
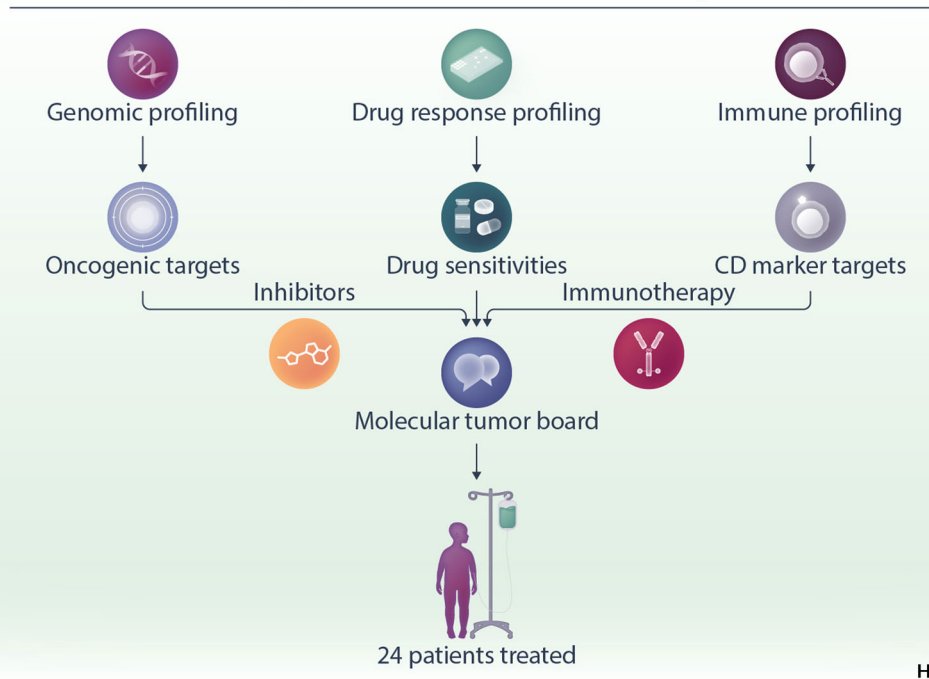


# Oncogenic and immunological targets for matched therapy of pediatric blood cancer patients: Dutch iTHER study experience


Judith M. Boer<sup>1,^</sup>  | Uri Ilan<sup>1,^</sup> | Aurélie Boeree<sup>1</sup> | Karin P. S. Langenberg<sup>1</sup> | Jan Koster<sup>2</sup> | Marco J. Koudijs<sup>1</sup> | Jayne Y. Hehir-Kwa<sup>1</sup> | Stefan Nierkens<sup>1,3</sup> | Corinne Rossi<sup>4</sup> | Jan J. Molenaar<sup>1</sup> | Bianca F. Goemans<sup>1,^</sup> | Monique L. den Boer<sup>1,5,^</sup> | C. Michel Zwaan<sup>1,5,^</sup>

## Graphical Abstract

56 pediatric patients with relapsed / refractory / high-risk hematological malignancies included in Dutch iTHER study



# Oncogenic and immunological targets for matched therapy of pediatric blood cancer patients: Dutch iTHER study experience

Judith M. Boer<sup>1,^</sup>  | Uri Ilan<sup>1,^</sup> | Aurélie Boeree<sup>1</sup> | Karin P. S. Langenberg<sup>1</sup> | Jan Koster<sup>2</sup> | Marco J. Koudijs<sup>1</sup> | Jayne Y. Hehir-Kwa<sup>1</sup> | Stefan Nierkens<sup>1,3</sup> | Corinne Rossi<sup>4</sup> | Jan J. Molenaar<sup>1</sup> | Bianca F. Goemans<sup>1,^</sup> | Monique L. den Boer<sup>1,5,^</sup> | C. Michel Zwaan<sup>1,5,^</sup>

Correspondence: Judith M. Boer ([j.m.boer-20@prinsesmaximacentrum.nl](mailto:j.m.boer-20@prinsesmaximacentrum.nl))

## Abstract

Over the past 10 years, institutional and national molecular tumor boards have been implemented for relapsed or refractory pediatric cancer to prioritize targeted drugs for individualized treatment based on actionable oncogenic lesions, including the Dutch iTHER platform. Hematological malignancies form a minority in precision medicine studies. Here, we report on 56 iTHER leukemia/lymphoma patients for which we considered cell surface markers and oncogenic aberrations as actionable events, supplemented with ex vivo drug sensitivity for six patients. Prior to iTHER registration, 34% of the patients had received allogeneic hematopoietic cell transplantation (HCT) and 18% CAR-T therapy. For 51 patients (91%), a sample with sufficient tumor percentage ( $\geq 20\%$ ) required for comprehensive diagnostic testing was obtained. Up to 10 oncogenic actionable events were prioritized in 49/51 patients, and immunotherapy targets were identified in all profiled patients. Targeted treatment(s) based on the iTHER advice was given to 24 of 51 patients (47%), including immunotherapy in 17 patients, a targeted drug matching an oncogenic aberration in 12 patients, and a drug based on ex vivo drug sensitivity in one patient, resulting in objective responses and a bridge to HCT in the majority of the patients. In conclusion, comprehensive profiling of relapsed/refractory hematological malignancies showed multiple oncogenic and immunotherapy targets for a precision medicine approach, which requires multidisciplinary expertise to prioritize the best treatment options for this rare, heavily pretreated pediatric population.

## INTRODUCTION

Among children treated for a hematological malignancy, 10%–30% suffer from relapsed disease. High-risk B-cell precursor (BCP) and T-cell relapsed acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma patients are often chemotherapy-resistant and have dismal outcomes.<sup>1,2</sup> Similarly, relapsed acute myeloid leukemia (AML), especially second relapses or refractory first relapses, have poor survival outcomes.<sup>3–5</sup> International treatment protocols have been

implemented for the first relapse of ALL and AML,<sup>6</sup> however, for further relapses or refractory disease, no standard treatment is available, and a personalized approach is needed. Recently, the use of immunotherapy has improved relapse treatment outcomes, such as blinatumomab for relapsed BCP-ALL<sup>7</sup> and gemtuzumab ozogamicin for relapsed AML.<sup>8</sup> In addition, targeted small molecules, directed to specific characteristics of cancer cells, can be implemented as single drugs or given in combination to re-sensitize relapsed cancer cells to chemotherapy.

<sup>1</sup>Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands

<sup>2</sup>Amsterdam UMC, University of Amsterdam, Center for Experimental and Molecular Medicine, Laboratory of Experimental Oncology and Radiobiology, Amsterdam, The Netherlands

<sup>3</sup>Center for Translational Immunology, UMC Utrecht, Utrecht, The Netherlands

<sup>4</sup>Department of Pediatric Oncology, Hematology, and Immunology, Heidelberg University Hospital, Heidelberg, Germany

<sup>5</sup>Department of Pediatric Oncology and Hematology, Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, The Netherlands

<sup>^</sup>Judith M. Boer and Uri Ilan contributed equally to this work. Bianca F. Goemans, Monique L. den Boer, and C. Michel Zwaan shared senior authors.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Author(s). *HemaSphere* published by John Wiley & Sons Ltd on behalf of European Hematology Association.

To identify targets for immunotherapy and small molecular inhibitors, leukemia, and lymphoma cells are analyzed by flow cytometry and targeted or comprehensive next-generation sequencing techniques. Recently, functional assays, including ex vivo drug response profiling, have been added to identify patient-specific vulnerabilities.<sup>9</sup> To facilitate the translation of tumor profiling results to clinical decision-making, institutional and national molecular tumor boards have been implemented. In these multidisciplinary platforms, relapsed and refractory pediatric cancer cases without standard-of-care treatment options are discussed to prioritize targeted drugs for individualized treatment based on actionable oncogenic lesions.<sup>10–15</sup> Pediatric patients with relapsed/refractory hematological malignancies form a minority in pan-pediatric cancer precision medicine programs, ranging from 9% to 21%.<sup>10,12,13,15</sup> On the one hand, this reflects the excellent outcomes for many subtypes of leukemia and lymphoma on first-line and first-relapse treatment protocols as well as the availability of effective immunotherapies for salvage therapy. On the other hand, the (multiple) relapse hemato-oncology cases enrolled in precision medicine programs are often challenging because they failed prior treatments, including allogeneic hematopoietic cell transplant (HCT) and immunotherapy including CAR T-cell therapy.

In the Netherlands, the Dutch individualized THERapy (iTHER) study was conducted from 2017 to 2022 as a prospective non-interventional study.<sup>10</sup> Here, we describe the results for the hematological malignancies, comprising 15% of the iTHER cases. We considered lineage-specific cell surface markers and oncogenic aberrations, supplemented with ex vivo drug sensitivity, as actionable events and reported on the matched targeted therapies.

## MATERIALS AND METHODS

### Study design

The design, patients, and sample inclusion of the iTHER study (Netherlands Trial Register NL5728) were described previously.<sup>10</sup> All patients or their parents provided written informed consent to participate in the study according to the Declaration of Helsinki. The consent included analysis of actionable events, drug response profiling, and discussion in a multidisciplinary tumor board. The iTHER study included 1 year of follow-up, during which time the administered targeted treatment and immunotherapy were registered. Patient status (alive or deceased) was available for all patients at the end of observation, August 2023. Follow-up time per patient was calculated from the date of molecular tumor board advice. Median follow-up for patients who were alive at the last observation was 33.5 months for patients ( $n = 12$ ) who received targeted treatment after molecular tumor board advice and 32.0 months for patients ( $n = 15$ ) who could not be profiled or did not receive targeted treatment after the molecular tumor board.

### Molecular profiling

Tumor DNA and RNA were isolated from bone marrow aspirates, peripheral blood, or fresh-frozen biopsies with  $\geq 20\%$  tumor cells. A matching germline sample was taken from a remission timepoint or a skin biopsy. In case of a relapse after allogeneic HCT, when available, an additional germline sample derived from donor DNA or a posttransplantation remission sample with 100% donor chimerism was included for DNA sequencing to subtract donor germline variants. For cases enrolled until March 6, 2020, molecular profiling was performed using whole exome sequencing, low coverage whole

genome sequencing, methylation profiling, and messenger RNA sequencing by the German INFORM program, which kindly provided the data.<sup>12,16</sup> Cases profiled after March 6, 2020, were analyzed using whole exome sequencing and whole transcriptome sequencing at the Laboratory for Childhood Cancer Pathology of the Princess Máxima Center. Whole exome sequencing data were filtered for 618 cancer genes selected by INFORM (Supporting Information S1: Table S1). The profiling data were visualized using the R2 platform (<https://r2.amc.nl>). Target identification, prioritization, and reporting at the Molecular Tumor Board were performed as described previously.<sup>10</sup> The pathogenicity and recurrence of aberrations were evaluated using the PeCan database and PeCan PIE (<https://pecan.stjude.cloud>), ClinVar,<sup>17</sup> and QIAGEN Clinical Insight. Somatic aberrations reported included (likely) pathogenic nucleotide variants with a variant allele frequency of  $>10\%$ , deletions with a  $\log_2$  ratio  $\leq -1$  (mono-allelic) or  $\leq -2$  (bi-allelic), amplifications with a  $\log_2$  ratio  $\geq 2$ , overexpression with  $z$ -score  $\geq 2.5$  (relative to the complete iTHER cohort), gene fusions, and rearrangements as described previously.<sup>10</sup> For AML patients, the methylation status of a specific region in the first intron of *FDFT1* was considered, which was described as a prediction marker for response to demethylating agents when unmethylated.<sup>18</sup>

### Immunoprofiling

Flow cytometry data with disease-specific flow panels was performed for routine clinical purposes in the Laboratory for Childhood Cancer Pathology of the Princess Máxima Center using standardized Euro-Flow operating procedures.<sup>19</sup>

### Drug response profiling

Ex vivo drug response profiling was performed for selected samples with sufficient cryopreserved cells ( $\geq 50$  million) available with  $>40\%$  blasts. Drug exposures were performed in co-culture assays essentially as described previously.<sup>20</sup> After thawing of viable frozen patient cells,  $1 \times 10^5$  leukemic cells were seeded with  $1 \times 10^4$  h-TERT immortalized human bone marrow-derived mesenchymal stromal cells. After 18 h, the co-cultures were exposed to a drug library at five different drug concentrations in duplicate. Readout of cell viability was performed on Cyquant stained cells using high-content automated microscopy discriminating leukemic cells from stromal support cells and related to cell viability in dimethyl sulfoxide control wells. Multiple aspects of the dose-response curves for each drug, including  $IC_{50}$ , slope, and minimum and maximum response, were combined into the drug sensitivity score (DSS) as described previously.<sup>21</sup> Drug response profiling results were reported in the molecular tumor board, including samples with minor quality issues, indicated in Supporting Information S1: Table S3.

### Target prioritization

Biological prioritization of actionable somatic events was performed using the INFORM 7-scale prioritization algorithm, as delineated by Worst et al.<sup>16</sup> The algorithm systematically prioritizes events based on druggability, genetic alterations, direct inhibitory potential, evidential support levels, and entity specificity. Resulting scores from the algorithm span a continuum from very high to very low, indicative of the degree of prioritization assigned to each event.

## RESULTS

### Baseline characteristics of hemato-oncology iTHER cases

Between November 2017 and April 2022, 56 patients with relapsed/refractory pediatric hematological malignancies were included in the iTHER study (Table 1 and Supporting Information S1: Table S2). The median patient age at inclusion was 13 years (range 0.9–23), with 17 patients (30.4%) aged 14–17 years and nine young adult patients (16.1%) relapsing from childhood leukemia. Disease entities included 25 acute lymphoblastic leukemia (ALL), 18 acute myeloid leukemia (AML) including a secondary AML after an initial diagnosis of BCP-ALL, 10 non-Hodgkin lymphoma (NHL), two Hodgkin lymphoma (HL) and one stem cell leukemia/lymphoma (SCLL). All T-ALL and lymphoma cases were included at the first relapse, primary refractory, or

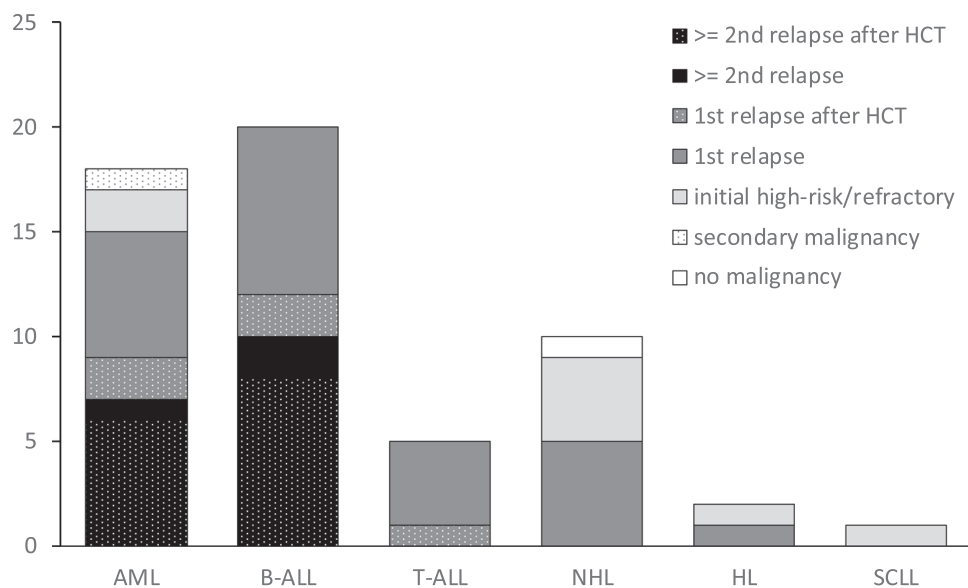
primary high-risk disease, while 39% of AML (7/18) and 50% of BCP-ALL (10/20) cases were included at the second or further relapse. (Figure 1). The iTHER-included cases were enriched for poor prognostic genetic subtypes: among AML, five *KMT2A*-rearranged cases and three acute megakaryoblastic leukemia cases with typical fusions (*CBFA2T3::GLIS2*, *NUP98::NSD1*, and *RBM15::MKL1*), and among BCP-ALL two *KMT2A*-rearranged, two intrachromosomal amplification of chromosome 21 (*AMP21*), three ABL-class fusions and one near haploid case were included. One case, presenting with B-cell lymphoma (NHL06), was a known Li-Fraumeni patient who was previously diagnosed with osteosarcoma. This patient and other pathogenic germline variants in iTHER patients have been described previously.<sup>10</sup> None of the other subjects in this hematological cohort had pathogenic germline variants.

Of the 56 cases, 17 (30%) were included upon their second or higher relapse and 29 (52%) at first relapse. The remaining patients

**TABLE 1** Overview of iTHER hemato-oncology patients included, profiled, and treated.

Characteristic	iTHER cohort N = 56 (100%)	iTHER profiled N = 51 (91%)	Inhibitor treatment N = 13 <sup>a</sup>	Immuno- therapy N = 17 <sup>a</sup>
<b>Sex</b>				
Female	16 (28.6%)	16 (31.4%)	4	3
Male	40 (71.4%)	35 (68.7%)	9	14
<b>Age (years)</b>				
Median (range)	13 (0.9–23)	13 (0.9–23)	13 (1–23)	14 (2–23)
0–5	10 (17.9%)	10 (19.6%)	4	3
6–13	20 (35.7%)	19 (37.3%)	5	5
14–17	17 (30.4%)	14 (27.5%)	2	5
18 and older	9 (16.1%)	8 (15.7%)	2	4
<b>Disease type</b>				
Acute myeloid leukemia	18 (32.1%)	17 (33.3%)	4	1
Acute lymphoblastic leukemia	25 (44.6%)	24 (47.1%)	7	15
B lineage	20 (35.7%)	19 (37.3%)	6	13
T lineage	5 (8.9%)	5 (9.8%)	1	2
Non-Hodgkin lymphoma	10 (17.9%)	9 (17.6%)	1	1
B lineage	6 (10.7%)	6 (11.8%)	0	1
T lineage	2 (3.6%)	2 (3.9%)	0	0
Other (NK, ALCL)	2 (3.6%)	1 (2.0%)	1	0
Hodgkin lymphoma	2 (3.6%)	0 (0%)		
Stem cell leukemia/lymphoma	1 (1.8%)	1 (2.0%)	1	0
<b>Disease stage</b>				
Initial high-risk disease	3 (5.4%)	3 (5.9%)	2	0
Primary refractory	5 (8.9%)	4 (7.8%)	1	0
First relapse	29 (51.8%)	26 (51.0%)	7	10
Without prior HCT	24 (42.9%)	22 (43.1%)	6	9
After HCT	5 (8.9%)	4 (7.8%)	1	1
Second or subsequent relapse	17 (30.4%)	17 (33.3%)	3	4
Without prior HCT	3 (7.3%)	3 (5.9%)	1	1
After HCT	14 (25.0%)	14 (27.5%)	2	3
Secondary disease	1 (1.8%)	1 (2.0%)	0	1
No malignancy	1 (1.8%)	0 (0%)		

<sup>a</sup>Six patients were treated with both targeted therapy and immunotherapy; in total 24 of 51 profiled patients (47%) were treated according to the molecular tumor board's advice.



**FIGURE 1** Types of hemato-oncology samples included in the iTHER study. Each bar represents a disease type, AML, acute myeloid leukemia; B-ALL, B-cell precursor acute lymphoblastic leukemia; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; SCLL, stem cell leukemia/lymphoma; T-ALL, T-cell acute lymphoblastic leukemia. Disease stages are indicated by patterns, white = no malignancy; white with pattern = secondary malignancy; light gray = initial high-risk or primary refractory; dark gray = first relapse; black = second or further relapse, dotted pattern indicates relapse after allogeneic stem cell transplantation. The Y-axis shows the number of cases.

were included because of initial high-risk or primary refractory disease or a secondary malignancy (Table 1). Nineteen patients (34%) had undergone allogeneic HCT prior to iTHER inclusion (Table 1 and Figure 1). Overall, 34 patients (61%) had received previous anti-CD marker immunotherapy, including 10 patients who received one or more CAR-T cell infusions. Five cases (9%) received targeted treatment prior to the iTHER molecular tumor board. Three types of actionable events were considered for the iTHER hematological malignancy cases. DNA and RNA sequencing were performed to identify oncogenic alterations, drug response profiling was performed to detect drug sensitivities, and flow cytometry was performed to detect surface expression of lineage markers (Figure 2A). Basic clinical characteristics and information on prior treatment, actionable events, and treatment given after molecular tumor board advice are summarized in Supporting Information S1: Table S2.

### Identification of actionable oncogenic alterations

For 49 of 56 patients, a bone marrow aspirate or peripheral blood sample with sufficient tumor cell percentage ( $\geq 20\%$ ) was available for molecular analyses at relapse or refractory disease (Table 1). For two additional cases, a tumor sample of the initial diagnosis was used for profiling: a T-ALL with molecular relapse after induction therapy and a BCP-ALL presenting at relapse with histiosarcoma and very low bone marrow involvement. For four patients, the tumor percentage of the sample was below 5%, and for one patient, no relapse or progression was found in the biopsy; these patients were not included in actionable event profiling. The median time between study registration and discussion in the iTHER molecular tumor board was 40 days (range 23–74 days) for iTHER1 (sample shipment and profiling at INFORM) and 25 days (range 3–54 days) for iTHER2 (profiling at the Princess Máxima Center) (Supporting Information S1: Figure S1).

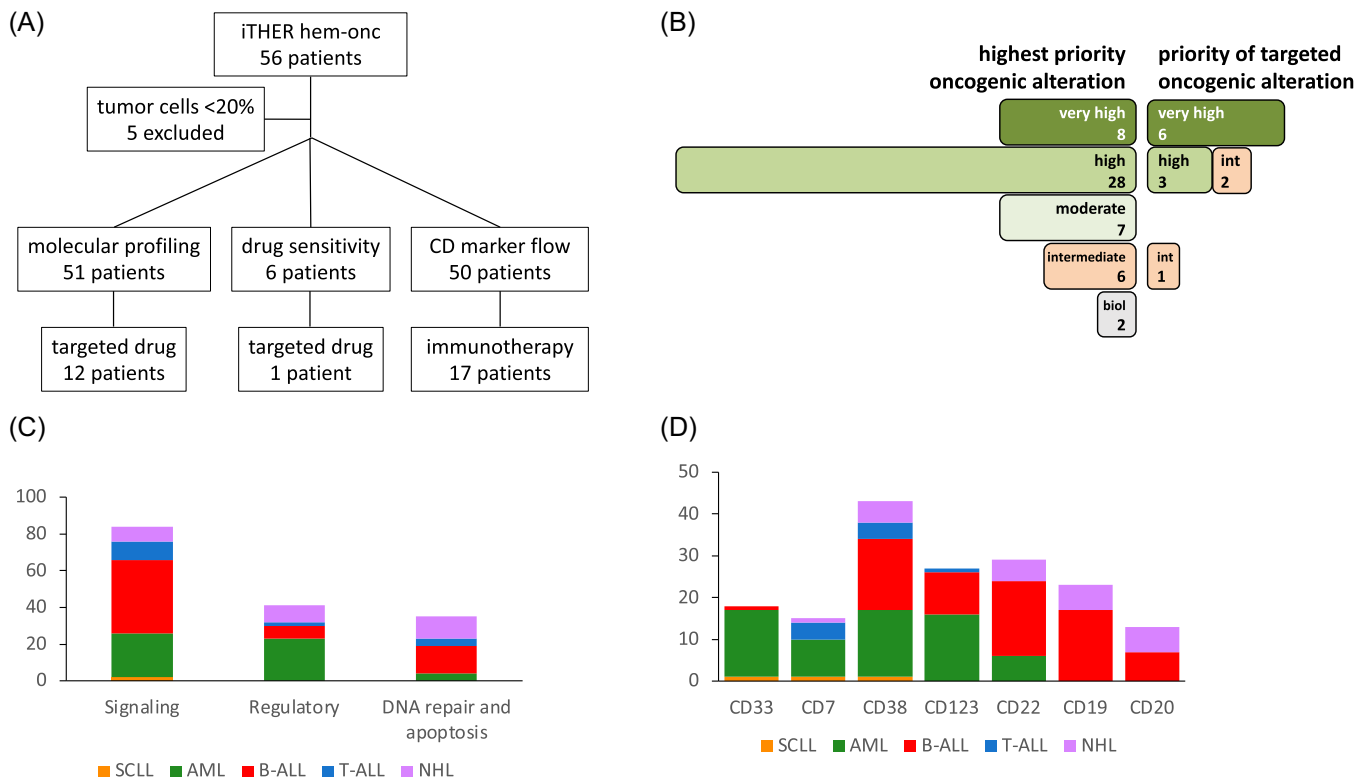
At the time of analysis, targetable oncogenic alterations were identified in 49 of 51 profiled cases with 1–10 (median 3) alterations

per case. For 2/51 (4%) of profiled cases, with 26% and 90% tumor cells, respectively, only nontargetable oncogenic alterations with biological relevance were identified: one patient with a *TP53* inactivating mutation and one patient with a *DUX4* rearrangement. The prioritization of the actionable events was evaluated using the 7-scale prioritization algorithm described previously,<sup>12,16</sup> favoring genetic aberrations overexpression changes and direct targeting over pathway inhibition. Among the 49 cases with at least one targetable oncogenic alteration, the highest prioritized target was “very high” (level 1 of 7) for 8 cases (16%), “high” (level 2 of 7) for 28 cases (57%), “moderate” (level 3 of 7) for 7 cases (14%), and “intermediate” (level 4 of 7) for 6 cases (12%). In summary, for 49 out of 51 (96%) of the profiled hemato-oncology cases, at least an intermediate priority target was identified (Figure 2B).

Specimens taken from two locations were profiled for four patients, for example, tumor cells from an involved lymph node and bone marrow for the SCLL patient. The actionable events corresponded to multiple biopsies from the same patient, suggesting that the targeted therapy would address the different disease locations (Supporting Information S1: Figure S2).

### Genes and pathways affected by oncogenic alterations

Somatic alterations were reported for targetability or biological relevance. Gene alterations included solely for biological relevance in our hematological cohort comprised of rearrangements of *DUX4* and *BCL6* as oncogenic drivers, *IKZF1* and *PAX5* as cooperating lesions, and *TP53* inactivation as treatment resistance markers. Targetable oncogenic alterations were identified in 55 genes (Figure 3). The affected genes were grouped according to affected pathway and class of targeted drugs (Figure 2C and Table 2), showing that the most frequently affected pathways were RAS-MAPK signaling (AML and ALL), JAK-STAT and ABL/SRC/other tyrosine kinase signaling (mostly



**FIGURE 2** Overview of profiling approaches and results. (A) Overview of numbers of patients profiled and resulting targeted and immune treatments given. (B) The highest priority target identified per patient with molecular profiling (bars to the left) and the priority of the target that was chosen for targeted treatment (bars to the right). Priorities according to the INFORM 7-scale decision tree. biol, not targetable but of biological relevance; int, intermediate. (C) Summary of pathways affected by oncogenic alterations for the five disease entities. (D) Summary of CD-marker positivity (including weak, partial, and heterogeneous staining) for the five disease entities.

AML and ALL), FLT3 signaling (AML and BCP-ALL), DNA damage sensing and PI3K/MTOR signaling (ALL and NHL), and *KMT2A/NUP98/DOT1L* aberrations (mostly AML). Aberrations predicting sensitivity to hypomethylating agents, affecting *WT1*, *SETD2*, *SUZ12*, *DNMT3B*, and *TERT*, were identified mostly AML and NHL. Somatic *TP53* aberrations occurred in 17.6% of the patients, including DNA binding domain mutations, which are potentially druggable with conformation-correcting drugs.<sup>22</sup>

### Sensitivities from drug response profiling

For six patients, sufficient cells were available to perform a high-throughput drug screen. The results were available within a range of 2–4 weeks. The drug sensitivity scores for commonly used chemotherapeutics and inhibitors targeting the identified somatic aberrations are summarized in Supporting Information S1: Table S3. Three of the six patients (AML17, BALL19, and SCLL) were positive for a tyrosine kinase fusion and showed ex vivo sensitivity to the matched inhibitor. Patient NHL09, with *CDKN2A* deletion identified as a targetable event, showed intermediate sensitivity to bortezomib in the drug screen.

### Targeted inhibitor treatment after iTHER advice

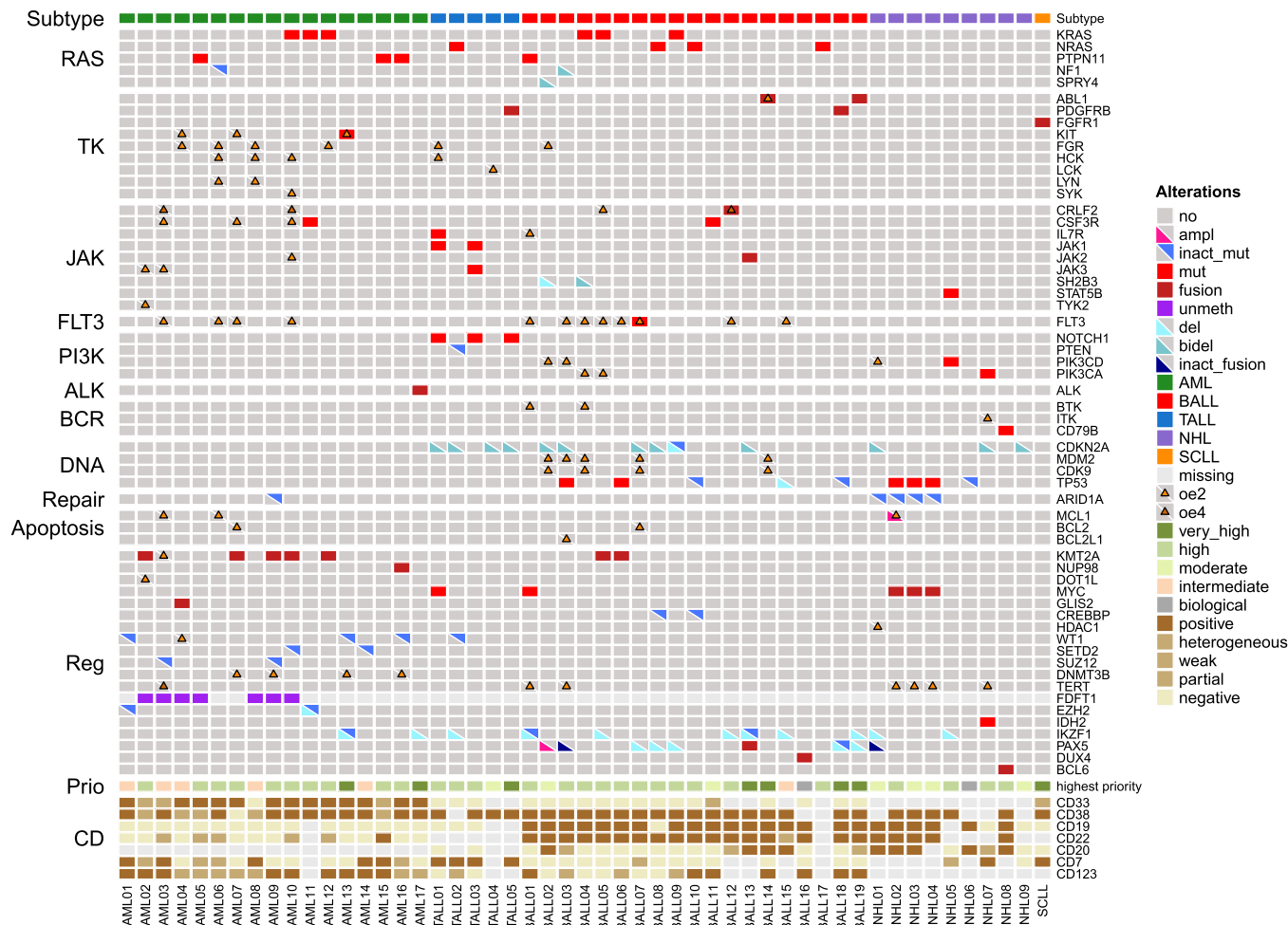
Targeted inhibitor treatment based on the iTHER advice was given to 25% (13/51) of the profiled patients; tyrosine kinase inhibitors and MEK inhibitors were most frequently used. The

targeted inhibitor was chosen solely based on ex vivo drug sensitivity in one patient (NHL09) and supported the choice for a targeted inhibitor in several other patients (Supporting Information S1: Table S3). The targeted drug matched an oncogenic aberration in 12 patients, including six patients with a gene fusion prioritized as “very high” (Supporting Information S1: Table S4). Targeted inhibitor therapy was combined with chemotherapy, for example, cytarabine in AML patients and dexamethasone or other induction therapy drugs in ALL patients (Supporting Information S1: Table S4). Complete remission was achieved in 9/13 patients (69%) treated with targeted inhibitors. Six patients proceeded to HCT, and three patients to CAR-T therapy (Figure 4).

### Targeted immunotherapy after iTHER advice

Cell surface expression determined by flow cytometry and/or RNA expression was routinely reported for CD33, CD123, CD38, CD19, CD22, CD20, and CD9, and targets for immunotherapy were identified in 49/50 (98%) profiled patients (Figures 2D and 3 and Table 2). Targeted immunotherapy treatment after molecular tumor board advice was given to 34% (17/50) of the profiled patients, the large majority BCP-ALL. Eight patients were treated sequentially with different immunotherapy treatments, resulting in 32 immunotherapy treatments given (Figure 4, Supporting Information S1: Tables S5 and S6). Most frequently used were anti-CD19 directed CAR-T cells (11 patients), the anti-CD22 directed antibody-drug conjugate inotuzumab ozogamicin (eight patients),





**FIGURE 3** Overview of actionable events in 51 hematological malignancies. Columns represent iTHER cases, ordered by disease type. Top row color code for disease type: green, AML; blue, T-ALL; red, BCP-ALL; purple, NHL; orange, SCLL. Upper rows represent recurrently aberrant genes, ordered by pathway. Row labeled Prio shows the highest priority actionable event detected by molecular profiling. Bottom rows represent cell surface positivity for seven CD markers. Abbreviations oncogenic aberrations: ampl, amplification with  $\log_2$  ratio  $\geq 2$ ; del, deletion with  $\log_2$  ratio  $\leq -1$ ; bidel, bi-allelic deletion with  $\log_2$  ratio  $\leq -2$ ; inact\_mut, loss-of-function mutation with variant allele frequency  $>10\%$ ; mut, activating mutation with variant allele frequency  $>10\%$ ; fusion, chimeric transcript or promoter/enhancer rearrangement; unmeth, unmethylated; oe2, overexpression with z-score  $\geq 2.5$ ; oe4, overexpression with z-score  $\geq 4$ .

and the anti-CD19/CD3 directed bispecific antibody blinatumumab (four patients). Six patients were treated both with an inhibitor targeting an oncogenic aberration and immunotherapy. Three patients received these types of treatments sequentially to treat a new relapse or progressive disease, while three other patients were treated with both treatment modalities in the same course to maximize the chances for response with acceptable toxicity. For example, two T-ALL patients received a combination of daratumumab and dasatinib, and a BCP-ALL patient was treated with inotuzumab ozogamicin and dasatinib. The choice for a targeted inhibitor or targeted immunotherapy after MTB advice for patients with both types of targets seemed to depend on the priority of the oncogenic event. For example, all six patients with BCP-ALL and a RAS pathway aberration received immunotherapy, and only patients BALL03 and BALL10 were also treated with a MEK inhibitor (priority high); vice versa, all three patients with BCP-ALL and an ABL-class fusion (priority very high) received a tyrosine kinase inhibitor and only BALL18 was also treated with immunotherapy (Figure 4). After immunotherapy, 16/17 patients reached CR (94%),

of which 10 patients subsequently underwent HCT within 1 year of MTB advice.

### Follow-up

Of the 24 patients starting targeted inhibitor and/or immunotherapy within 1 year after molecular tumor board advice, 18 (75%) were alive after 1 year. Of the 32 patients who were included in iTHER but could not be profiled or did not receive targeted inhibitor and/or immunotherapy after the molecular tumor board, 20 (63%) were alive after 1 year. Of these 32 patients, in one patient, no malignancy was found, two patients died before the advice was known, and four patients had progressive disease. For the remaining 26 patients, the disease responded to the therapy that was started before iTHER registration or while waiting for the profiling results: immunotherapy only for seven patients, of which 3 CAR-T, immunotherapy plus chemotherapy for eight patients, and chemotherapy only for 10 patients. Overall, around 50% of the patients were in complete

**TABLE 2** Summary of actionable events in hematological malignancies. (continued on next page)

Target	Event	AML (n = 17)	B-ALL (n = 19)	T-ALL (n = 5)	NHL/SCLL (n = 10)	Tot (n = 51)	Targeted therapy
RAS-MAPK signaling	KRAS, NRAS, PTPN11 activation; NF1, SPRY4 inactivation	7	9 <sup>3</sup>	1	0	17	MEK1/2 inhibitor
ABL, SRC, other TK signaling	ABL1, PDGFRB, FGFR1, JAK2 fusions; KIT activation	1	4 <sup>3</sup>	1 <sup>1</sup>	1 <sup>1</sup>	7	Tyrosine kinase inhibitor
	KIT, FGR, HCK, LCK, LYN, SYK, PDGFRB overexpression	6 <sup>1</sup>	1	2	0	9	
JAK-STAT signaling	JAK1/2/3, IL7R, CSF3R, CRLF2, STAT5B activation/rearrangement; SH2B3 inactivation	1	5	2	1	9	JAK1/2 inhibitor
	JAK1/2/3, IL7R, CSF3R, CRLF2, TYK2 overexpression	4	3	0	0	7	
FLT3 signaling	FLT3 activation	0	1	0	0	1	FLT3 inhibitor
	FLT3 overexpression	4	8	0	0	12	
PI3K-AKT-MTOR signaling	PIK3CD, PIK3CA, NOTCH1, PTEN activation	0	0	4	3	7	PI3K/MTOR inhibitor
	PIK3CD, PIK3CA overexpression	0	4	0	1	5	
ALK signaling	ALK fusion	1 <sup>1</sup>	0	0	0	1	ALK inhibitor
BCR signaling	BTK, ITK overexpression	0	2	0	2	4	BTK inhibitor
	CD79B activation	0	0	0	1	1	
DNA damage sensing	CDKN2A/B/ARF4 inactivation	0	6	4	3	13	CDK4/6, MDM2, CDK2/ 9 inhibitor
	MDM2, CDK9 overexpression	0	5	0	0	5	
TP53	DNA binding domain mutation	0	2	0	3	5	Conformation correction
DNA repair	ARID1A inactivation	1	0	0	4	5	PARP inhibitor
Apoptosis	MCL1 amplification	0	0	0	1	1	Antiapoptosis inhibitors
	BCL2, BCL2L1, MCL1 overexpression	3	2	0	1	6	



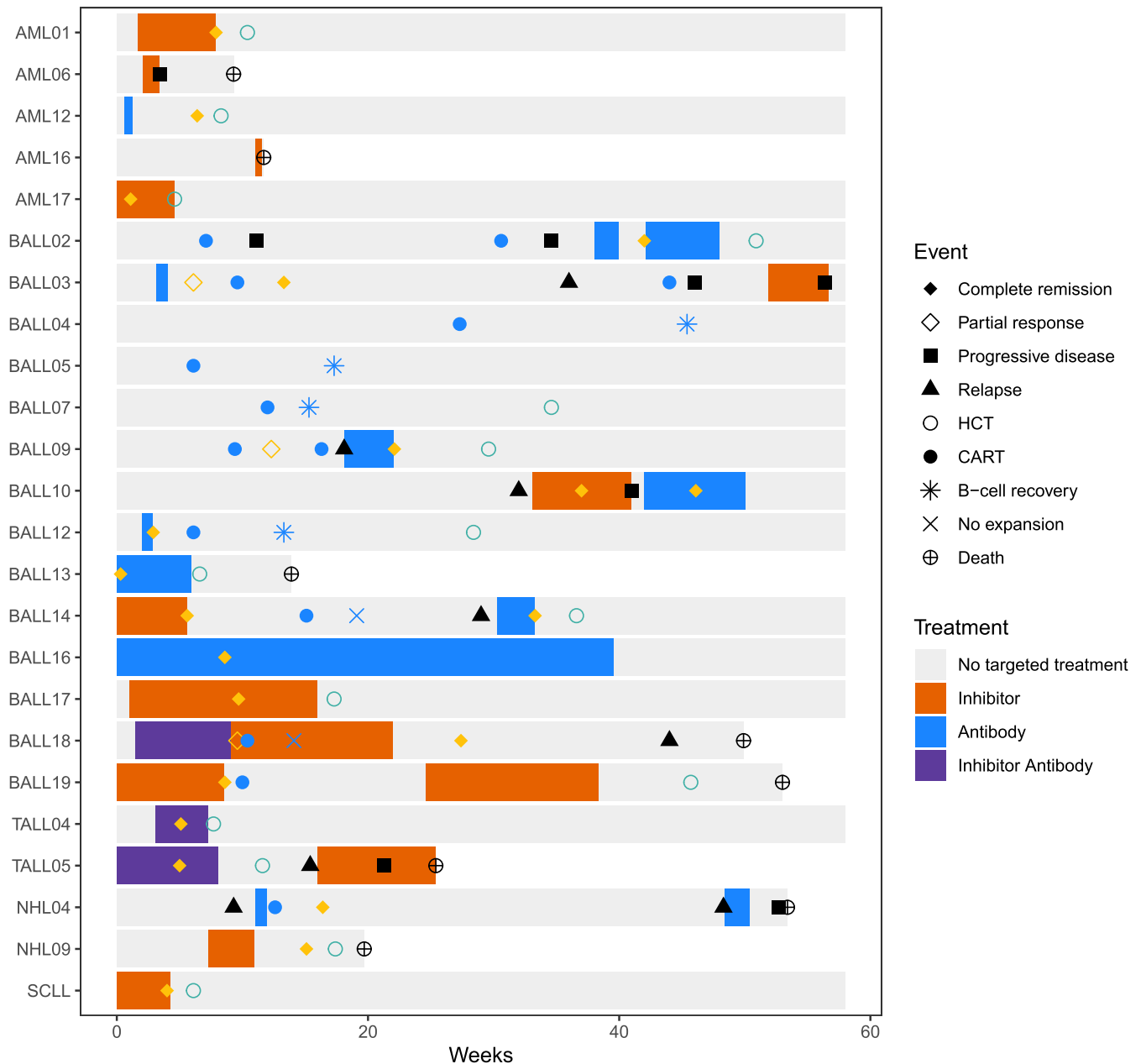
TABLE 2 (Continued)

Target	Event	AML (n = 17)	B-ALL (n = 19)	T-ALL (n = 5)	NHL/SCLL (n = 10)	Tot (n = 51)	Targeted therapy
Transcription, epigenetic, regulatory	KMT2A, NUP98 fusion	6	2	0	0	8	Menin inhibitor
	KMT2A, DOT1L overexpression	2	0	0	0	2	
	MYC activation; CREBBP inactivation	0	3	1	3	7	HDAC inhibitor
	HDAC1 overexpression	0	0	0	1	1	
	WT1, SETD2, SUZ12 inactivation	7 <sup>1</sup>	0	1	0	8	Hypomethylating agent
	WT1, DNMT3B, TERT overexpression	6	2	0	4	12	
	FDFT1 unmethylated intron 1	7/8	NA	NA	NA	7	
	EZH2 inactivation	2 <sup>1</sup>	0	0	0	2	Proteasome inhibitor
	IDH2 activation	0	0	0	1	1	IDH2 inhibitor
	CD-marker surface expression	16 <sup>1</sup>	1	0	1	18	Anti-CD33
	16	17	4 <sup>2</sup>	6	43	Anti-CD38	
	0	17 <sup>12</sup>	0	6 <sup>1</sup>	23	Anti-CD19	
	6	18 <sup>7</sup>	NA	5 <sup>1</sup>	29	Anti-CD22	
	NA	7	0	6 <sup>1</sup>	13	Anti-CD20	
	9	0	4	2	15	Anti-CD7	
	16	10	1	NA	27	Anti-CD123	

Note: Table includes genes/pathways/CD markers with  $\geq 1$  alteration prioritized  $\geq$  intermediate. Per row, a patient is counted once, for example, if multiple JAK-STAT pathway genes are overexpressed.

Colors indicate priority score of oncogenic targetable event: dark green, very high; green, high; light green, moderate; light orange, intermediate; dark orange, borderline; light red, low. Immunotherapy targets were not prioritized.

Superscript number indicates number of patients treated with matching targeted therapy after molecular tumor board recommendation.



**FIGURE 4** Swimmerplot showing targeted treatments started within a year from iTHER molecular tumor board advice. Patients are represented in rows; the x-axis indicates weeks after the iTHER molecular tumor board. Targeted treatments are shown as colored bars: orange, targeted inhibitor; blue, targeted antibody; purple, combination of inhibitor and antibody.

remission at the end of the observation period in the targeted treatment and in the remaining patients group (Supporting Information S1: Figure S3).

## DISCUSSION

### Profiling hematological malignancies is feasible

The experience of the iTHER molecular tumor board with pediatric hematological malignancies demonstrated that surface marker and molecular profiling was a feasible approach to identify actionable

events for targeted therapy in this rapidly proliferating disease. Profiling was successful for all patient samples with tumor cell percentages  $\geq 20\%$  (91%), a threshold based on sensitivity to detect fusion genes in diagnostic practice. The remaining 9% of the patients had tumor percentages below 5%, which was too low to attempt enrichment for tumor cells. Drug response profiling was feasible as well, although it was used complementarily for selected patients and not prioritized in the iTHER study. During the iTHER study, next-generation sequencing was implemented as a routine part of diagnostics in our national center, which helped to reduce turn-around-time to a median of 25 days, comparable with the turn-around-time reported by the INFORM study.<sup>12</sup>

## Actionable lesions in the majority of hematological malignancies

From 1 to 10 actionable oncogenic events with at least intermediate priority were identified per patient in 96% of the profiled hemato-oncology cases. These results compare favorably with two reported pediatric pan-cancer profiling studies using the same 7-scale target prioritization algorithm: 67% of 523 INFORM cases had an actionable target prioritized intermediate or higher<sup>12</sup> and 78% of 226 iTHER cases.<sup>10</sup> Focusing on hemato-oncology cases in reported molecular tumor board studies, using different types of molecular profiling and prioritization tools, 70%–84% of profiled cases had at least one actionable oncogenic event, and matching treatment was given to 15%–19% of these cases.<sup>11,12,14,23</sup> In the iTHER study, cell surface markers that could be targeted with immunotherapy were detected by flow cytometry in all profiled leukemia and lymphoma patients. Matched therapy with an inhibitor targeting an oncogenic aberration was given to 13 of the profiled iTHER patients (25%); one of these patients was enrolled in an early clinical trial. One or more CD-marker-directed immunotherapies were given to 17 patients (33%); 6 out of 28 immunotherapy treatments were given in the context of a clinical trial. In total, 24 profiled patients (47%) received targeted treatment according to iTHER molecular tumor board advice, which led to objective responses and served as a bridge to HCT. Twelve of these patients (50%) were alive at the end of the observation time, with a median follow-up time of 33 months. These results show that both immunological and genetic vulnerabilities are valuable targets in relapsed/refractory hematological malignancies.

## Combination treatment rather than single-agent

The small molecular inhibitors for individualized treatment of patients in the iTHER cohort were without exception combined with conventional chemotherapy based on tolerability shown in previous studies, for example, imatinib or dasatinib with induction chemotherapy,<sup>24</sup> trametinib with dexamethasone,<sup>25</sup> and venetoclax with azacitidine.<sup>26</sup> Similarly, the antibodies gemtuzumab, ozogamicin and daratumumab were given in combination with chemotherapy based on previous clinical studies.<sup>27,28</sup> In iTHER molecular tumor board-discussed patients, also immunotherapy and small molecular inhibitors were combined in three cases to maximize the chances of a response when no additional toxicity was expected. This may be a promising strategy to avoid nonresponse or resistance to either therapy, supported by preclinical<sup>29</sup> and early clinical<sup>30,31</sup> studies. Also, alternating treatment with anti-CD22 and anti-CD19 immunotherapy such as applied in patient BALL16 is a promising and less toxic option for chemotherapy-resistant BCP-ALL relapse.<sup>32</sup> The treatment decisions taken in the iTHER hemato-oncology patients illustrate the need for early clinical trials combining targeted inhibitors and/or immunotherapies with chemotherapy rather than as single agents. In relapsed and refractory solid pediatric cancer, combinations of targeted therapy with chemotherapy or other targeted therapy have been shown to be successful in the MAPPYACTS eSMART studies.<sup>13,33</sup> An example of early clinical trial platform testing targeted inhibitors in combination with dexamethasone and/or venetoclax is the HEM-iSMART trial for relapsed or refractory T-ALL (NCT05658640; ITCC-101). Further international sharing of clinical experiences with combinations of targeted small molecule/immune therapy and chemotherapy is essential to move forward with additional promising combinations for T-ALL and other hematological malignancies. Molecular and surface profiling of patients enrolled in these targeted trials is important to obtain insight into the difference between responders and nonresponders.<sup>34</sup>

## Additional value of combined expertise

Disease-specific biological expertise is needed to evaluate actionable events in their genetic and immunophenotypic context to determine whether they are targetable. For example, a patient with diffuse large B-cell lymphoma had mutations in both *CD79B* and *MYD88*, which would predict intense sensitivity to BTK inhibitors; however, co-occurrence with inactivation of *TNFAIP3* was described to reduce ibrutinib response.<sup>35</sup> Effective targeting may also be subtype-specific, for example, JAK-mutated T-ALL but not BCP-ALL responds better to MEK inhibitors than to JAK inhibitors,<sup>36</sup> and inhibitor specificity may depend on the specific mutation.<sup>37</sup> Combining whole exome and transcriptome sequencing allowed for better interpretation of variants, especially mutations predicted to cause splicing aberrations and confirmation of the activation of downstream pathways, such as *CDK6* upregulation in *KMT2A*-rearranged cases. In addition to oncogenic targets, immunotherapy targets, and drug sensitivities were included in the targeted treatment recommendation of hemato-oncology cases by the iTHER molecular tumor board. Target prioritization for hematological malignancies would benefit from a decision tree integrating these different actionable events, as is currently being explored in the international leukemia/lymphoma target board (NCT05270096; ITCC-107).

Experimental treatment expertise is needed to select the best fitting treatment for the individual patient given multiple targets and treatment modalities, previous (salvage) treatments, and other patient-specific factors. In our study, 9% of patients had been treated with a small molecule inhibitor, and 61% of patients received immunotherapy prior to iTHER enrollment. Previously described profiling platforms for pediatric cancer included a minority of hematological malignancies (e.g., INFORM, MappyActs, Zero Childhood Cancer, iTHER). International tumor boards dedicated to hematological malignancies, such as the International Leukemia/Lymphoma Target Board (iLTB for ALL, AML, and lymphoma) and FEDRRAL (ALL), provide structured platforms to obtain treatment advice from an international panel of clinical and disease-biology experts for this rare pediatric relapse population. This approach aims to optimize patient enrollment in early clinical trials to evaluate the safety and efficacy of potential new anti-cancer drugs and may eventually lead to better cure rates of relapsed, refractory, and high-risk leukemia and lymphoma in children.

In conclusion, the analysis of molecular tumor board-discussed pediatric blood cancer patients showed targetable events in all profiled patients, with targets overlapping between different disease entities. The molecular tumor board advice resulted in one or multiple targeted treatments in almost half of the patients, with durable responses observed in half of these patients. Our experience strengthened the idea that sharing disease-specific biological, genomic, and clinical expertise improves advice for targeted treatment recommendations for this rare pediatric relapse population.

## ACKNOWLEDGMENTS

We thank all patients and their families who participated in this study. We are grateful for the excellent support provided by the Laboratory for childhood cancer pathology, especially Eric Strengman and Marc van Tuil. We are grateful for the support provided by the Trial and Data Center of the Princess Máxima Center, especially trial manager Miriam Strumpf, central data manager Ria Koolma, local data manager Saskia Rasser, and research nurse Nurten Deniz and colleagues. We thank Jean-Pierre Bourquin and Peter Horvath for the drug response profiling data. We thank all clinicians and researchers who contributed to the Molecular Tumor Board discussions. The INFORM program is financially supported by the German Cancer Research

Center (DKFZ), several German health insurance companies, the German Cancer Consortium (DKTK), the German Federal Ministry of Education and Research (BMBF), the German Federal Ministry of Health (BMG), the Ministry of Science, Research and the Arts of the State of Baden-Württemberg (MWK BW); the German Cancer Aid (DKH), the German Childhood Cancer Foundation (DKS), RTL television, the aid organization BILD hilft e.V. (Ein Herz für Kinder) and the generous private donation of the Scheu family.

## AUTHOR CONTRIBUTIONS

The project was conceived by Jan J. Molenaar, Monique L. den Boer, Bianca F. Goemans, and C. Michel Zwaan. Data curation was performed by Judith M. Boer and Uri Ilan. Experimental and computational analyses were performed by Judith M. Boer, Uri Ilan, Aurélie Boeree, Stefan Nierkens, Jayne Y. Hehir-Kwa, Marco J. Koudijs, Monique L. den Boer, and Corinne Rossi. Data interpretation was performed by Judith M. Boer, Uri Ilan, Karin P. S. Langenberg, and Jan J. Molenaar. Data visualization was performed by Jan Koster, Marco J. Koudijs, Judith M. Boer, and Uri Ilan. The manuscript was written by Judith M. Boer, Uri Ilan, Monique L. den Boer, and C. Michel Zwaan, and approved by all authors.

## CONFLICT OF INTEREST STATEMENT

C. Michel Zwaan has received research funding from Syndax, Abbvie, Takeda, Jazz Pharmaceutical, and Pfizer. C. Michel Zwaan has been involved as a consultant for BMS, Gilead, Kura Oncology, Novartis, Incyte, and Pfizer; C. Michel Zwaan has performed an advisory role for Sanofi and Novartis. C. Michel Zwaan is a member of the board of directors of the ITCC Hem Malignancies Committee. The other authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in EGA at <https://web2.ega-archive.org/>.

## FUNDING

This study was financially supported by ZonMw (project number 848 101 004 to J.J.M. and C.M.Z.), the Dutch Foundation Children Cancer-free (KiKa core funding to J.J.M. and M.L.B.), the Oncode Institute (M.L.B.), and the Pediatric Cancer Foundation Rotterdam (M.L.B.).

## ORCID

Judith M. Boer  <https://orcid.org/0000-0003-4848-7789>

## SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

## REFERENCES

- Eckert C, Parker C, Moorman AV, et al. Risk factors and outcomes in children with high-risk B-cell precursor and T-cell relapsed acute lymphoblastic leukaemia: combined analysis of ALLR3 and ALL-REZ BFM 2002 clinical trials. *Eur J Cancer*. 2021;151:175-189. doi:10.1016/j.ejca.2021.03.034
- Burkhardt B, Reiter A, Landmann E, et al. Poor outcome for children and adolescents with progressive disease or relapse of lymphoblastic lymphoma: a report from the Berlin-Frankfurt-Muenster group. *J Clin Oncol*. 2009;27(20):3363-3369. doi:10.1200/JCO.2008.19.3367
- Rasche M, Steidel E, Zimmermann M, et al. Second relapse of pediatric patients with acute myeloid leukemia: a report on current treatment strategies and outcome of the AML-BFM Study Group. *Cancers*. 2021;13(4):789. doi:10.3390/cancers13040789
- Rasche M, Zimmermann M, Steidel E, et al. Survival following relapse in children with acute myeloid leukemia: a report from AML-BFM and COG. *Cancers*. 2021;13(10):2336. doi:10.3390/cancers13102336
- White T, Kaspers G, Abrahamsson J, et al. Clinical outcomes of second relapsed and refractory first relapsed paediatric AML: a retrospective study within the NOPHO-DB SHIP consortium. *Br J Haematol*. 2022;197(6):755-765. doi:10.1111/bjh.18039
- Ceolin V, Ishimaru S, Karol SE, et al. The PedAL/EuPAL project: a global initiative to address the unmet medical needs of pediatric patients with relapsed or refractory acute myeloid leukemia. *Cancers (Basel)*. 2023;16(1):78.
- Locatelli F, Zugmaier G, Rizzari C, et al. Effect of blinatumomab vs chemotherapy on event-free survival among children with high-risk first-relapse b-cell acute lymphoblastic leukemia: a randomized clinical trial. *JAMA*. 2021;325(9):843-854. doi:10.1001/jama.2021.0987
- Niktoreh N, Lerijs B, Zimmermann M, et al. Gemtuzumab ozogamicin in children with relapsed or refractory acute myeloid leukemia: a report by Berlin-Frankfurt-Münster study group. *Haematologica*. 2019;104(1):120-127. doi:10.3324/haematol.2018.191841
- Frismantas V, Dobay MP, Rinaldi A, et al. Ex vivo drug response profiling detects recurrent sensitivity patterns in drug-resistant acute lymphoblastic leukemia. *Blood*. 2017;129(11):e26-e37. doi:10.1182/blood-2016-09-738070
- Langenberg KPS, Meister MT, Bakhuizen JJ, et al. Implementation of paediatric precision oncology into clinical practice: the individualized therapies for children with cancer program 'iTHER'. *Eur J Cancer*. 2022;175:311-325. doi:10.1016/j.ejca.2022.09.001
- Pikman Y, Tasian SK, Sulis ML, et al. Matched targeted therapy for pediatric patients with relapsed refractory or high-risk leukemias: a report from the LEAP consortium. *Cancer Discov*. 2021;11(6):1424-1439.
- van Tilburg CM, Pfaff E, Pajtlér KW, et al. The pediatric precision oncology INFORM registry: clinical outcome and benefit for patients with very high-evidence targets. *Cancer Discovery*. 2021;11(11):2764-2779. doi:10.1158/2159-8290.CD-21-0094
- Berlanga P, Pierron G, Lacroix L, et al. The European MAPPYACTS Trial: precision medicine program in pediatric and adolescent patients with recurrent malignancies. *Cancer Discovery*. 2022;12(5):1266-1281. doi:10.1158/2159-8290.CD-21-1136
- Marks LJ, Oberg JA, Pendrick D, et al. Precision medicine in children and young adults with hematologic malignancies and blood disorders: the columbia university experience. *Front Pediatr*. 2017;5:265. doi:10.3389/fped.2017.00265
- Wong M, Mayoh C, Lau LMS, et al. Whole genome, transcriptome and methylome profiling enhances actionable target discovery in high-risk pediatric cancer. *Nat Med*. 2020;26(11):1742-1753. doi:10.1038/s41591-020-1072-4
- Worst BC, van Tilburg CM, Balasubramanian GP, et al. Next-generation personalised medicine for high-risk paediatric cancer patients—the INFORM pilot study. *Eur J Cancer*. 2016;65:91-101. doi:10.1016/j.ejca.2016.06.009
- Landrum MJ, Chitipiralla S, Brown GR, et al. ClinVar: improvements to accessing data. *Nucleic Acids Res*. 2020;48(D1):D835-D844. doi:10.1093/nar/gkz972
- Sun W, Triche Jr. T, Malvar J, et al. A phase 1 study of azacitidine combined with chemotherapy in childhood leukemia: a report from the TAEL consortium. *Blood*. 2018;131(10):1145-1148. doi:10.1182/blood-2017-09-803809
- Kalina T, Flores-Montero J, van der Velden VHJ, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. *Leukemia*. 2012;26(9):1986-2010. doi:10.1038/leu.2012.122
- Fischer U, Forster M, Rinaldi A, et al. Genomics and drug profiling of fatal TCF3-HLF-positive acute lymphoblastic leukemia identifies

- recurrent mutation patterns and therapeutic options. *Nat Genet.* 2015;47(9):1020-1029. doi:10.1038/ng.3362
21. Yadav B, Pemovska T, Szwajda A, et al. Quantitative scoring of differential drug sensitivity for individually optimized anticancer therapies. *Sci Rep.* 2014;4:5193. doi:10.1038/srep05193
  22. Demir S, Boldrin E, Sun Q, et al. Therapeutic targeting of mutant p53 in pediatric acute lymphoblastic leukemia. *Haematologica.* 2020;105(1):170-181. doi:10.3324/haematol.2018.199364
  23. Eaton A, Wong V, Schiff D, et al. Tumor genomic profiling and ex vivo drug sensitivity testing for pediatric leukemia and lymphoma patients. *J Pediatric Pharmacol Therap.* 2022;27(2):123-131. doi:10.5863/1551-6776-27.2.123
  24. Biondi A, Gandemer V, De Lorenzo P, et al. Imatinib treatment of paediatric Philadelphia chromosome-positive acute lymphoblastic leukaemia (EsPhALL2010): a prospective, intergroup, open-label, single-arm clinical trial. *Lancet Haematol.* 2018;5(12):e641-e652. doi:10.1016/S2352-3026(18)30173-X
  25. Menne T, Slade D, Savage J, et al. Selumetinib in combination with dexamethasone for the treatment of relapsed/refractory RAS-pathway mutated paediatric and adult acute lymphoblastic leukaemia (SeluDex): study protocol for an international, parallel-group, dose-finding with expansion phase I/II trial. *BMJ Open.* 2022;12(3):e059872. doi:10.1136/bmjopen-2021-059872
  26. Winters AC, Maloney KW, Treece AL, Gore L, Franklin AK. Single-center pediatric experience with venetoclax and azacitidine as treatment for myelodysplastic syndrome and acute myeloid leukemia. *Pediatr Blood Cancer.* 2020;67(10):e28398. doi:10.1002/pbc.28398
  27. Cooper TM, Alonzo TA, Tasian SK, et al. Children's Oncology Group's 2023 blueprint for research: myeloid neoplasms. *Pediatr Blood Cancer.* 2023;70(Suppl 6):e30584. doi:10.1002/pbc.30584
  28. Stone RM, Moser B, Sanford B, et al. High dose cytarabine plus gemtuzumab ozogamicin for patients with relapsed or refractory acute myeloid leukemia: Cancer and Leukemia Group B study 19902. *Leuk Res.* 2011;35(3):329-333. doi:10.1016/j.leukres.2010.07.017
  29. Kirchhoff H, Karsli U, Schoenherr C, et al. Venetoclax and dexamethasone synergize with inotuzumab ozogamicin-induced DNA damage signaling in B-lineage ALL. *Blood.* 2021;137(19):2657-2661. doi:10.1182/blood.2020008544
  30. Foà R, Bassan R, Vitale A, et al. Dasatinib–Blinatumomab for Philadelphia acute lymphoblastic leukemia in adults. *N Engl J Med.* 2020;383(17):1613-1623. doi:10.1056/NEJMoa2016272
  31. Jabbour E, Short NJ, Jain N, et al. Ponatinib and blinatumomab for Philadelphia chromosome-positive acute lymphoblastic leukaemia: a US, single-centre, single-arm, phase 2 trial. *Lancet Haematol.* 2023;10(1):e24-e34. doi:10.1016/S2352-3026(22)00319-2
  32. Jabbour E, Sasaki K, Ravandi F, et al. Chemoimmunotherapy with inotuzumab ozogamicin combined with mini-hyper-CVD, with or without blinatumomab, is highly effective in patients with Philadelphia chromosome-negative acute lymphoblastic leukemia in first salvage. *Cancer.* 2018;124(20):4044-4055. doi:10.1002/cncr.31720
  33. Geoerger B, Paoletti X, Bautista F, et al. AcSé-ESMART, a European precision cancer medicine proof-of-concept platform trial. *Nat Med.* 2023;29(12):2985-2987. doi:10.1038/s41591-023-02580-5
  34. Janssen M, Schmidt C, Bruch PM, et al. Venetoclax synergizes with gilteritinib in FLT3 wild-type high-risk acute myeloid leukemia by suppressing MCL-1. *Blood.* 2022;140(24):2594-2610. doi:10.1182/blood.2021014241
  35. Ondrisova L, Mraz M. Genetic and non-genetic mechanisms of resistance to BCR signaling inhibitors in B cell malignancies. *Front Oncol.* 2020;10:591577. doi:10.3389/fonc.2020.591577
  36. Canté-Barrett K, Spijkers-Hagelstein JAP, Buijs-Gladdines JGCAM, et al. MEK and PI3K-AKT inhibitors synergistically block activated IL7 receptor signaling in T-cell acute lymphoblastic leukemia. *Leukemia.* 2016;30(9):1832-1843. doi:10.1038/leu.2016.83
  37. Wagle N, Emery C, Berger MF, et al. Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. *J Clin Oncol.* 2011;29(22):3085-3096. doi:10.1200/JCO.2010.33.2312