

Advancing Chemogenomic Strategies for Functional Precision Medicine in Relapsed/Refractory T-ALL and ETP-ALL: The Gimema ALL2720 Trial

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Abstract

Despite the vast amount of cancer genomic data, linking mutations to drug efficacy remains challenging. National efforts integrating *ex vivo* drug response profiling (DRP) with clinical genomics are rare. To address this gap, we designed the GIMEMA ALL2720 trial (NCT04582487), a multicenter study evaluating a chemogenomic-driven approach in T-cell acute lymphoblastic leukemia (T-ALL), a subtype still lacking effective salvage therapies. 29 patients with *de novo* or relapsed/refractory (R/R) T-ALL or ETP-ALL were enrolled in the study to receive genomic profiling and DRP. Integrated patient-specific reports were generated within a median of 7 days. DRP revealed recurrent vulnerabilities and four response clusters. Of 28 patients, 15 (53.6%) received chemogenomic-guided therapy, achieving an overall response rate of 60%. Four responders proceeded to transplantation. This prospective study demonstrates the nationwide feasibility and clinical relevance of integrating genomic and functional profiling in T-ALL, supporting chemogenomic-guided precision medicine as a promising strategy to broaden rescue options and improve outcomes.

Introduction

Despite advances in cancer genomics, linking mutations to drug efficacy remains challenging. T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) exemplifies this gap: despite two decades of target discovery and genomic characterization¹, effective salvage treatments are lacking². Current options for relapsed or refractory (R/R) disease are limited to cytotoxic agents such as nelarabine, with a median survival of under six months^{3,4}. Meanwhile, chimeric antigen receptor-T cell (CAR-T) and other immunotherapies are only in early clinical development, underscoring the need for novel strategies⁵⁻⁷.

The lack of innovative therapies for T-ALL may be due to two main factors. First, T-ALL is relatively uncommon, accounting for approximately 12–15% of pediatric and 20–25% of adult ALL². Moreover, first-line therapy is curative in most patients, making R/R T-ALL a niche disease less attractive to pharmaceutical investment². Second, its genomic landscape is dominated by overexpression of transcription factors, such as *TAL1*, *TLX1*, *TLX3*, *HOXA*, *LMO2*, *BCL11B*, and *NKX2-1*, which define disease subtypes but are not directly druggable⁸. However, their downstream effects, along with additional cooperating alterations, deregulate several targetable signaling pathways, including CDKN2AB, IL7R, JAK1-3, STAT5, PI3K–AKT, and Notch. Disease heterogeneity, limited patient numbers, and disappointing efficacy of some agents have hampered efforts to design clinical trials based solely on these genomic alterations⁹.

An alternative strategy for R/R T-ALL is the use of functional precision medicine (FPM) approaches, which directly profile drug responses (DRP) on patient-derived leukemic cells to guide therapeutic decisions¹⁰⁻¹². For example, we designed a combination of venetoclax and bortezomib as a potential salvage regimen based on DRP patterns in R/R early T-cell precursor (ETP) ALL^{10,13}. Other groups have shown that venetoclax-resistant cases are more likely to respond to dasatinib, potentially identifying a

broader subset of responsive patients¹⁴. However, these and other studies have so far involved a limited number of patients and are restricted to a few specialized centers, where technological infrastructures can meet the demand of such assays. Given the potential to identify specific drugs and to reduce costs and toxicities associated with ineffective treatments, national initiatives integrating *ex vivo* DRP with clinical genomics remain rare¹⁵⁻¹⁹.

We present the first Italian prospective multicenter trial, GIMEMA ALL2720, evaluating the feasibility of integrating DRP with advanced genomic profiling to guide treatment within a clinically informative timeframe in R/R T-ALL.

Materials and Methods

Study Design

ALL2720 (NCT04582487, registration date 2020-09-24) is a multicenter, prospective, and feasibility study that aims to integrate the genomic landscape of patients with R/R T-ALL/LBL and ETP-ALL with *ex vivo* DRP to guide an FPM approach. The primary objective was to determine the rate of chemogenomic-driven treatment identification for R/R T-ALL patients with limited therapeutic options, within a clinically meaningful timeframe. A combined molecular–cytogenetic and functional strategy was applied to prioritize actionable targets and dependencies for personalized therapy.

Secondary objectives included evaluating correlations and reproducibility between genomic and drug response profiles, assessing treatment outcomes, and evaluating the feasibility of allo-hematopoietic stem cell transplantation (alloHSCT) across the cohort and among patients receiving DRP-guided or standard therapy.

Ethical approval

for this study was obtained from the Comitato Etico Regionale Umbria (CER Umbria) Ethics Committee (3884/21, approval date December 17th, 2020); from the Area Vasta Emilia Nord (AVEN) Ethics Committee (59/2022/TESS/AOUPR, approval date June 21st, 2022); and by the ethics committees of all participating centers. The study was conducted in accordance with the Declaration of Helsinki and written informed consent was obtained from all participants. This study was conducted within a national multicentre clinical trial network and did not involve international partnerships requiring additional inclusion and ethics considerations beyond standard institutional ethics approvals and informed consent.

A detailed description of materials and methods is provided in the *Supplementary Information* section.

Results

ALL2720, Chemogenomic Pipeline

We prospectively generated chemogenomic profiles from leukemia cells collected from bone marrow (BM), peripheral blood (PB), or pleural effusion. After enrolment, samples were shipped to genomic and functional labs within 24 hours. Combined interphase (CI)-FISH, drug testing, and single-nucleotide polymorphism array (SNPa) analyses were successfully performed in all cases, while next-generation sequencing (NGS) mutational profiling succeeded in 96.5% (28/29) and was completed within 28 days of sample receipt. NGS runs were performed every 15 days, with reports delivered in an average of 28 days (range 21–35) (**Figure 1a**).

Seventy-six compounds from a T-ALL-focused library were tested per sample (**Figure 1b**; **Supplementary Data 1**). DRP success rate was 96.5% (28/29), with one sample excluded due to low cell count. Cytogenetic and molecular workflows were feasible in all patients, enabling oncogenic subclassification in 38% (11/29) (**Figure 1c**). Patient-specific DRP and CI-FISH reports were issued the same day and returned within a median of 7 days (range 4–15) (**Figure 1d**). The FPM Review Board (RB) supported treatment decisions and identified at least one actionable compound or drug class for every patient.

ALL2720, Patients, and Profiling

Between 2021 and 2024, 34 patients with R/R T-ALL/LBL and *de novo* or R/R ETP-ALL from 15 GIMEMA Hematology Centers were referred to the trial. Samples were centralized for DRP assay and genomic assessment (**Figure 2a**). Thirty-two patients (94.1%) were eligible and enrolled in the study. Three additional patients were excluded due to insufficient material for genomics and functional studies, leaving 29 evaluable for complete chemogenomic assessment (**Figure 2b**). Demographic and disease characteristics are listed in **Table 1** and **Supplementary Table 1**. Median age of the patient cohort was 55 years (range 21-77 years). The male-to-female ratio was 1.6.

Six patients (20.7%) had a *de novo* ETP-ALL, while 23 (79.3%) were R/R patients, including 13 ETP-ALL and 10 T-ALL/LBL (9 = cortical EGIL T-III phenotype; 1 = pre-T EGIL T-II phenotype). Median white blood cells (WBC) count at diagnosis was $15,080 \times 10^3/\text{mL}$ (range 1,360-228,000), and 17.2% of patients (5/29) had $\text{WBC} > 100,000 \times 10^3/\text{mL}$. Primary leukemic cells were obtained from BM (23/29; 79.3%), PB (3/29; 10.3%), and pleural effusion (3/29; 10.3%).

Among patients with R/R (23/29) disease, the most frequent first-line treatment was based on the GIMEMA LAL1913 trial, administered to 15 of 23 R/R patients (65.2%), followed by therapy based on NILG ALL 10/07 trial (4/23; 17.4%) (**Figure 2c**; **Supplementary Table 2**). Twelve patients (52.2%) had undergone allo-HSCT before enrolment, including one patient who had received two allo-HSCTs. Fifty-two percent of patients had received more than one line of therapy at enrolment, with nelarabine accounting for 30.4% of all lines of therapy, and the median number of previous lines of treatment was 1 (range 1-4). (**Supplementary Table 3**). Similarly, among ETP-ALL patients enrolled at diagnosis (6/29), GIMEMA LAL1913 and LAL1104-based treatments were the preferred first-line regimens, accounting for 50% each (**Supplementary Table 4**). All patients who received LAL1913 underwent allo-HSCT in first remission.

Oncogenic classification obtained for all 29 patients identified 21% *HOXA*, 10% *TLX1*, and 3% each for *TAL/LMO* and *TLX3*. Sixty-two percent of cases were “unclassified” (**Table 1; Figure 2d**). Genomic characterization revealed *NOTCH1* mutation as the top hit in our cohort, present in 65.5% of patients (19/29), with 17 patients harboring a *NOTCH1* mutation, and two a *NOTCH1* fusion (**Figure 2e; Supplementary Table 5; Supplementary Data 2**). *FBXW7* mutations were restricted to the *NOTCH1*-altered group. *CDKN2AB* and *CDKN1B* deletions were the second most recurrent aberrations in our cohort, accounting for 31% and 27.5%, respectively. Genomic analysis revealed that the most frequently altered pathways/class of genes were Notch1 signaling (65.5%), transcription factors (62%), epigenetic modulators (44.8%), RAS/MEK/ERK, and cell cycle/apoptosis (41.4% each) (**Figure 2f**).

Chemogenomic assessment identifies clusters of response

Hierarchical clustering of samples and compounds was performed on drug sensitivity score (DSS) values scaled to the average response to all drugs within each sample (patient-wise z-score, **Figure 3a**). This transformation method mitigated the influence of broad responder or non-responder cases and avoided artificially inflating clustering relevance of drugs with a narrower activity spectrum. This approach split the cohort in four patient groups (**Figure 3b**): two clusters consisted exclusively of immature cases (“I1” and “I2”), one was predominantly typical (“T”) and a fourth was phenotypically heterogeneous (“H”). Indeed, a marked separation of samples by phenotype at earliest branching points is also evident when clustering unscaled DSS data (**Supplementary Figure 1a**) and tumor phenotype appears as a major axis of variation in sensitivity profiles. Interestingly, all cases in cluster I2 were not classifiable using CI-FISH/SNPa assessment. Genetic alterations showed limited association with clusters, although cluster H had fewer *NOTCH1*-altered cases (33%) and cluster I1 included all cases with concomitant *ETV6* and *WT1* alterations.

Clusters showed distinct patterns of selective compound activity (**Figure 3c, Supplementary Data 3**). Dinaciclib and mitoxantrone were broadly potent, with cluster I2 more sensitive to dinaciclib than cluster I1 (FDR < 0.01). BCL2 inhibitors venetoclax and navitoclax were highly active in immature T-ALL clusters, with venetoclax showing higher activity in cluster I1 compared to T and H (FDR < 0.01) and in I2 compared to H (FDR < 0.05). HDAC inhibitors such as givinostat and AR-42 showed higher median DSS in clusters T and I2; interestingly, cluster I2 was more sensitive than the other immature cluster I1 and the heterogeneous cluster H (FDR < 0.05). Considering HSP90 inhibitors, cluster I1 was less sensitive to onalespib than any other cluster (FDR 0.009 - 0.019), while also being less sensitive to luminespib than I2 and H (FDR < 0.01). Dasatinib was less active in immature clusters I1 and I2 than in cluster T, which is predominantly typical (FDR < 0.05), consistent with previously reported activity patterns^{11,12}. Clusters also differed in their sensitivity to conventional chemotherapeutics: daunorubicin showed low activity in clusters I1 and H while displaying a broad DSS range in T and I2 (FDR < 0.01 for T-H and I2-H). Together, these findings demonstrate that patient clustering based on DSS profiles recapitulates phenotypic subgroups through distinct patterns of activity across drug classes, while also revealing potentially meaningful subdivisions in response profiles within each phenotype.

We also evaluated, as a secondary objective, associations between specific genetic or phenotypic characteristics and drug responses (**Figure 3d**). When contrasting phenotypic subgroups (**Supplementary Data 4**), dasatinib was more potent in typical than in immature cases (FDR < 0.01), while venetoclax, as expected^{14,20}, was more effective in the immature group (FDR<0.05). Dexamethasone resistance is associated with *IKZF1* alterations in B-ALL, but analogous data in T-ALL cases are limited^{21,22}. Indeed, in our cohort, *IKZF1*-altered cases were more resistant to dexamethasone ($p < 0.01$). We also investigated the association between *PTEN* alterations and higher sensitivity to PI3K/mTOR/AKT pathway inhibitors (**Supplementary Data 5**): data for MK-2206 were significant (FDR < 0.05), while other compounds showed a similar trend but did not reach significance. *NOTCH1* alterations were not significantly associated with specific resistances or sensitivities in our cohort.

Finally, we investigated the sensitivity profile of cases with *FBXW7* alterations (*-alt*), which co-occurred with *NOTCH1* alterations (**Supplementary Data 6**). Interestingly, *FBXW7-alt* cases showed increased sensitivity to nine kinase inhibitors (**Supplementary Figure 1b, Supplementary Data 6a-b**). By comparing *FBXW7-alt* cases to both *NOTCH1-wt* and *NOTCH1-alt-FBXW7-wt* cases, we confirmed that *FBXW7-alt* group was more sensitive to dasatinib than the others (FDR < 0.05) (**Supplementary Figure 1b**), further showing similar trends for five additional kinase inhibitors (**Supplementary Figure 1c, Supplementary Data 6c**). Finally, since dasatinib sensitivity was also significantly associated to phenotypic subgroups, we confirmed this finding using a Freedman-Lane permutation-based ANOVA. Results confirmed that both *FBXW7* alterations and phenotype were independently associated with higher sensitivity to dasatinib, respectively in *FBXW7-alt* and in typical cases ($p < 0.05$) (**Supplementary Data 6d**).

N-of-1 chemogenomic-driven approach

The secondary objective was to evaluate the correlation and reproducibility of chemogenomic predictivity with clinical application and the feasibility of a tailored approach using genomic and functional profiling. Treating physician, guided by experience, drug availability, and off-label regulations, made the final decision to follow or not a DRP-driven approach. Of 29 patients, 28 (96.5%) were fully profiled, and 64.3% (15/28) received a chemogenomic-driven treatment based on FPMRB suggestions (**Table 2**). Of 15 patients who received an N-of-1 treatment (40%), six had previously received a standard-of-care therapy after study enrollment but were subsequently rescued with a chemogenomic-guided approach following early relapse or refractory disease. One patient received two consecutive chemogenomic-driven treatments. The median time from the transmission of the FPMRB report to the start of the chemogenomic-driven approach as first treatment was 26 days (range 2–35). The patients' median number of previous lines of therapy was 2 (range 1–4). Only one patient enrolled at diagnosis was treated according to FPMTB suggestions after a primary induction failure. Seven patients (46.7%) received consolidative allo-HSCT before the chemogenomic-driven approach, and six patients (40%) had previously received nelarabine (**Supplementary Table 6**).

Considering biological features, 73% (11/15) of patients in the chemogenomic-driven cohort showed an immature phenotype, while 4 (26%) were typical T-ALL. The oncogenic classification revealed that 66.7%

were unclassified, 4 were *HOXA* (26.7%), and 1 was *TLX3* (6.7%). Sixty percent of patients (9/15) showed *NOTCH1* aberrations, 40% *RUNX1* aberrations, and 33.3% *ETV6* or *EZH2* aberrations (**Figure 4a**).

Among chemogenomic-driven treatment selection, *BCL2* inhibitors, such as venetoclax and navitoclax, alone or in combination, were the most frequently used for *N-of-1* treatments, accounting for 68.7% of the treatments, followed by bortezomib (37.5%). The most frequent chemogenomic-driven treatment combinations included navitoclax and venetoclax (43.7%), venetoclax and bortezomib (18.7%), and dasatinib and bortezomib (12.5%) (**Table 3**). In one patient, two consecutive chemogenomic-driven treatments, venetoclax-bortezomib-dexamethasone and decitabine-venetoclax-gilteritinib, were selected to achieve complete remission with MRD-negative status, as analyzed by multi-parametric flow cytometry (MFC), to enable a bridge-to-alloHSCT strategy.

When evaluating the efficacy of the chemogenomic-driven cohort, overall response rate (ORR) was 46.7%, and all patients achieved a complete response (CR), with five patients MRD-negative. Nearly half of patients who achieved a response (4/9; 44%) were bridged to alloHSCT, all with ETP phenotype (**Figure 4b**, **Supplementary Table 6**). Among patients who received standard-of-care treatment (n = 14/29), 35.7% achieved CR (3/5 MRD-negative), 28.6% were refractory to the subsequent line of therapy, and 35.7% had unassessed or unknown response. The five patients who achieved a CR were bridged to alloHSCT (**Supplementary Table 7**, **Supplementary Figure 2a**).

As proof-of-concept, we report two cases of very high-risk ETP-ALL that were treated according to FPMRB's recommendation. A 28-year-old young man (#006-2) was diagnosed with T-ALL with an immature phenotype. The disease showed a gdTCR restriction at MFC evaluation and was classified as *HOXA* subgroup with *KMT2A* rearrangement. He received a first-line therapy based on GIMEMA LAL1913 schedule, achieving a CR with molecular MRD positivity, followed by consolidation with haploidentical allo-HSCT, which resulted in molecular MRD negativity. The disease relapsed after 55 months with an aggressive course, refractory to a second-line therapy with two cycles of Hyper-CVAD. The third line of treatment, consisting of two cycles of nelarabine followed by a second haploidentical allo-HSCT, induced a CR with MRD negativity. However, an early relapse occurred after seven months (**Figure 4c**). Once enrolled in the study, genomic characterization showed a t(6;11)(q27;q23) with *KMT2A::AFDN* rearrangement (**Supplementary Figure 2b-c**) and mutations in *NOTCH1* p.(Leu1678Pro), *PHF6* p.(Ala238Valfs*4), *EED* p.(Arg313*), *BCL11B* p.(Gly630Thrfs*91), *NRAS* p.(Gly13Val), and *JAK3* p.(Ala573Val). A chemogenomic-guided combination of navitoclax and venetoclax was proposed (**Figure 4c**). Twenty-three days after the DRP/CI-FISH report, the patient started venetoclax 50 mg and navitoclax 50 mg qd. After 6 days, PB evaluation showed no blasts. CR with MFC-MRD negativity was achieved after two cycles, and the patient relapsed with extramedullary disease after 8 months of progression-free survival (PFS), slightly longer than post-second transplant.

A second illustrative case is that of a 42-year-old woman (#038-1) with a prior clear-cell renal carcinoma treated with right nephrectomy, who later developed T-ALL with a near-ETP phenotype. She received GIMEMA LAL1913-based chemotherapy and achieved sustained MRD negativity. Four years later, a

molecular relapse emerged, followed two months later by 35% lymphoid blasts in the BM. She was subsequently referred to the Hematology and BMT Unit of Parma University Hospital. Flow cytometry showed relapse of the primary leukemic clone and concurrent emergence of a distinct, 15% myeloid clone. Cytogenetic/FISH analysis confirmed this bilinear event: a t(10;11)(p12-13;q14-21) *PICALM::MLLT10* in the lymphoid population, and monosomy 7 and a *MECOM* rearrangement, t(3;?) (q26;q?), in the myeloid clone (**Supplementary Figure 2d-e**). NGS analysis showed *NOTCH1* p.(Gln2361Argfs*4), *SUZ12* p.(?), *RUNX1* p.(Gly170Glu fs*8), *WT1* p.(Arg370Leufs*3) and CNVs *BRAF* (ex.15) and *EZH2* (ex. 2-20). CT-scan and ¹⁸FDG-PET identified a mediastinal lymphoid mass (48x27x55 mm). CSF analysis was negative. DRP showed sensitivity to bortezomib, HDAC, and BCL-2 inhibitors (**Supplementary Figure 2f**). Exceptional activity was also observed for fludarabine, mitoxantrone, actinomycin D, and CAD204520 (a Notch1 inhibitor). The patient was treated following FPMRB's suggestion with venetoclax plus bortezomib as a chemo-free regimen to bridge to alloHSCT as an intention-to-treat. Intrathecal prophylaxis was added. BM evaluation after two cycles exhibited a rapid clearance of the ETP clone with *IG/TCR* MRD negativity and a residual myeloid clone quantified in the recurrence of *MECOM* rearrangement (**Figure 4d, Supplementary Figure 2g**). The CT scan showed a reduction of mediastinal mass, with a negative ¹⁸FDG PET. She underwent haploidentical allo-HSCT after a fludarabine-based conditioning regimen. The patient continued immunosuppression without graft-versus-host disease and achieved normal immune reconstitution. Thirty months post-transplant, she was in CR with full donor chimerism. This case demonstrates the value of early chemogenomic assessment for identifying exceptional responders who benefit from a bridge-to-transplant approach.

Discussion

Over the past decade, FPM has emerged as a complement to genomics for personalizing cancer treatment²³. *Ex vivo* DRP can improve this approach by revealing unexpected therapeutic vulnerabilities and guiding treatment, especially in R/R cases with limited options and undefined or undruggable oncogenic drivers.

Recent prospective clinical trials in hematological malignancies, such as the SMARTrial, have validated this concept, demonstrating that functional profiling can reveal patient-specific vulnerabilities otherwise undetectable by genomic screening alone^{15-18,24}. In the EXALT trial, *ex vivo* DRP was feasible in > 95% of samples and guided therapy in 39% of cases. Nearly 55% achieved clinical benefit, with ≥ 1.3-fold improvement in PFS, and 40% of responders had exceptional responses¹⁵. A follow-up study (NCT04470947) is underway, with encouraging preliminary findings¹⁹. In parallel, Malani et al. reported on the implementation of an FPM tumor board for real-time treatment decision in R/R acute myeloid leukemia (AML). They demonstrated the real-time clinical utility of an FPM tumor board in R/R AML. Among 186 profiled patients, DRP identified actionable sensitivities in 97% of cases, and 59% of treated patients achieved an objective response¹⁸. These and other studies demonstrate that integrating DRP into routine practice is technically and logistically achievable across institutions. However, the

incorporation of functional testing alongside genomic profiling in clinical decision-making remains limited to a small number of Cancer Centers, particularly in Europe.

Functional stratification in ALL has primarily been limited to retrospective studies, despite providing insights beyond genomic features via *ex vivo* DRP. In a pioneering study, Frismantas et al. profiled 60 compounds in 68 relapsed or R/R ALL (24 T-ALL, 44 B-ALL), revealing disease-relevant sensitivities without prior knowledge of genetic lesions. Notably, a subset of T-ALL without *ABL1* mutations showed unexpected sensitivity to dasatinib, suggesting potential drug repurposing for high-risk cases¹¹. Another study used mitochondrial BH3 profiling to show that T-ALL and ETP-ALL maturation stage dictates BCL-2 versus BCL-XL dependence, explaining venetoclax's selective activity in immature T-ALL¹⁴. Recent studies on large retrospective ALL cohorts integrating multi-omics and limited DRP identified pharmacotype clusters linked to survival and showed that *ex vivo* resistance predicts clinical outcomes and treatment vulnerabilities^{25,26}.

No national study has yet applied a chemogenomic strategy specifically to T-ALL/ETP-ALL. ALL2720 is, to our knowledge, the first prospective trial to systematically evaluate T-ALL using an integrated chemogenomic pipeline. Coordinated by GIMEMA, it centralized clinical and laboratory workflows nationwide, with fresh samples delivered within 24 hours and reports returned in a median of 7 days, supporting timely decisions in this aggressive disease. This platform surpasses previous genomic-only studies, such as BEAT AML²⁷, and aligns with larger investigations where DRP or chemogenomic turnaround ranged from 3–7 days^{15,16,18,19}.

More importantly, we demonstrated that translation is feasible even in countries where drug access is restricted by registration. Fifty-three percent of patients received an *N-of-1*–guided treatment, positioning this trial, despite the small cohort, among reports with the highest proportion of clinical application in a disease-specific setting^{15,17,18}.

When considering outcomes, patients treated with a chemogenomic-guided approach achieved an ORR of 46.7%, enabling nearly half of responders to proceed to transplantation. In R/R T-ALL, the registered option is nelarabine. Phase II trials on single-agent nelarabine in first relapse, both in children and adults, showed an ORR of 41–55%^{4,28}. However, efficacy decreased substantially in patients with ≥ 2 prior relapses, with ORRs of 27% and 36%, respectively^{4,28}. Emerging studies on novel agents, including venetoclax-based combinations and daratumumab, have shown encouraging activity in R/R patients^{5,10,29}. In FPM approaches, however, both the quality and speed of CR matter. Unlike standard regimens that often require hospitalization, strategies such as venetoclax with bortezomib or dasatinib can be administered in the outpatient setting, reducing costs and improving quality of life, which is essential in R/R cases where rapid remission is needed for alloHCST.

Several limitations should be noted. Our functional analyses required large cell numbers ($\approx 2 \times 10^7$) and relied on samples with high blast percentages ($> 35\%$), limiting applicability in patients with low blast burden or extramedullary disease. This issue could be mitigated by using platforms able to distinguish

leukemic from normal cells^{15,19,24}. Second, although the trial shows proof-of-concept feasibility, treatment decisions were at physicians' discretion, and off-label drug access varied across centers, introducing potential heterogeneity and delays. Despite being the largest prospective T-ALL cohort, confirmatory studies are needed to validate predictive sensitivity patterns, and limited follow-up prevents conclusions on long-term survival or durable post-transplant remissions.

In conclusion, the ALL2720 trial demonstrates that a coordinated national platform can provide functional genomic data to support timely therapeutic decisions, successfully rescuing nearly 40% of R/R T-ALL cases through tandem DRP-alloHSCT. Despite limitations such as small cohort size, N-of-1 decision-making, and limited follow-up, its prospective, multi-center, and standardized design provides a robust framework for managing aggressive diseases such as R/R T-ALL/ETP-ALL.

Declarations

AUTHOR CONTRIBUTIONS

Conceptualization and Design: L.P., M.M., A.P., E.C., G.M., M.V., P.F., C.M., R.L.S., G.R.; Methodology and Experiment Execution: L.P., R.R., M.G., R.Z., V.B., K.T., A.M., A.G.; Data Collection and Analysis: L.P., M.G., R.R., V.B., M.M., G.M.; Data Access and Verification: L.P., R.R., M.M., A.P., G.M., C.M., G.R.; Investigation and Clinical Data: L.P., M.G., B.C., S.C., F.G., F.C., C.P., M.P., F.L., S.S., P.Z., L.B., E.B., E.A., Er.Bo., M.B., R.B., M.D.T., An.Pe. A.R., Lu.Pr., C.M., R.L.S., G.R.; Writing, Review, and Editing: L.P., R.R., M.G., A.M., M.M., G.R., R.L.S., C.M.; Data Curation: L.P., R.R., M.G., A.M., C.M., G.R.; Supervision: G.R., C.M. All the authors read and discussed the manuscript.

COMPETING INTERESTS

Er.Bo. reports honoraria and advisory board fees from AbbVie, Otsuka, Incyte, Amgen, and Pfizer. L.B. reports honoraria from AbbVie, Jazz Pharmaceuticals, and Incyte. All other authors declare no competing interests. The remaining authors declare no competing interests.

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DATA AVAILABILITY

Data generated or analyzed during this study are included in this published article and its Supplementary Information, including the Supplementary Data files. This includes processed/summary results from targeted NGS, CI-FISH, and SNP-array analyses required to reproduce the reported findings. Raw and/or individual-level genomic data (including sequencing and array files) and drug response profiling (DRP) data are not publicly available but may be made available upon reasonable request to the corresponding author and subject to approval by the relevant ethics committees and execution of an appropriate data-transfer agreement, in accordance with applicable regulations.

CODE AVAILABILITY

All custom code used for data processing, statistical analyses, and figure generation in this study is publicly available in the GitHub repository *bioruffo/ALL2720* (<https://github.com/bioruffo/ALL2720>).

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Tables

Table 1 | Patients’ characteristics at enrollment

Characteristics	n = 29
Median age (years, range)	55 (21-77)
Sex (n, %)	
Male	18 (62.1)
Female	11 (37.9)
M:F ratio	1.6
Status at enrollment (n, %)	
Diagnosis	6 (20.7)
Relapse/Refractory	23 (79.3)
WBC at diagnosis > 100*10 ³ /mL (n, %)	5 (17.2)
NA	3 (10.3)
Phenotypic subgroup (n, %)	
Immature (ETP/near-ETP)	19 (65.5)
Typical (EGIL T-II/T-III)	10 (34.5)
Oncogenic subgroup (n, %)	
HOXA	6 (20.7)
TAL/LMO	1 (3.4)
TLX1	3 (10.3)
TLX3	1 (3.4)
Unclassified	18 (62.1)
Number of prior lines of treatment (n, %)	
0	6 (20.7)
1	11 (37.9)
2	5 (17.2)
≥3	7 (24.1)
Sample source (n, %)	
Bone marrow	23 (79.3)
Peripheral blood	3 (10.3)
Pleural effusion	3 (10.3)

CI-FISH assessment (n, %)	29 (100)
Drug response profiling (DRP) assessment (n, %)	28 (96.5)
Median percentage of blast on DRP sample (n, range)	70 (18-100)
Median time to combined DRP-FISH report (n, range)	7 (4-15)

Table 2 | Patients' characteristics of the Chemogenomic-guided Cohort

Characteristics	n = 15
Median age (years, range)	57 (22-69)
Sex (n, %)	
Male	9 (60)
Female	6 (40)
M:F ratio	1.5
Phenotypic subgroup (n, %)	
ETP	11 (73.3)
EGIL-T II	1 (6.7)
EGIL-T III	3 (20)
Oncogenic subgroup (n, %)	
HOXA	4 (26.7)
TLX3	1 (6.7)
Unclassified	10 (66.7)
Number of prior lines of treatment (n, %)	
1	2 (13.3)
2	7 (46.7)
≥3	6 (40)
Previous allo-HSCT (n, %)	
Yes	7 (46.7)
No	8 (53.3)

Table 3 | Treatment regimens adopted as N-of-1 treatment according to FPMRB's suggestions.

Regimen (n,%)	Schedule	Patients (n = 15)
Navitoclax-Venetoclax* ¹	Navitoclax 50 mg qd Venetoclax 400 mg qd	7 (46.7%)
Venetoclax-Bortezomib ²	Venetoclax 800 mg qd Bortezomib 1.3 mg/m ² day 1,4,8,11 (28 day/cycle)	3 (20.0%)
Dasatinib-Bortezomib ³	Dasatinib 140 mg/day Bortezomib 1.3 mg/m ² day 1,4,8,11 (28 day/cycle)	2 (13.3%)
Decitabine-Venetoclax-Gilteritinib	Decitabine 20 mg/m ² day 1-5 Venetoclax 200 mg qd Gilteritinib 120 mg qd	1 (6.7%)
Mitoxantrone-Etoposide-Bortezomib	Mitoxantrone 8 mg/m ² day 1-5 Etoposide 100 mg/m ² day 1-5 Bortezomib 1.3 mg/m ² day 1,4,8,11	1 (6.7%)
Cyclophosphamide-Ruxolitinib	Cyclophosphamide 500 mg qd day 1-3 Ruxolitinib 10 mg, then 5 mg bid	1 (6.7%)
Actinomycin D	Actinomycin D 12.5 mg/m ² qd day 1-5	1 (6.7%)

* The use of venetoclax involved a ramp-up phase as specified in the standard schedule.

¹ In one patient, venetoclax was administered at 50 mg qd. In one patient, nelarabine (1,500 mg/m² day 1, 3, 5 on a 21-day/cycle) was added to the regimen. In one patient, vincristine (2 mg on days 1, 8, 15, 22), dexamethasone (20 mg/m² on days 1-4,15-18), and peg-asparaginase (2000 UI/m² on day 3) were added to the regimen.

² In one patient, venetoclax was administered at a 200 mg qd dose, and mitoxantrone (10 mg/m² day 1-3) was added to the regimen.

³ In one patient, dexamethasone (8 mg qd) was added to the regimen.

Figures

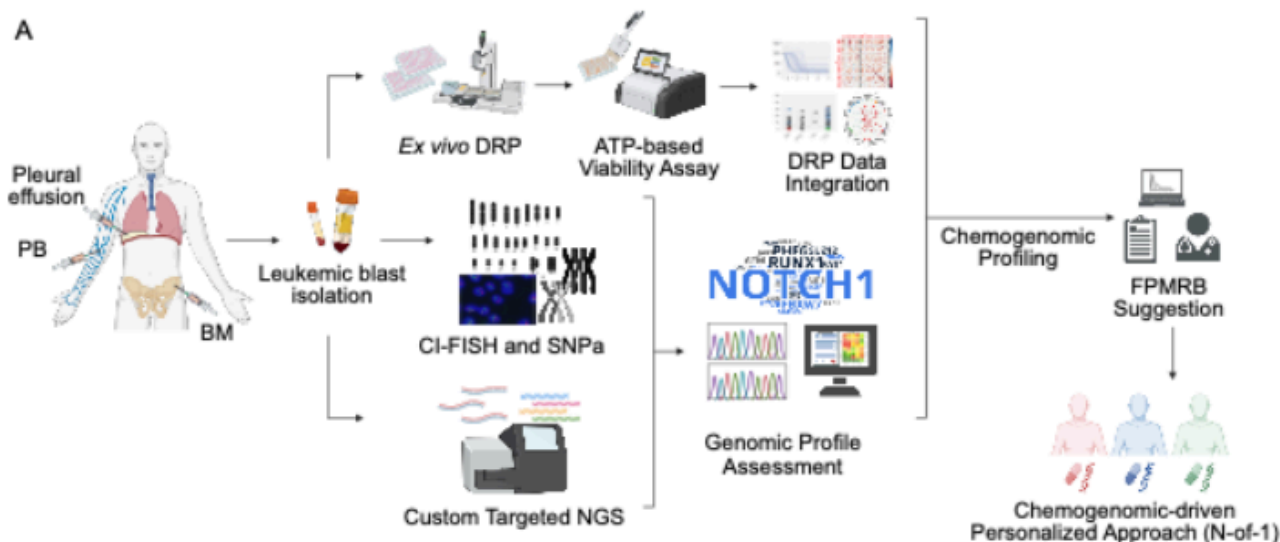
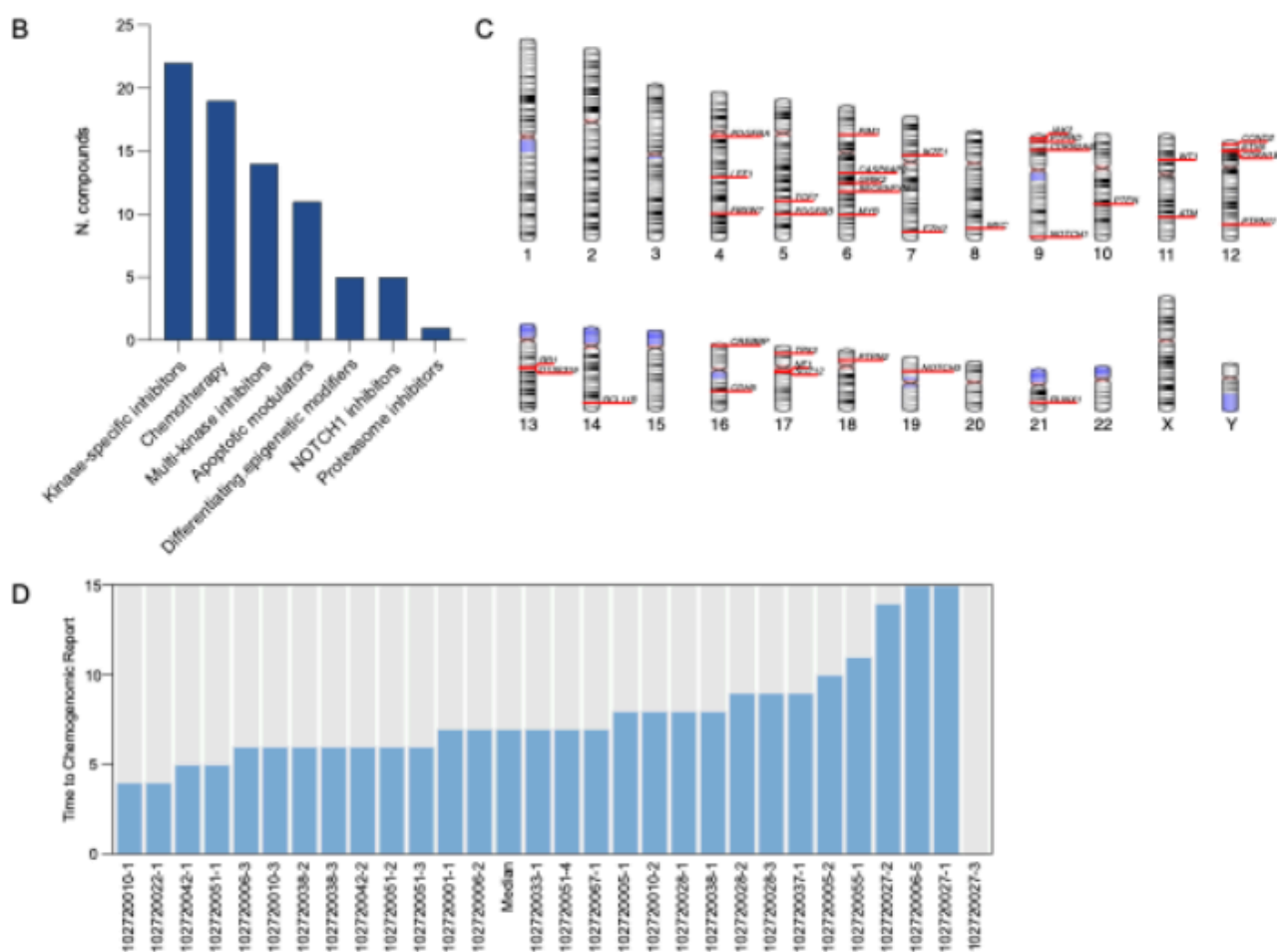


Figure 1

The ALL2720 study serves as a proof-of-principle for the routine application of chemogenomic assessment in T-cell acute lymphoblastic leukemia.

a) Outline of the ALL2720 chemogenomic platform. Briefly: samples from peripheral blood (PB), bone marrow (BM), or pleural effusion were collected and sent to the reference laboratories for

chemogenomic characterization through drug response profiling (DRP), combined-interphase FISH (CI-FISH), SNP array, and custom targeted NGS analysis. Chemogenomic data were then integrated and discussed by a functional precision medicine review board (FPMRB) to build a patient-specific report and suggest a personalized approach. The final therapeutic decision was left to the discretion of the local investigator.

- b) Overview of the drug classes in the compound library based on their primary target or mechanism of action.
- c) Chromosomal localization of major genetic lesions assessed by the genomic study.
- d) Time from sample arrival to issuance of a chemogenomic report, sorted by patient.

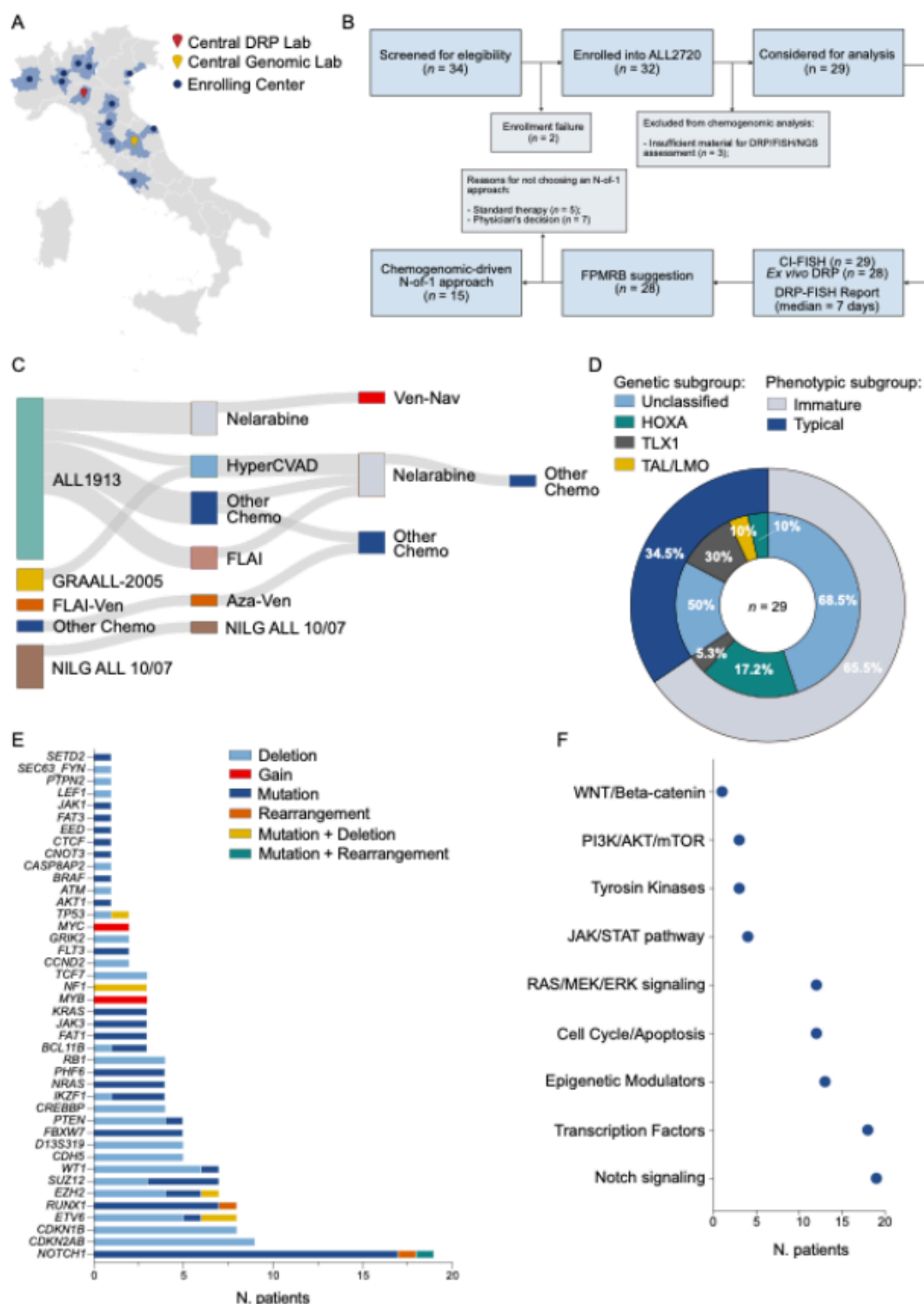


Figure 2

Flow diagram and patient's characteristics at enrollment.

a) Italy map showing the GIMEMA enrolling Centers and the reference laboratories for functional and genomic assays.

b) Flow diagram of study participants.

c) Treatment sequencing in T-ALL/ETP-ALL patients. The Sankey diagram shows the treatment transition among regimens used as first line in patients before enrollment in the ALL2720. Aza: azacytidine; Nav: navitoclax; Ven: venetoclax.

d) Doughnut chart showing the frequency of the phenotypic and genetic subgroups in the ALL2720 cohort.

e) Genomic landscape of aberrations identified through CI-FISH and targeted NGS profiling.

f) Dot plot illustrating the frequency of genomic alterations across major oncogenic signaling pathways. The x-axis represents the number of patients harboring alterations, while the y-axis denotes the affected pathways.

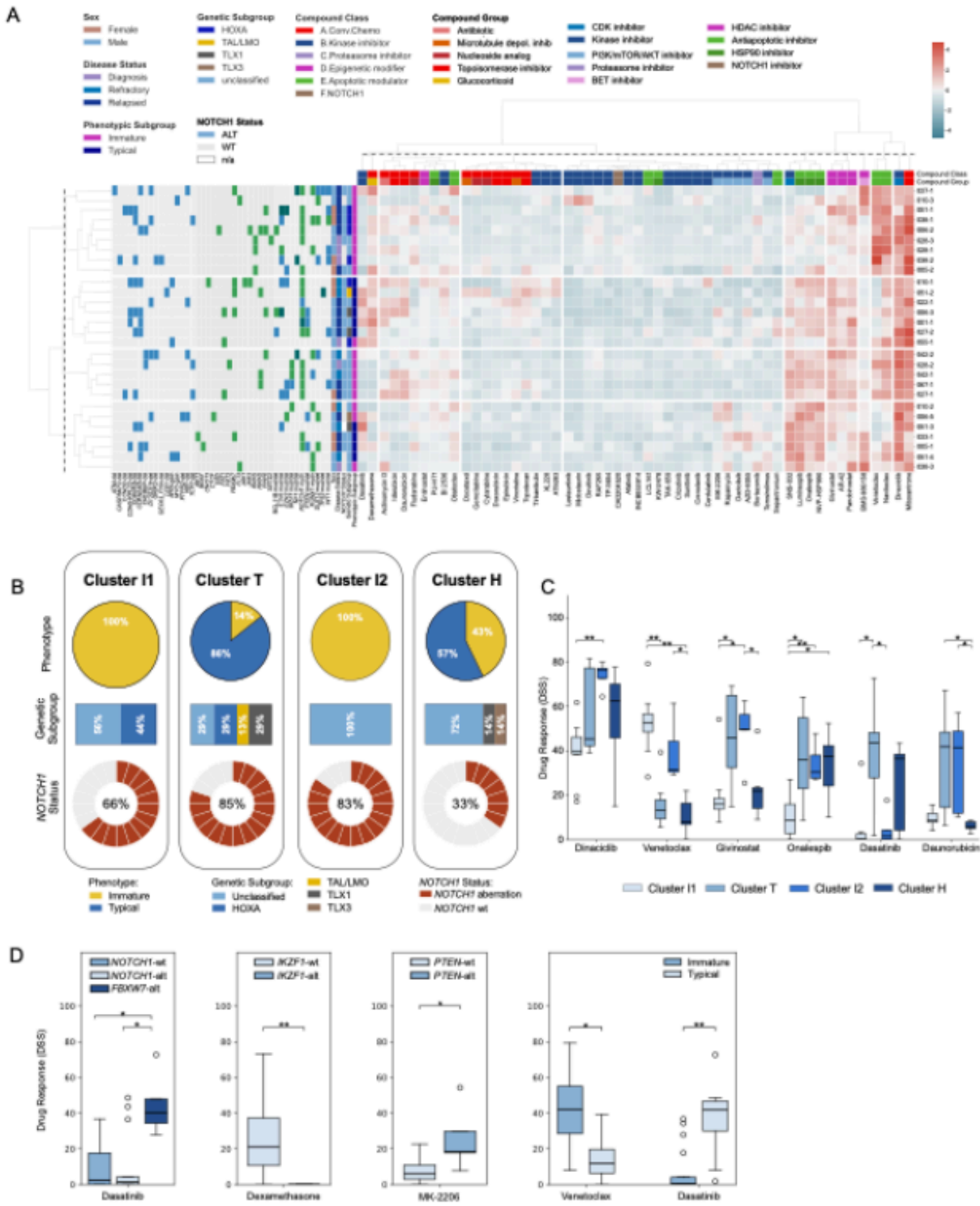


Figure 3

Drug response profiles are associated with clinical and genetic characteristics.

a) ClusterMap of scaled DSS values (sample-wise z-scores) highlighting patterns of relative drug sensitivity. Rows and columns are hierarchically clustered using Ward's method. Dendrograms are split at selected thresholds (red lines); the corresponding clusters are shown separated by gaps. Row

annotations: alterations detected by CI-FISH (blue), NGS (green), or both (dark teal) and clinical data. Column annotations: drug classes and groups as per legend.

b) Main features of the 4 clusters identified in A) as for phenotypic subgroup, genetic subgroup, and *NOTCH1* status.

c) Selected associations between clusters and selected drug response. Post-hoc Mann-Whitney U (MWU) test with FDR correction; significance levels: * FDR < 0.05, ** FDR < 0.01.

d) Selected associations between genetic/phenotypic characteristics and drug response. MWU as follows: dasatinib (in *NOTCH1* and *FBXW7* groups): post-hoc MWU with FDR correction; dexamethasone: single-drug MWU; MK-2206, venetoclax and dasatinib (in immature/typical phenotype): MWU with FDR correction for testing on all drugs. Significance levels: * $p < 0.05$, ** $p < 0.01$.

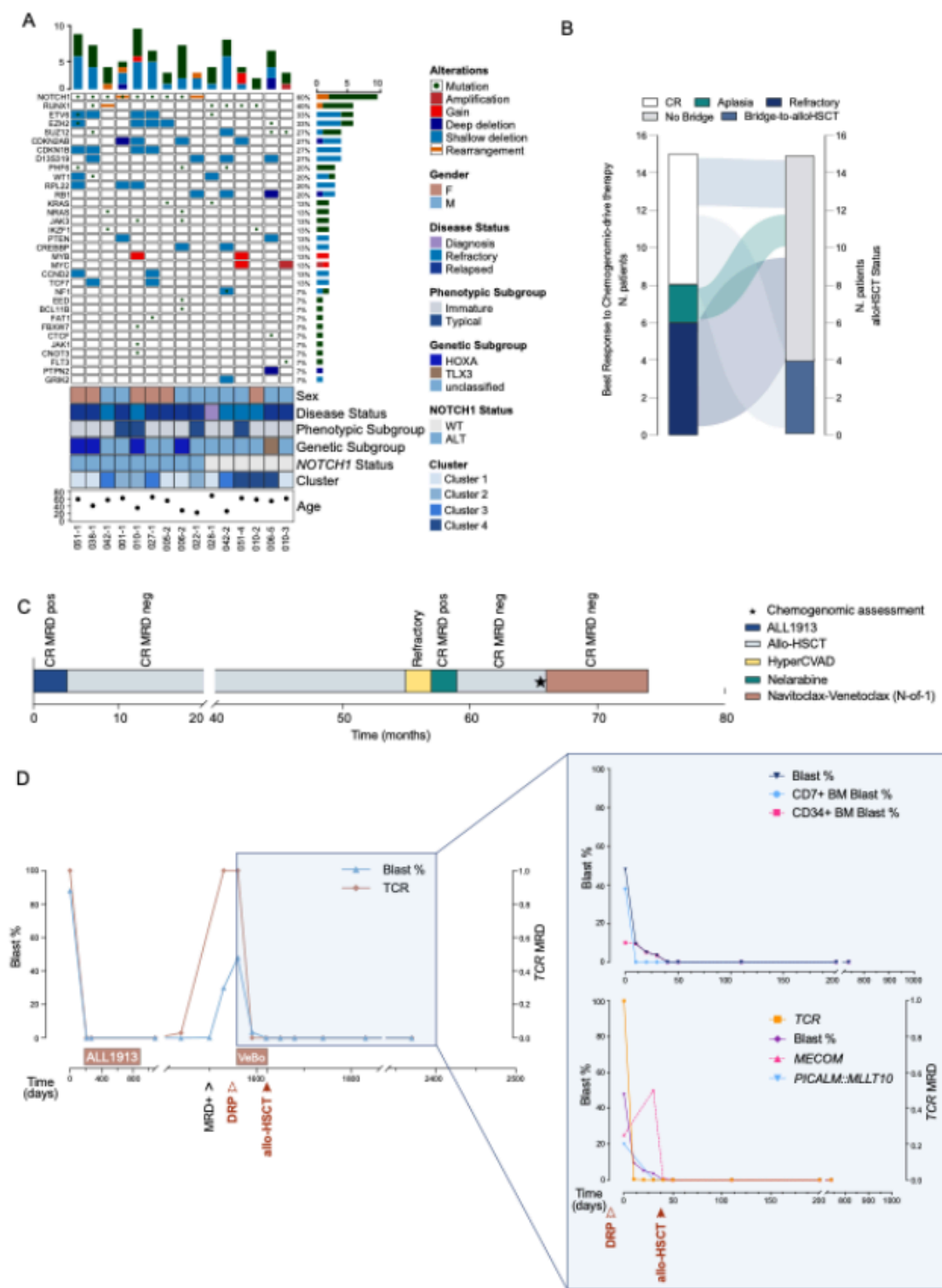


Figure 4

A chemogenomic-driven strategy induces deep responses in high-risk T-ALL/ETP-ALL patients.

a) The OncoPrint illustrates the distribution of gene alterations affecting individual patients. Each row in the OncoPrint displays the percentage distribution of relative gene alterations in the entire cohort (shown in the right histogram panel). Each column represents the total number of mutations for each patient,

with a specific indication of the gene alteration type (upper histogram panel). The OncoPrint also provides relevant characteristics of the patient samples collected for the study, such as sex, disease status, phenotypic and genetic subgroups, *NOTCH1* status, and age (lower panel).

b) The Sankey plot depicts the type of response to a chemogenomic-driven approach, such as complete remission (CR; white), aplasia (teal), and refractory (blue) (bar chart on the left) and the relative feasibility of an allogeneic hematopoietic stem-cell transplantation (allo-HSCT) (bar chart on the right). Patients who received an *N-of-1*-based approach as a bridge-to-transplant are shown in light blue; patients not suitable for alloHSCT are shown in grey.

c) Swimmer plot showing the time to next treatment for R/R ETP-ALL patient treated according to the chemogenomic assessment after relapse, after a second allo-HSCT. Colored bars indicate the therapeutic regimen as shown in the legend. The star indicates the *ex vivo* chemogenomic assessment.

d) Timeline depicting the course of disease and treatment choice coupled with measurable residual disease assessment for an R/R T-ALL patient. Colored lines show the MRD assessment.

Supplementary Files

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