Articles

Genomics and drug profiling of fatal TCF3-HLF–positive acute lymphoblastic leukemia identifies recurrent mutation patterns and therapeutic options

TCF3-HLF–positive acute lymphoblastic leukemia (ALL) is currently incurable. Using an integrated approach, we uncovered distinct mutation, gene expression and drug response profiles in TCF3-HLF–positive and treatment-responsive TCF3-PBX1–positive ALL. We identified recurrent intragenic deletions of PAX5 or VPREB1 in constellation with the fusion of TCF3 and HLF. Moreover somatic mutations in the non-translocated allele of TCF3 and a reduction of PAX5 gene dosage in TCF3-HLF ALL suggest cooperation within a restricted genetic context. The enrichment for stem cell and myeloid features in the TCF3-HLF signature may reflect reprogramming by TCF3-HLF of a lymphoid-committed cell of origin toward a hybrid, drug-resistant hematopoietic state. Drug response profiling of matched patient-derived xenografts revealed a distinct profile for TCF3-HLF ALL with resistance to conventional chemotherapeutics but sensitivity to glucocorticoids, anthracyclines and agents in clinical development. Striking on-target sensitivity was achieved with the BCL2-specific inhibitor venetoclax (ABT-199).

One of the hallmarks of pediatric ALL is the presence of subtype-defining chromosomal translocations that cause gene fusions involving master regulators of hematopoietic development. These initiating lesions often cooperate with specific somatic aberrations, including monosomy deletions of B cell developmental genes, such as PAX5, IKZF1 and EBF1 (ref. 1). Other cooperative liaisons are represented by trisomy 21q22 with CRLF2 activation2–4 or near-haploid ALL with activation of receptor tyrosine kinase or RAS signaling5. RAS pathway mutations appear in high-risk ALL but are often lost with disease progression, which suggests involvement of additional tumorigenic factors6,7. The patterns of recurrent genomic alterations need to be better understood, because apart from tyrosine kinase inhibitor–supplemented treatment of BCR-ABL1–positive ALL, the only proven successful first-line treatment strategies for high-risk ALL are chemotherapy intensification and early allogeneic hematopoietic stem cell transplantation8.

The translocation t(1;19)9 that results in a fusion of the transcriptional activation domain of the B cell developmental transcription factor TCF3 to the DNA-binding domain of PBX1 occurs in about 5–10% of precursor B cell (pre-B cell) ALL patients and is associated with a median five-year event-free survival probability of 78–85%10. In contrast, the translocation t(17;19)(q22;p13), resulting in the fusion gene TCF3-HLF, defines a rare subtype of ALL (<1% of pediatric ALL) that is typically associated with relapse and death within two years from diagnosis11,12. Both translocations disrupt one allele of TCF3, which drives the B cell differentiation program upstream of the transcription factor PAX5 (ref. 13). As an initiating event, expression of TCF3-HLF leads to transcriptional reprogramming in pre-leukemic cells. Possible direct targets of TCF3-HLF include the transcription factor gene LMO2, which is implicated in initiation of T cell ALL14,15, and the transcriptional repressor SNAI1 (SLUG), which regulates embryonic development and apoptosis16,17. Further targets have been proposed, including BCL2 (ref. 14). The TCF3-HLF fusion likely requires additional events to cause leukemia, because TCF3-HLF transgenic and knock-in mice did not recapitulate the human phenotype18,19.

Here we report that the genomic and transcriptomic landscape of TCF3-HLF–positive ALL differs markedly from TCF3-PBX1–positive ALL. The TCF3-HLF fusion likely occurs in B lymphoid progenitors in the context of PAX5 haploinsufficiency and is associated with transcriptional reprogramming toward an immature, hybrid hematopoietic state. Drug response profiling in patient-derived xenografts, which maintained the genomic and global transcriptome landscapes of the corresponding primary leukemic samples, identified resistance patterns to drugs commonly used for the treatment of TCF3-HLF–positive patients. A general trait of TCF3-HLF–positive ALL in our study is extreme sensitivity toward the BCL2-specific inhibitor ABT-199 (venetoclax), indicating new therapeutic options for this fatal ALL subtype.

RESULTS

The TCF3-HLF ALL patient cohort

We applied high-throughput sequencing analysis integrating short and large insert size paired-end whole-genome, whole-exome and transcriptome sequencing to a discovery cohort consisting of five diagnostic pre-treatment samples of TCF3-PBX1–positive ALL (samples 1a–5a) and TCF3-HLF–positive ALL (samples 6a–9a and 11a). As nontumor controls we used matched bone marrow samples collected after induction treatment for minimal residual disease (MRD) evaluation (maximum leukemic cell load ≤10−3; samples
**TCF3-HLF ALL and impaired pro- to pre-B cell transition**

Pre-B cell ALL is frequently associated with somatic copy number alterations affecting B cell developmental genes. PAX5 deletions are generally observed in 13% of ALL cases and in up to 28% of high-risk ALL.\(^2\) We observed enrichment for monoallelic PAX5 deletions in TCF3-HLF-positive ALL, identifying such events in 67% of the cases. (Fig. 1c and Supplementary Table 2). Illegitimate RAG-mediated recombination appears to be implicated in the generation of such events in TCF3-HLF-positive ALL, given the close proximity to RSS motifs (Supplementary Table 5). In most samples without PAX5 deletion, we identified hemi- and homozygous deletions of VPREB1, which encodes a component of the surrogate light chain of the pre-B cell receptor (Supplementary Fig. 3a, b). Among additional DNA samples from seven TCF3-HLF-positive cases (diagnostic samples 10a, 12a, 13a, 14a, 15a, and Supplementary Table 2), we identified deletions of PAX5, BTG1, and VPREB1 in 24 TCF3-PBX1-positive cases (Supplementary Tables 2 and 3). In most cases TCF3-HLF-positive ALL responded to induction chemotherapy but remained MRD-positive. Nine children included in this study died owing to disease progression and treatment-related toxicities within 2 years on average, and only one patient is in remission after a short follow-up time, reflecting the dismal prognosis of TCF3-HLF-positive ALL.

**TCF3 breakpoints suggest a committed lymphoid cell of origin**

Consistent with previous reports\(^3,4\) and our results, all TCF3 translocation breakpoints were restricted to three hotspot regions (Fig. 1a, b and Supplementary Fig. 1). Those were associated with small non-template nucleotide insertions in the transcription factor SCL, LMO2, and LDB1 bound to DNA. Upon LMO2 binding, bonds are formed between TCF3 and SCL, including a hydrogen bond (dashed line) between D561 and R230, reducing the DNA binding capacity of the complex. Inset, D561V introduces a hydrogen bonding, thus altering the DNA-binding properties of the complex.

1b–9b and 11b; Supplementary Table 1).

For validation, we used additional DNA samples from seven TCF3-HLF-positive cases (diagnostic samples 10a, 12a, 13a, 14a, 15a, 16a, 17a, remission samples 10b, 12b, 13b) and 24 TCF3-PBX1-positive cases (Supplementary Tables 2 and 3). In most cases TCF3-HLF-positive ALL responded to induction chemotherapy but remained MRD-positive. Nine children included in this study died owing to disease progression and treatment-related toxicities within 2 years on average, and only one patient is in remission after a short follow-up time, reflecting the dismal prognosis of TCF3-HLF-positive ALL.

**Figure 1** Genetic lesions identified in pediatric TCF3-HLF- and TCF3-PBX1-positive ALL. (a) Breakpoints in TCF3, PBX1 and HLF cluster in genomic hotspot regions. Boxes correspond to exon regions; arcs represent fusions in patient samples. (b) TCF3 breakpoints cluster in two TCF3 intronic regions: between exons 16 and 17 (type I) and between exons 15 and 16 (type II). On the transcript level, type I translocations join TCF3 exon 16 to HLF exon 4, including inserted non-templated and intronic sequences and new splice acceptor sites (patients 8 and 9). Type II translocations occur downstream of exon 15 and exclude TCF3 exon 16 from the fusion transcript (patients 6, 7 and 11). (c) Schematic of somatic structural and nucleotide variations in samples. TCF3-HLF-positive ALL is characterized by mutually exclusive PAX5, BTG1 and VPREB1 deletions and nonsynonymous nucleotide variations in TCF3 (p.Asp561Val, ‘D561V’ in patient 8). Indel, insertion-deletion. Recurrantly affected genes are indicated by bold symbols. (d) Models of wild-type and mutant TCF3 based on the crystal structure of TCF3 in complex with the transcription factors SCL, LMO2 and LDB1 bound to DNA.\(^5\) Upon LMO2 binding, bonds are formed between TCF3 and SCL, including a hydrogen bond (dashed line) between D561 and R230, reducing the DNA binding capacity of the complex. Inset, D561V introduces a hydrophobic valine residue close to polar residues that may interfere with hydrogen bonding, thus altering the DNA-binding properties of the complex.

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Recurrent RAS pathway mutations in TCF3-HLF ALL

We identified only a few additional somatic alterations affecting protein-coding sequences in both TCF3-PBX1- and TCF3-HLF–positive ALL (Fig. 1c, and Supplementary Tables 6 and 7), involving among others, genes associated with pre-B cell ALL26 (TCF3, PAX5 and LEF1) and transcriptional and chromatin regulation (ZNZF263, MLL2, HIST1H3A and C6orf89). We observed a prominent association of TCF3-HLF–positive ALL with activating mutations in RAS signaling pathway genes (NRAS, KRAS and PTPN11), detectable in four of five discovery cases (Fig. 1c) and in three of five additional TCF3-HLF–positive validation samples (PTPN11 and SPHK1) (Supplementary Table 2). We identified no RAS pathway mutations in the TCF3-PBX1–positive discovery cohort and only one oncogenic NRAS mutation in the 24 TCF3-PBX1–positive validation cases (Supplementary Table 3). NRAS and KRAS mutations were generally detected in subclones (Supplementary Table 7). We discovered a new fusion gene, KHDRBS1-LCK, due to an interstitial chromosomal deletion in one TCF3-HLF–positive sample (6a), triggering the overexpression of the LCK tyrosine kinase (Supplementary Fig. 4). This was also present in three of 74 randomly selected ALL samples, demonstrating that KHDRBS1-LCK fusion is recurrent in ALL (Supplementary Fig. 5). LCK is a drug target in RAS-dependent cancer cells that have higher LCK expression27, suggesting a possible interplay with RAS-related signaling networks in TCF3-HLF–positive ALL. Oncogenic activation of LCK associated with (1;7)(p34;q34) translocation had been reported in the T cell leukemia cell line HS9 (ref. 28). Our data indicate a frequent association of proliferation-driving mutations in TCF3-HLF–positive ALL in the context of stalled B cell differentiation.

Figure 2 TCF3-HLF programs leukemia to a hybrid hematopoietic transcriptional state. (a) Heatmap of the 401 differentially expressed genes between the two ALL subtypes (edgeR, log2(fold change)) ≥ 1, false discovery rate (FDR) ≤ 0.001). (b) Enriched hematopoietic stages in TCF3-HLF–positive (orange) and TCF3-PBX1–positive (green) ALL. Stages show include hematopoietic stem cells (HSC), common myeloid progenitors (CMP), lymphoid-specific progenitors (GMP and MEP), neutrophils (NEUTRO), monocytes (MONO), multipotent progenitor (MLP), early T cell precursors (ETP), pro-B cells (PROB), T cells (TCELL) and B cells (BCELL). Gene set enrichment analysis was carried out using a Genomatix genome analyzer and gene set enrichment analysis (GSEA) (GSEA: FDR ≤ 0.02; Genomatix genome analyzer: adjusted P ≤ 0.02). The source of the significantly enriched gene sets is noted by the superscript: 1, curated gene sets of hematopoietic precursors23; 2, human immunologic gene signatures (MSigDB v4.0)24; 3, text mining–based tissue-specific gene sets25. (c) Enrichment plot for the HSC signature (Fig. 2c). FDR, false discovery rate. NES, normalized enrichment score. (d) Components of the TCF3-HLF–positive ALL signature reveal functional annotation related to stem cells and their cellular location (Genomatix genome analyzer: P = 4.65 × 10−4; adjusted P < 0.001).

Reprogramming toward a more immature state in TCF3-HLF ALL

Consistent with the occurrence of TCF3-HLF and TCF3-PBX1 translocations in lymphoid precursors, both leukemia subtypes had in common a gene expression signature of B lymphoid cells (including PAX5, BLK, CD19, CD22, CD79B, TCF3, EBFI, VPREB1, RAG1, ROR1, BLNK and DNTT; Supplementary Tables 9 and 10), but differential expression of 401 genes (false discovery rate ≤ 0.001) strongly distinguished chromosome in one TCF3-HLF–positive case (8a). Mutations at this position have been reported in sporadic Burkitt lymphoma29 and may reduce binding to its negative regulator ID3 (ref. 29). Based on available crystal structure data, p.Asp561Glu may affect the interaction of TCF3 with the transcription factor SCL (also known as TAL1; Fig. 1d), possibly altering TCF3 protein complexes. We detected a second TCF3 mutation (p.Ser467Gly) in another TCF3-HLF–positive case (13a, Supplementary Table 2). The functional consequences of this mutation are currently unclear. We could not detect any somatic mutations in TCF3 by targeted sequencing of 1,033 unselected ALL patients from the European multicenter trial AIEOP-BFM ALL 2000, suggesting a specific association with TCF3-HLF–positive ALL (Supplementary Table 8). Thus, deregulation of normal TCF3 function may also contribute to TCF3-HLF–positive ALL. Corroborating our findings, a recent study included a single TCF3-HLF case, as part of a cohort comparing diagnostic and relapse ALL samples, which showed a PAX5 deletion and two mutations in TCF3 (p.His460Tyr and p.Gly470fs), all of which were conserved at relapse30. The relapse sample featured a VPREB1 deletion as well as a shift in subclonal mutations in NRAS (p.Gly12Asp and p.Gly12Val), reinforcing the idea of cooperative effects between TCF3-HLF, and alteration of PAX5 and VPREB1 gene dosage. Taken together, seven of 11 TCF3-HLF cases were hemizygous for PAX5, whereas five samples featured VPREB1 deletions (Supplementary Fig. 6).

Mutations affecting the second TCF3 allele in TCF3-HLF ALL

We identified a mutation in the basic helix-loop-helix region of TCF3 (p.Asp561Val, D561V, Fig. 1c,d) affecting the non-translocated
the two TCF3-translocated subtypes (Fig. 2a, and Supplementary Tables 11 and 12). In silico prediction of transcription factor binding sites in the corresponding promoter regions revealed enrichment for PBX (Z score = 3.72) and HLF (Z score = 2.99) binding motifs associated with TCF3-PBX1 and TCF3-HLF gene signatures, respectively (Supplementary Tables 13 and 14). Further, PBX1 and HLF were the only transcription factors among those with enriched binding motifs that were significantly differentially expressed between the two ALL subtypes, and between leukemia and remission samples. The chimeric HLF transcript was strongly induced in TCF3-HLF, but we detected no wild-type HLF expression. We predicted 39 potential HLF targets, including the known target SNAI2 (SLUG)\textsuperscript{16}, GPC4 and BMP3 involved in stem cell proliferation, which showed induced expression in TCF3-HLF samples (Supplementary Table 15). Other potential TCF3-HLF targets that regulate developmental programs and cell survival, such as LMO2 (ref. 14) and BCL2 (ref. 14), were not predicted. However, their expression was increased in TCF3-HLF–positive ALL.

Gene set enrichment analysis using gene sets from sorted human hematopoietic stem cells and early progenitor populations\textsuperscript{31} as well as curated oncogenic (C6) and human immunologic (C7) signatures from MsigDB\textsuperscript{32} revealed an enrichment for stem cell and myeloid signatures in TCF3-HLF–positive ALL. In contrast, lymphoid features were more prominent in TCF3-PBX1–positive ALL (Fig. 2b and Supplementary Table 16). The hematopoietic stem cell signature\textsuperscript{31} ranked among the top gene sets enriched in TCF3-HLF–positive ALL (Fig. 2c and Supplementary Table 17). We obtained similar results using an independent method based on text mining notations (Fig. 2d, and Supplementary Tables 18 and 19). We also consistently detected high expression of the stem cell marker LGR5 (ref. 33) in TCF3-HLF–positive ALL, suggesting a reactivation of immature features shared with other stem cell populations. Consistent with previous reports, the myeloid marker CD33 was expressed in TCF3-HLF–positive blasts, which provides a target for antibody-directed therapy\textsuperscript{12,34}. Other differentially expressed genes, such as BMP2 (ref. 35), could present additional therapeutic targets.

Our results are consistent with a model in which TCF3-HLF arises in lymphoid cells and promotes transcriptional reprogramming toward a hybrid hematopoietic state. We also detected features of mesenchyme-derived tissues in TCF3-HLF–positive ALL, which may indicate a profound cellular reprogramming toward a drug-resistant state.

Mutation profiles of TCF3-HLF ALL are conserved in xenografts

We generated leukemia xenografts in nonobese diabetic severe combined immunodeficiency (NOD/SCID)/IL2rnull (NSG) mice for all cases included in this study (Supplementary Table 20)\textsuperscript{36,37}. We also established for the first time to our knowledge leukemia xenografts from follow-up samples with MRD, some with less than 0.1% ALL cells after induction chemotherapy (Fig. 3a, and Supplementary Tables 1 and 20). Leukemia and MRD engraftment was rapid with conserved and predictable kinetics for xenografts derived from the same patient (Supplementary Fig. 7), suggesting that no major adaptation to the mouse microenvironment was needed for proliferation. Most SNVs and intra-chromosomal deletions that had been present at diagnosis were conserved in the corresponding xenografts (Fig. 3b and Supplementary Table 7). Only deletions detected in the relapse sample 11c were not conserved in the corresponding xenografts, and a deletion in BTG1 emerged in one MRD-derived sample (7b, Supplementary Fig. 3c,d). A few mutations were lost in MRD or relapse xenograft samples, including GNB1 and DDX3X, indicating that these are probably dispensable or may cause drug sensitivity. Mutations in the RAS pathway were largely maintained in xenografts. However, the NRAS mutation p.Gln61His identified in the primary
MRD sample 7b was not detected in the corresponding xenograft. Instead, we identified a heterozygous damaging mutation in KRAS (p.Lys147Glu) associated with Noonan syndrome. In patient 9a, we identified two subclones displaying either a KRAS (p.Gly13Asp) or an NRAS (p.Gly12Ser) mutation. The corresponding xenograft retained only the KRAS mutated subclone. Thus, maintenance and acquisition of RAS pathway mutations in xenografts support the notion that they occur later during selection at a multiclonal level and confer a selective advantage in TCF3-HLF–positive ALL. No other SNVs emerged \textit{de novo} in the xenografts. In summary, the molecular characteristics of both leukemia subtypes were largely conserved in the xenografts, confirming the validity of this model.

**TCF3-HLF–associated gene expression is maintained in xenografts**

Hierarchical clustering based on the gene signature specifying the two leukemia subtypes showed that the expression profile and the subtype specificity of the primary leukemia were maintained in the xenografts (Fig. 4). The genes most significantly upregulated in matched patient and xenograft samples from TCF3-HLF–positive leukemia specified stem cell features (Supplementary Tables 21 and 22). Similar to the case in patient samples, we detected features of mesenchyme-derived tissues in xenografts derived from TCF3-HLF–positive ALL. TCF3-HLF–positive leukemias and xenografts displayed systematic downregulation of PAX5 expression to halved levels. Though monallelic deletions of PAX5 were a prominent feature of TCF3-HLF–positive ALL, we also saw reduced expression in diploid cases, hinting at alternative molecular mechanisms. The recapitulation of this pattern in the xenograft samples enforces the notion that TCF3-HLF–positive leukemia emerges in a specific cellular context with reduced native molecular mechanisms. The recapitulation of this pattern in the xenograft samples enforces the notion that TCF3-HLF–positive leukemia emerges in a specific cellular context with reduced native molecular mechanisms. The recapitulation of this pattern in the xenograft samples enforces the notion that TCF3-HLF–positive leukemia emerges in a specific cellular context with reduced native molecular mechanisms. The recapitulation of this pattern in the xenograft samples enforces the notion that TCF3-HLF–positive leukemia emerges in a specific cellular context with reduced native molecular mechanisms. The recapitulation of this pattern in the xenograft samples enforces the notion that TCF3-HLF–positive leukemia emerges in a specific cellular context with reduced native molecular mechanisms. The recapitulation of this pattern in the xenograft samples enforces the notion that TCF3-HLF–positive leukemia emerges in a specific cellular context with reduced native molecular mechanisms. The recapitulation of this pattern in the xenograft samples enforces the notion that TCF3-HLF–positive leukemia emerges in a specific cellular context with reduced native molecular mechanisms. The recapitulation of this pattern in the xenograft samples enforces the notion that TCF3-HLF–positive leukemia emerges in a specific cellular context with reduced native molecular mechanisms. The recapitulation of this pattern in the xenograft samples enforces the notion that TCF3-HLF–positive leukemia emerges in a specific cellular context with reduced native molecular mechanisms. The recapitulation of this pattern in the xenograft samples enforces the notion that TCF3-HLF–positive leukemia emerges in a specific cellular context with reduced native molecular mechanisms. The recapitulation of this pattern in the xenograft samples enforces the notion that TCF3-HLF–positive leukemia emerges in a specific cellular context with reduced native molecular mechanisms.

**Drug activity profiling of TCF3-HLF and TCF3-PBX1 ALL**

To determine drug sensitivity and resistance profiles, we established ALL cocultures on human mesenchymal stromal cells under serum-free conditions. Both subtypes depend on stroma for survival (Supplementary Fig. 9). TCF3-PBX1–positive ALL had a higher proportion of cells in S phase than TCF3-HLF–positive ALL on such cultures, reflecting consistent biological differences. By screening 98 bioactive agents, including many agents in clinical development (Supplementary Table 23), on an automated microscopy–based platform, we unambiguously discriminated the two translocations based on their drug sensitivity profiles, using either single (log half maximal inhibitory concentration (IC$_{50}$), Fig. 5a and Supplementary Fig. 10) or multiple response parameters (logIC$_{50}$, logEC$_{50}$ and area under the curve (AUC), Fig. 5b and Supplementary Table 24). To capture informative differences, we compared the responses of xenografts derived from TCF3-HLF–positive ALL to xenografts derived from other high-risk pre-B and T ALL patients on the same platform (Fig. 5c and Supplementary Table 25). This provided information about the activity range of each drug on the respective ALL subtype. TCF3-HLF–positive cases were consistently more resistant to various drugs from the same class, including nucleotide analogs (for example, cytarabine), mitotic spindle inhibitors (for example, vincristine), polo-like and aurora kinase inhibitors. Given the importance of cytarabine and vincristine in standard ALL therapy, the implications of these observations need to be further explored. TCF3-HLF–positive ALL was very resistant to dasatinib in this assay, whereas TCF3-PBX1–positive ALL responded well. This partly challenges a recent report, which had proposed dasatinib as an alternative for the treatment of these leukemias based on strong \textit{in vitro} activity in one TCF3-HLF– and ten TCF3-PBX1–positive primary ALL samples. However, \textit{in vivo} studies will be required to verify these differences in drug response, as differences in cell-cycle activity may influence the pattern of response \textit{in vitro}.

TCF3-HLF–positive ALL were sensitive to glucocorticoids (prednisone and dexamethasone) and to other drugs that could be relevant for the treatment of resistant ALL, including mTOR inhibitors, anthracyclines, bortezomib, the HSP90 inhibitor AUY922 and panobinostat. However, in spite of the good response of patients with TCF3-HLF–positive leukemia to prednisone therapy and the observed responsiveness of TCF3-HLF–positive ALL cells to glucocorticoids and anthracyclines that are commonly used in ALL treatment, patients who undergo this treatment relapse. Our transcriptome data suggested that resistance to apoptosis due to high expression of the anti-apoptotic oncoprotein BCL2 might promote cancer cell survival and constitute a druggable target (Supplementary Fig. 11). BCL2 is a putative transcriptional target of TCF3-HLF$^{41}$. Of note, PAX5, commonly deleted in our cohort, normally represses BCL2 transcription$^{41}$.

TCF3-HLF ALL is extremely sensitive to the BCL2 antagonist venetoclax

To assess the role of BCL2 overexpression in TCF3-HLF–positive ALL and to provide preclinical evidence for therapeutic activity,
we tested the BCL2-targeting drug venetoclax (ABT-199) in our xenograft model (Fig. 5c). This BH3-mimetic compound is a highly specific small molecule inhibitor that competes with pro-apoptotic BCL2 family proteins for binding to BCL2, and shifts the balance of pro-death and pro-survival signals inside the cell in favor of cell death. Venetoclax is in clinical development (phase II and III trials) for chronic lymphocytic leukemia and lymphoma, and holds promise for ALL and acute myeloid leukemia.

TCF3–HLF-positive ALL samples were more sensitive to venetoclax than TCF3–PBX1-positive samples (Fig. 6a), which correlated with higher BCL2 transcript and protein expression (Fig. 6b). A two-week treatment course of daily venetoclax administration delayed leukemia progression significantly in ALL xenografts from three different TCF3–HLF-positive cases (Fig. 6c,d). Treatment of mice in the control arm that reached maximal leukemia burden resulted in very rapid reduction of the leukemic load (Fig. 6e). Xenografts from MRD or relapse remained sensitive to venetoclax (Supplementary Fig. 11). Profiling of primary cells from two additional cases with refractory ALL confirmed exquisite sensitivity to venetoclax (Supplementary Fig. 12). Combined treatment of patient-derived xenografts from patients 6–11 with venetoclax and either vincristine or dexamethasone indicated a potentially synergistic effect in some of those patients (Supplementary Fig. 13 and Supplementary Table 26). Our data identified BCL2 dependency in TCF3–HLF ALL as a druggable target, and illustrate how integration of drug response profiling and molecular genetic analyses can inform the development of innovative treatment strategies in patients with unmet therapeutic needs.

**DISCUSSION**

To our knowledge, a long-term cure has never been achieved for patients with TCF3–HLF–positive ALL. Our study revealed a recurrent pattern of TCF3–HLF accompanied by abnormalities that affect transcriptional regulation of lymphoid development. We found frequent deletions of PAX5 and VPREB1 in association with TCF3–HLF, but did not detect deletions of Ikaros family members, which are commonly affected in ALL. We also uncovered recurrent mutations of the transcription factor TCF3, which acts upstream of PAX5 in

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**Figure 5** Drug activity profiling of TCF3-translocated leukemia reveals relevant differences in drug sensitivity. (a) Unsupervised clustering based on the drug activity profile of 98 compounds (log IC₅₀). Numbers identify the compounds shown in (b). (b) Principal component analysis of the response variables IC₅₀, EC₅₀, EC₅₀ and AUC (Supplementary Table 24) show TCF3–PBX1–positive and TCF3–HLF–positive ALL in two distinct clusters. The separation of TCF3–PBX1–positive and TCF3–HLF–positive ALL is determined by responses to topoisomerasers, BCL2 inhibitors, glucocorticoids and antimitotic agents, which correlate with the first three principal components. (c) Selection of drugs based on differences in sensitivity or resistance in TCF3–PBX1–positives and TCF3–HLF–positives. For comparison, the corresponding drug activity is indicated for 25 additional ALL samples tested on the same platform, including standard risk (SR, n = 5), medium risk (MR, n = 4) and high risk (HR, n = 16) cases (Supplementary Table 25). Boxplots extend from the first to the third quartiles (hinges) of the response range for each compound. Whiskers correspond to values from the hinge to the lowest or highest values within 1.5× of the distance between the first and third quartiles, respectively. Drugs with differential activity include docetaxel, paclitaxel, vincristine, AT9283, barasertib, BI2536, torin-1, dasatinib, lestaurtinib and XL228 (P ≤ 0.05).

Drugs which are active across the patients include doxorubicin, idarubicin, mitoxantrone, bortezomib, panobinostat, NVP-AUY922, ABT-199 (venetoclax) and navitoclax. Asterisks indicate drugs currently in clinical use.
lymphoid development, potentially impairing structural interactions with other transcription cofactors\(^{29}\). PAX5 expression was reduced by twofold in all TCF3-HLF–positive cases, underscoring the possibility of an interaction between TCF3-HLF, TCF3 and PAX5. PAX5 is required for B lymphoid lineage commitment and maturation\(^{43}\), and is frequently deleted in high-risk ALL with complex patterns of copy number abnormalities\(^{23}\). Similarly, deletions in IKZF1, which is required for the development of B and T lymphoid lineages and has additional stem cell–like functions\(^{44}\), are detected both in high-risk BCR-ABL1–positive and –negative ALL, and in the more favorable ERG-altered ALL subtype\(^{45}\), but never in TCF3-HLF–positive ALL. We also detected focal deletions of VPREB1 in TCF3-HLF ALL, which may lead to a developmental arrest associated with lack of pre-B cell receptor formation and the resulting loss of negative feedback on RAG-mediated recombination\(^{46}\). VPREB1 deletions were present at a similar frequency compared to other high-risk ALLs, such as BCR-ABL1–like and BCR-ABL1–positive ALL (~30–40% of cases)\(^{47}\) or hypodiploid ALL (~30%)\(^{48}\), associated with poorer overall survival in high-risk pre-B cell ALL patients\(^{49}\). However, specific ALL subtypes associated with good prognosis (for example, ETV6-RUNX1–positive ALL) also present high frequencies of VPREB1 deletions\(^{50}\), suggesting an important impact of the genomic context\(^{51}\). Thus, distinct patterns of association emerge that are likely to reflect important underlying biological mechanisms. Based on our results, we propose that a reduction of PAX5 gene dosage constitutes a favorable context for the oncogenic activity of TCF3-HLF.

As observed for hypodiploid ALL\(^{5}\) and in subsets of MLL-rearranged ALL\(^{48}\), we identified mutations in NRAS, KRAS and PTPN11 in TCF3-HLF–positive ALL. In our xenograft models we detected variable persistence of NRAS and a switch to KRAS mutations, indicating that RAS mutations are multiclonal and might not be strictly required for disease progression in TCF3-HLF–positive ALL. Indeed, mutations in the RAS pathway are enriched at relapse in ALL\(^{7,30,48}\) but mostly in a subclonal pattern with losses or switches in NRAS and KRAS from diagnosis to relapse. These represent secondary events, possibly compensating functional effects of the initiating events. Mutations in the RAS pathway might not represent optimal therapeutic targets, given their volatility and the potential to select for slower-proliferating, more resistant subclones. The TCF3-HLF gene expression signature, enriched for components of stem cell and myelomonocytic stages, was very similar among leukemias and maintained in xenografts, specifying additional, novel markers associated with stem cell function, such as LGR5, which marks epithelial stem cells\(^{49}\) and embryonic and fetal hematopoietic progenitor cells in mice\(^{50}\). Thus, in analogy to experimental induction of pluripotent stem cells\(^{51,52}\), TCF3-HLF likely induces a whole set of factors that carry out reprogramming and leukemic transformation in the context of low PAX5 expression. Deletion of PAX5 in early B cell progenitors induced dedifferentiation to a state with myeloid and T cell potential\(^{53,54}\). Moreover, rescue with low-level expression of PAX5 in knockout mice generates a stalled biphenotypic B-lymphoid/myeloid state\(^{54}\). Together with an activating mutation in STAT5, PAX5 haploinsufficiency initiates ALL in mice\(^{41}\). Based on these data, we propose that the initiating TCF3-HLF fusion results in severe transcriptional reprogramming with dedifferentiation. The favorable context for transformation is secured through
secondary cooperating lesions in early B cell differentiation genes including TCF3 and PAX5. A central question remains pertaining to the cell of origin in different ALL subtypes. Our study provides important clues that should be further addressed using disease models. The molecular analysis of the TCF3-HLF and TCF3-PBX1 fusion gene breakpoints indicated that the TCF3-HLF, like the TCF-PBX1 translocation, originates in cells already committed to lymphoid differentiation. Furthermore, we found the associated somatic structural variants to be RAG-mediated, which is comparable to patterns identified recently in ETV6-RUNX1–positive ALL, the most frequent pre-B cell ALL subtype, which is consistent with expression of RAG in TCF3-HLF–positive ALL.\(^5\) We favor the hypothesis that the TCF3-HLF translocation occurs in a B cell progenitor and that the specific lineage constraint is constrained further in a restricted developmental stage by additional mutations. The detection of TCF3-HLF being restricted to leukemic cells supports this idea, although initiation in a more immature compartment cannot be formally excluded.

The molecular landscapes of TCF3-HLF–positive ALL were largely conserved in xenografts, providing a valuable, well-characterized, model for preclinical testing. Drug activity profiling revealed that TCF3-HLF–positive cases were more resistant to several standard ALL drugs, such as nucleotide analogs (for example, cytarabine) and mitotic spindle inhibitors (for example, vincristine). We detected activity for other related drug classes, such as mTOR inhibitors, the proteasome inhibitor bortezomib, the HSP90 inhibitor AUY922 and the HDAC inhibitor panobinostat. The BCL2 inhibitor venetoclax (ABT-199)\(^5\) was highly active in all TCF3-HLF–positive cases analyzed, which we confirmed using primary ALL cells from two additional cases with refractory disease. These results refine data obtained using the broader spectrum BH3 mimic ABT-737 in TCF3-HLF–positive cell lines.\(^4,14\) Given the activity of venetoclax also in other ALL subsets including immature T cell ALL (refs. 56,57 and our own unpublished data) and the lack of on-target thrombocytopenia caused by ABT-737, venetoclax should be explored for experimental therapy in refractory ALL in selected cases based on such functional data. Thus integrated genomic and functional analyses of TCF3-HLF–positive ALL provide insight into the molecular context and associated components and offer unprecedented possibilities to investigate new agents for the treatment of these children who currently lack effective therapeutic options.

URLs Information on the two image processing programs used for in vitro drug screening and automated microscopy can be found at http://acc.ethz.ch/.

METHODS Methods and any associated references are available in the online version of the paper.

Accession codes. Sequencing data are available from the public POPGEN repository (2015-UFO-NG-1; Christian Albrechts University, Kiel) upon written request accompanied by a positive internal review board vote for research addressing leukemia-related questions. Sequencing data transfer can proceed upon positive review and signing of a material transfer agreement.

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ONLINE METHODS

Study individuals and sample selection. Samples and associated clinical information from patients included in sequencing and validation analyses were collected from different countries within the International BFM Study Group (I-BFM-SG). All patients were enrolled in multicenter trials on treatment of pediatric ALL conducted by individual member groups of the I-BFM-SG: the AIEOP-BFM study group (Austria, Germany, Italy and Switzerland), the FRALLE study group (France) and the United Kingdom (UK) National Cancer Research Institute (NCRI) Childhood Cancer and Leukemia Group99,60. All treatment trials were approved by the respective national institutional review boards, and informed consent for the use of spare specimens for research was obtained from study individuals, parents or legal guardians. The specific research project reported here was approved by the Ethics Committee of the Medical Faculty of the Christian Albrechts University, Kiel, Germany (vote D508/13). Depending on consent and availability of samples, all enrolled patients were positive for the rare TCF3-HLF gene fusion were included. These patients were matched with TCF3-PBX1–positive patients.

Cell isolation and nucleic acid purification. Mononuclear cells were isolated by Ficoll–Paque gradient centrifugation (Pharmacia) from bone marrow or peripheral blood samples followed by extraction of nucleic acids according to standardized protocols using Qiagen DNA Blood Kits (Qiagen) for DNA and Qiagen RNeasy columns (Qiagen) for RNA. The quantity of nucleic acids was determined by spectrophotometry; DNA quality was assessed visually by inspection of agarose gel electrophoresis while RNA integrity was evaluated by using the Bioanalyzer 2100 (Agilent). Nucleic acids isolated from bone marrow aspirates collected in morphological remission served as individual germ-line surrogates/references.

Sequencing. Whole genome sequencing. For structural variants, Illumina v2 mate-pair libraries with 5 kbp insert size and 2 × 101 bp reads were prepared from 10 µg of DNA and sequenced on the Illumina HiSeq 2000 platform (Illumina) to obtain a physical coverage of 30×. For copy number alterations, breakpoints and short variants (SNVs, short indels), Illumina TruSeq paired-end libraries with 2 × 101 bp reads were prepared from 1 µg of DNA and sequenced on HiSeq 2000/2500 instruments to a coverage of 40× for reference samples and 80× for tumor samples.

Whole exome sequencing. To increase the sensitivity of detecting short variants in coding regions, 1 µg of DNA each from the diagnostic leukemic and a corresponding remission sample of patients was used for whole exome sequencing. Whole exome capture employed a TruSeq enrichment kit (Illumina) and paired-end libraries with 2 × 101 bp reads on a HiSeq 2500 according to the manufacturer’s protocol.

Whole transcriptome sequencing. Illumina TruSeq custom stranded paired-end libraries with 2 × 51 bp reads were prepared from 1 µg RNA using the Ribo-Zero Gold Kit (Epicentre) and sequenced on a HiSeq 2000 with a loading of one library per lane.

Sanger sequencing validation. Structural variant breakpoints from whole-genome sequencing approaches and SNVs from exome sequencing were validated by Sanger sequencing.

Targeted sequencing of TCF3 and RAS pathway candidate genes. TCF3 binding domain (E47 isoform, exon 18) mutations were screened for in 1,033 ALL patients using Sanger sequencing. Primer sequences are listed in Supplementary Table 2. Sanger sequencing was also applied for validation of relative absence of RAS pathway mutations in 24 TCF3-PBX1–positive ALL samples. The latter analysis included KRAS exon 1, NRAS exons 1 and 2, FLT3 exons 14 and 20, PTPN11 exons 3 and 13, and was conducted as described62.

Multiplex ligation-dependent probe amplification. Detection of genomic aberrations in B cell differentiation–associated and other genes frequently deleted in ALL (PAX5, IKZF1, ET维尔13, RBL1, BTG1, EBF1, CDKN2A, CDKN2B and P2RY8-CRLF2) were investigated by the Multiplex Ligation-dependent Probe Amplification (MLPA) assay SALSA P335 kit (MRC-Holland) using 125 ng of genomic DNA. The assays were performed according to the manufacturer’s protocol as described62. An intensity ratio between 0.75 and 1.3 was considered to represent normal copy number, a ratio between 0.25 and 0.75 was considered a monoallelic deletion and a ratio <0.25, a biallelic deletion.

Bioinformatics analysis. DNA data processing. DNA reads were aligned to the human reference genome hg19 (downloaded from the UCSC Genome browser) using Elandv2 (ref. 63; mate pairs) and BWA64 (paired ends). For xenograft samples, the human DNA reads were deconvoluted after mapping to a combined reference consisting of human hg19 and mouse mm9.

Structural variant detection. Structural variants were detected using DELLY65 and BIC-seq66 (DNA data) and TopHat2 (ref. 67) /deFuse68 (RNA data).

SNV detection. Somatic protein-changing SNVs were detected using established pipelines incorporating GATK69, MuTect70, pibase71, Picard, SAMtools72 and VarScan2 (ref. 73).

Indel detection. Somatic indels in coding regions were detected using SAMtools followed by Dindel74.

Transcriptome data analysis. RNA reads were aligned to hg19 using BWA and SAMtools and used for integrated data analysis. For xenograft samples, the human RNA reads were deconvoluted after mapping to a combined reference consisting of human hg19 and mouse mm9. Mapped reads were annotated using Ensembl v70. Gene expression levels were quantified in reads per kilobase of exon model per million mapped reads (RPKM)75. RPKM calculation and differential gene expression (DGE) analysis was performed using the R package edgeR75. To identify DGE between ALL subtypes, and between leukemia and remission the following set-up was performed: TCF3-PBX1 vs. TCF3-HLF (comparison 1), TCF3-PBX1 vs. remission (comparison 2), TCF3-HLF vs. remission (comparison 3). The results were filtered by fold change (FC; \log_{2}(\text{FC}) ≥ 1) and false discovery rate (FDR, FDR ≤ 0.001). The final list of 401 genes was created by combining the intersection between comparison 1 and comparison 2 as well as between comparison 1 and comparison 3. The functional analyses of gene lists were done using gene set enrichment analysis (GSEA)77 and the Genomatix genome analyzer (v. 3.00801; Genomatix Software GmbH). The GeneRanger tool in Genomatix was used to test for enriched gene sets, which were based on gene-tissue annotations obtained by text mining78. For GSEA, protein-coding genes were filtered by a minimum expression of 1 RPKM in at least four samples among the primary pre-B cell ALLs. The remaining 11,315 genes were tested for DGE between the ALL subtypes using edgeR. The provided FDR and fold-change values were used to obtain a ranking score to measure the degree of differential expression between the ALL subtype. A pre-ranked classic GSEA was performed using the ranking score, a gene set permutation and a FDR ≤ 0.02. The analysis included gene sets for hematopoietic stages79 and signatures from MsigDB80 pathways (C2): KEGG, BIOCARTEA, REACTOME; curated oncogenic signatures (C6); human immunologic signatures (C7).

In silico transcription factor binding site (TFBS) analysis. TFBSs in promoter regions of genes (2 kbp upstream region) corresponding to the specific transcriptome signatures of TCF3-PBX1- and TCF3-HLF–positive ALL, respectively, were analyzed using the Genomatix Genome Analyzer (v3.10124). Based on a matrix of known TFBS motifs, the software tool predicted TFBSs in the investigated promoters and compared their frequency against (i) the background of TFBSs in the promoter regions of all known protein-coding genes in the Ensembl database (v.70, 22864 genes) and (ii) the background of TFBSs in the whole genome. A Z score was calculated based on the TFBS frequency in the investigated promoters and the expected frequency and s.d. were estimated from the background81. The resulting lists were filtered by the Z scores based on the two backgrounds (\{genomic Z ≥ 2, promoter Z ≥ 2\). TFBSs overrepresented in genes upregulated in both TCF3-PBX1- and TCF3-HLF–positive ALL were filtered out, to retain only TFBS specifically enriched in the respective subtypes.

Integrated data analysis. SNVs and indels were orthogonally validated by integrating genome, exome and transcriptome data of patients and xenografts, and further confirmed by Sanger sequencing. Structural variants were validated by integrating whole genome paired-end and mate-pair data and whole-transcriptome data, and finally by Sanger sequencing. Ensembl v70 and ANNOVAR82 were used to annotate the variants. Silent variants and known germline variants in the 1000 Genomes Project81 population data, in 136 North German healthy controls (publicly available through GrabBlur82), or in the International Cancer Genome Consortium’s internal healthy controls were eliminated. All final somatic non-synonymous variants were inspected using IGV83.
Preclinical characterization. Xenograft model. Animal experiments were approved by the veterinary office of the Canton of Zurich, Switzerland. Approval for experiments with human samples in the mouse xenograft model was obtained from the ethics commission of the Canton Zurich (approval number 2014-0383). In brief, primary ALL cells were recovered from cryopreserved samples and transplanted intrafemorally to NSG mice as previously described36. Mice were 5–10 weeks old; both males and females were randomly used. Leukemia progression was monitored by flow cytometry with rat anti-mouse CD45 (eFluor450, clone 30-F11, REF 48-451-82, eBioscience), mouse anti-human CD45 (Alexa Flour 647, clone H130, REF 304018, BioLegend), and mouse anti-human CD19 (PE, clone HIB19, REF 302208, BioLegend). ALL cells recovered from spleens of NSG mice were used for molecular characterization in in vitro and in vivo experiments.

Immunophenotyping. Immunophenotyping of patient and xenograft-amplified human ALL cells after recovery from the spleen was performed as described before64. All included xenograft samples consisted of at least 95% human leukemic cells.

Cell culture. Human hTERT immortalized primary bone marrow mesenchymal stromal cells (MSC; provided by D. Campana, St. Jude Children’s Research Hospital, Memphis, USA) were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, t-glutamine (2 mM), penicillin/streptomycin (P/S; 100 IU/ml) and hydrocortisone (1 µM). Xenograft-amplified human ALL cells were co-cultured on MSC in AIM V medium (Gibco by Life Technologies) at a ratio of 10:1. All cultured cells were kept in the incubator at 37 °C, 5% CO2. For cryopreservation, cells were frozen in heat-inactivated FBS with 10% dimethylsulfoxide and subsequently stored in liquid nitrogen.

Cell viability assay. MSCs were seeded in 24-well plates at a number of 50,000 cells per well in RPMI 1640 medium (10% heat-inactivated FBS). After 24 h primary ALL cells were thawed and seeded as suspension culture alone or in co-culture with MSC at a number of 400,000 cells per well in AIM-V medium. Three days later, all cells were collected from monoluculture or co-culture by scraping and stained with 7-AAD (BD Pharmingen). Cell viability (7-AAD negative population) was measured by FACS using counting beads (SPHERO Accu Count Blanc Particles, Spherotech Inc.) for cell counts normalization. Viabilities shown are average viabilities of duplicate wells (normalized to input) and s.d.

Cell cycle assay. MSCs were seeded in 96-well tissue culture plates at a concentration of 10,000 cells per well in 100 µl AIM-V medium. After 24 h all cells were added at a concentration of 100,000 cells per well in 90 µl AIM-V. The Click-iT Edu Alexa Fluor 488 Flow Cytometry Assay Kit (Life Technologies) in combination with propidium iodide was used to measure proliferation and to identify the different phases of the cell cycle on days 1 and 3. Co-cultured cells were incubated with Edu (10 µM) for 20 h before cell cycle read-out with flow cytometry. The cell cycle assay was performed in triplicate, and at least two independent experiments were performed for each sample. Similar variances were obtained between the groups that were statistically compared.

In vitro drug screening and automated microscopy. MSCs were seeded in 384-well plates at a concentration of 2,500 cells per well in 30 µl AIM-V medium. After 24 h, ALL cells were added at a concentration of 25,000–30,000 cells per well in 27.5 µl AIM-V. Drugs were added as single agents after an additional 24 h using the pipetting robot epMotion 5070 (Eppendorf). Drug response was normalized to ALL cells treated with the drug vehicle alone. Experiments were performed in duplicate in five different dilutions (1, 10, 100, 1,000 and 10,000 nM). For two samples comparable results were obtained in two independent drug screening experiments. After 72 h or 96 h of drug incubation, cells were stained using the CyQUANT direct cell proliferation assay (Life Technologies). 20 µl staining mix (AIM-V medium, CyQUANT 1:300, repressor (1:20)) was added into each well followed by an incubation time of 1 h at 37 °C, 5% CO2. Subsequently, automatically measured was performed using the ImageXpress Micro microscope (Molecular Devices) equipped with a CoolSNAP HQ camera (Photometrics) and a 10× plan fluor objective with 0.3 NA (Nikon). Nine images were taken per well, covering 50% of each well and captured employing the MetaXpress software (Molecular Devices). Images were processed using CellProfiler software (Broad Institute). Cells were classified and counted using the Advanced Cell Classifier software. This software uses random forest classification to assign ALL cells properly.

Immunohistochemistry. Whole cell extracts were prepared from 1 × 106 cells using radioimmunoprecipitation assay (RIPA) buffer (20 mM Tris-Cl pH 7.5, 150 mM NaCl, 1% NP-40, 1 mM EDTA pH 8.0, 0.1% SDS) supplemented with Complete mini protease inhibitor cocktail (Roche Life Science) for 20 min on ice, sonicated as necessary, and diluted with SDS loading buffer (250 mM Tris pH 6.8, 4% SDS, 0.02% bromophenol blue, 40% glycerol, 4% (vol/vol) β-mercaptoethanol). After SDS-PAGE, proteins were blotted onto nitrocellulose membranes. Membranes were blocked in 5% non-fat dry milk and incubated with primary Bcl-2 (clone 124; Dako) and tubulin antibodies diluted 1:1,000 in milk. Horseradish peroxidase–labeled anti-mouse antibodies were used for signal detection with chemiluminescence substrate and direct scanning.

In vivo experiments. ALL cells were recovered from cryopreserved xenograft samples, and per thawed sample 12 to 16 mice were transplanted with 1,000,000 cells per mouse. After three days, randomized cohorts were treated with 100 mg/kg of ABT-199 (ABBVIE) or vehicle control with 6 to 8 mice per treatment arm65. ABT-199 or vehicle control were administered orally daily for two weeks. Mice of the ABT-199 group transplanted with sample 7a were additionally treated with a second block (100 mg/kg of ABT-199 for 14 d) starting at day 66, when the frequency of circulating leukemia cells started to increase again. Follow-up of circulating leukemia cells was performed every 7 d by flow cytometry with rat anti-mouse CD45, mouse anti-human CD45, and mouse anti-human CD19; frequency of leukemia cells as ratio of mCD45+ hCD45+ hCD19+ count to total lymphocytes. The investigator was blinded to the group allocation during the assessment of outcome. To evaluate the ability of ABT-199 to decrease tumor burden, four mice in the control group were treated when the frequency of leukemia cells in the peripheral blood was equal or higher than 50%. Follow-up of circulating leukemia cells was performed every 4 – 7 d. In vivo experiments were terminated when the frequency of circulating leukemia cells reached 50% or earlier if the mice showed abnormal behavior. One in vivo experiment was performed per each sample.

Statistical analysis. Differences in the distribution of categorical variables among patient subsets were analyzed using Fisher’s exact or chi-squared test. Comparisons of continuous variables between groups were performed by t-test or Mann-Whitney U test.

Drug responses were evaluated by fitting DMSO-normalized response data with the four-parameter log-logistic function of the form:

\[ f(x) = \text{base} + \frac{\text{max} - \text{base}}{1 + (x/x_0)^{\text{Coef}}} \]

as implemented in the drc package of R (version 2.3-96). Outliers were detected and removed before curve fitting using Bayesian change point analysis25 (R package bcp, version 3.0.1). Non-convergent cases (for example, drugs with no activity) were identified based on linear fit parameters. Hierarchical clustering was performed to group patients according to their drug-response profiles (R package gplots version 2.14.2). Drugs with differential activity in patients with TCF3-PBX1 – compared to TCF3-HLF – positive ALL were identified using a t-test (P ≤ 0.05). In in vivo experiments, 25% of circulating leukemia cells or termination of the experiment if 25% of leukemia was not reached were considered as an event in the Kaplan-Meier analysis. For sample 9a, 50% was used because of the rapid engraftment. Differences in the survival of mice receiving ABT-199 or vehicle control were determined by the Mantel-Cox test and verified by the Gehan-Breslow-Wilcoxon test.

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