologist. Potential matching drugs and trials were also discussed together, but the registry did not give treatment recommendations (giving treatment recommendations would be an intervention and would be an interventional clinical trial according to the German Medicinal Products Act). The use of target information for clinical decision-making on an individual basis remained exclusively the responsibility of the treating pediatric oncologist. Thereafter, the targets were deposited in the globally accessible web portal as the final target report. The treating physician had password-secured access to their patients' data deposited in the web portal. They could use the molecular target information for clinical decision-making on an individual basis. Reporting and discussion of the molecular target information and potential matched treatments and trials with patients and their families was performed by the treating physician, too. Disease evaluations were performed according to routine standard of care in line with (inter)national disease-specific guidelines. Clinical and molecular baseline and follow-up data, including (concomitant) treatments, of all patients were prospectively documented in the web portal by the treating center. Clinical outcome data were centrally monitored in the web portal for plausibility and completeness by INFORM research physicians supported by data management. If necessary, queries for the local sites were generated in the web portal. At baseline, medical history and treatment were checked for plausibility and completeness by research physicians in preparation for the online molecular tumor board. During follow-up, data management monitored for completeness at regular intervals. After follow-up was finished and documentation was completed by the local sites, research physicians checked treatment and response data for plausibility. No site monitoring was performed.

A predisposition to cancer (CPS) may be critical for the affected patient as it may influence treatment decisions but could also affect carriers within the family. Prior to any analyses of constitutional DNA, patients and/or legal guardians received genetic counseling. To allow for a more precise interpretation of somatic variants detected in tumors, constitutional DNA isolated from white blood cells, alternatively from saliva, was sequenced in parallel. Variants identified in tumor tissues were reviewed in the constitutional genetic code from normal blood cells to infer true somatic mutations. If the patient and/or legal guardians had consented to investigation for a genetic cancer predisposition during the informed consent process, constitutional DNA of each patient was screened for damaging alterations in a predefined list of 157 known cancer predisposition genes (Supplementary Table S7b). Potential constitutional alterations were assessed by human geneticists according to American College of Medical Genetics and Genomics (ACMG) criteria (34), and only likely pathogenic (ACMG class 4) or pathogenic (ACMG class 5) variants with a probable relation to the patient's cancer diagnosis were reported to the treating physician, and genetic counseling of the patient and the family was recommended. Constitutional variant findings were incorporated in the target prioritization only if they represented an actionable target.

Molecular Profiling and Prioritization Algorithm

Within the INFORM pilot phase between October 2013 and January 2015, logistic and analytic pipelines necessary for rapid and comprehensive molecular profiling in a clinical setting have been established and described (18). In brief, fresh-frozen tumor material and nonmalignant DNA (e.g., from blood sample) was subjected to WES, lcWGS, RNA sequencing, RNA-based gene expression array, and DNA-methylation. RNA-sequencing data were used for identification of actionable fusion genes and for assessment of outlier gene expression of actionable genes in a given sample compared with a within- and across-malignant disease type reference series. The platform has an overall validation rate of 99.3% (range, 98.2%–100%) across the three test systems (WES, RNA sequencing, WGS) used. Only those variants receiving a "high-confidence" score based on an in-house quality assessment were considered. This metric takes into account features such as mappability of the region, coverage at the position, strand bias of reads, and number of variant reads in the control sample. No strict cutoffs in terms of variant allele fraction are applied, but in practice the quality score gives a lower variant allele fraction limit of approximately 0.05 for reported variants.

As previously described (18), actionable and tumor biologically relevant findings were prioritized in a standardized way based on a seven-scale score ranging from "very high" to "very low," depending on the alteration type and its disease-specific relevance. As new evidence for certain treatments or relevance of specific alterations emerged during the study period, the target priority level could have changed over time. For the present analysis, the priority levels reported back to the treating physician after the respective interdisciplinary review were used.

Statistical Analysis

Survival time was defined as time since all necessary samples were received for molecular analysis. Patients who died or had a disease progression in the time between registration and sample receipt completion were excluded from the PFS analyses. Patients who died in the time between registration and sample receipt completion were excluded from OS analyses. OS is defined as time since all necessary samples were received to death from any cause. PFS is defined as time since all necessary samples were received until progression or death. PFS and OS comparisons were performed by log-rank tests.

Data Availability

WES, lcWGS, RNA-sequencing, and methylation data generated by this study are available from the European Genome Archive, accession number EGAS00001005112.

Authors' Disclosures

Authors' Contributions

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Note

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