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Relapsed childhood T-cell acute lymphoblastic leukemia and lymphoblastic lymphoma

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Abstract

While outcomes for pediatric acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LBL) have improved dramatically in recent decades, relapsed and refractory disease remain a significant therapeutic challenge. This is particularly true for patients with T-cell ALL and LBL, where survival for patients with relapsed/refractory disease remains dismal. Recent efforts to comprehensively profile the genomics of T-ALL/LBL to improve understanding of disease biology have enhanced our ability to identify high-risk patients at diagnosis who are more likely to relapse and have also identified novel targets for precision medicines. Novel immunotherapies have transformed the treatment landscape for patients with B-cell ALL (B-ALL). Many immunotherapies are under investigation in clinical trials for patients with T-ALL/LBL and early results are very promising. Given these insights into disease biology and the development of targeted and immune-based treatments, it is reasonable to hope for improved patient outcomes, although challenges still exist. In this review, we summarize the present state of understanding of the risk factors for relapse of T-ALL/LBL, established treatment regimens, and the promising small molecule inhibitors and immunotherapies with the potential to revolutionize the treatment of relapsed/refractory T-ALL/LBL.

1. Introduction

T-cell acute lymphoblastic leukemia (T-ALL) and lymphoblastic lymphoma (T-LBL) are rare diseases with yearly incidence of approximately 5-6 new cases per million people aged <20 years in the United States.[1] T-ALL accounts for approximately 15% of pediatric ALL and T-LBL accounts for approximately 30% of pediatric non-Hodgkin lymphoma.[2] Over the past few decades, a succession of multi-institutional trials orchestrated largely by cooperative groups have resulted in the creation of risk-stratified multi-agent therapies able to achieve long-term overall survival (OS) rates approaching 90% for newly diagnosed children, adolescent and young adults (AYA) with T-ALL and T-LBL.[3] Historically, patients with relapsed and refractory (r/r) disease have had a dismal prognosis. While outcomes for B-cell ALL have improved significantly in recent years, the outcomes for r/r T-ALL/T-LBL have remained poor with OS <30%.[4]

A major improvement in the treatment of B-ALL has been the advent of immunotherapy, including monoclonal antibodies, bispecific antibodies, and cell therapies. Unfortunately, the translation of immunotherapies for patients with T-ALL has been less robust. Nevertheless, several promising immunotherapies are currently in clinical trials with promising early results, raising optimism that the next decade will see similar improvements in outcome for patients with r/r T-ALL/LBL.

T-LBL is conventionally differentiated from T-ALL based on the degree of marrow involvement, using a historical cutoff of 25%. Over the past 20 years the treatment of

patients with T-ALL and T-LBL has largely been identical, as T-LBL patients were shown to have improved outcomes with leukemia-type regimens as compared with lymphoma-type regimens.[5] This is reinforced by the World Health Organization (WHO), which classifies T-LBL as the same disease as T-ALL.[5] Yet, differential response to novel therapies has been seen with several recent trials, highlighting that while T-ALL and T-LBL share many clinical and biologic features, they are different diseases.

In this manuscript, we summarize the current understanding of T-ALL/LBL disease biology and approaches for its treatment with particular attention on emerging therapies and the r/r population.

2. Risk factors for relapse

As survival is poor for T-ALL/T-LBL patients with relapsed disease, a major goal has been preventing relapse. One approach to reducing the risk of relapse is to identify patients who are more likely to relapse before they relapse and treating those patients with alternative or more intensive therapy through risk-stratification. Risk-stratification often incorporates clinical and demographic features, leukemia biology, and response to therapy. Risk-stratification has been instrumental in the improvement in outcomes in B-ALL. For T-ALL however, few features have been identified that can accurately risk-stratify patients independent of treatment response. Moreover, while individual

cooperative groups have identified potential biomarkers that are prognostic independent of treatment response, these biomarkers have not been validated in independent studies. Our group and others recently performed integrated genomic analyses in larger cohorts of patients with more robust sequencing technologies, allowing us to identify prognostic biomarkers that may be translatable into the clinic in the near future.[6]

2.1 Clinical and demographic factors

The only clinical features found to be independently prognostic of treatment response in T-ALL are age and central nervous system (CNS) involvement at diagnosis. While very rare, infants <1 year have inferior outcomes in T-ALL, similar to B-ALL.[7] Older adults also have inferior outcomes.[8] However, recent Children's Oncology Group (COG) phase 3 studies AALL0434 and AALL1231 found T-ALL patients aged 1-30 years have similar outcomes.[9] This is in stark contrast to B-ALL where adolescents and young adults fare worse than younger children.

Presence of blasts in the CNS is associated with worse outcomes, however the degree of involvement that associates with outcome depends on the treatment regimen and techniques used to identify leukemic blasts. Multiple studies have demonstrated patients with CNS3 disease have inferior outcomes (CNS status definitions are detailed in Table 1). However, patients with CNS2 had similar outcomes to those with no CNS involvement (CNS1) when treated on AALL0434/AALL1231. In contrast, patients with CNS2 had inferior outcomes to CNS1 on UKALL2003 and UKALL2011.[10, 11]

White blood cell (WBC) count in peripheral blood at diagnosis correlates with outcome in T-ALL, however, unlike B-ALL, the prognostic significance of WBC is mitigated when considering treatment response, and many groups no longer use WBC count at presentation for risk stratification for T-ALL.[12] Recent evidence shows that very high WBC counts, for example WBC >100,000/uL, or >200,000/uL for non-early T-cell precursor (non-ETP) T-ALL, may be predictive of worse outcomes.[13]

T-ALL is twice as common in males as females, likely due the presence of several genes on the X-chromosome being involved in leukemogenesis,[2, 14] however, outcomes for males and females are similar. T-ALL is more prevalent in patients who self-identify as Black or African American, however outcomes are not worse for the patients who self-identify as Black or African American or Hispanic patients compared to patients who self-identify as White.[15] This is also in contrast to B-ALL, where patients who self-identify as White have better outcomes.[16]

T-LBL is rarer than T-ALL, and so it is less clear which if any clinical and demographic features, aside from CNS status, are prognostic independent of treatment response in T-LBL. Disease involvement in bone marrow or peripheral blood at diagnosis, referred to as minimal disseminated disease (MDD), has been used in risk stratification by some groups. In evaluating a relatively large number of pediatric patients with T-LBL treated on the prednisone reference arm of EURO-LB02, MDD >1% had no prognostic significance, however surprisingly MDD <0.1% was associated with un*favorable* outcomes compared with MDD ≥0.1%, specifically in disease without *NOTCH1* and/or

FBXW7 mutation (5-year event-free survival (EFS) 94% versus 38%).[17] MDD has not been found to be prognostic by other groups on recent trials, possibly due to differences in therapy intensity.[18]

2.2 Response factors

Response to therapy is the only factor that is consistently identified as prognostic across trials for patients with T-ALL/LBL.[13] The Berlin-Frankfurt-Munster (BFM) cooperative group utilizes a regimen that starts therapy with a one-week course of prednisone, and reduction in disease burden at the end of this prednisone pre-phase is used for risk stratification: patients with a peripheral blood blast count of <1,000/μL are classified as prednisone good responders (PGR), differentiating them from prednisone poor responders (PPR). The PGR group has lower rates of relapse and improved overall survival (OS) compared to PPR.[19]

On COG trial AALL0434, flow cytometry-based measurable residual disease (MRD, also called minimal residual disease) in peripheral blood after one week of induction therapy was prognostic by univariate, but not multivariate, analysis when integrated with bone marrow MRD at end of induction.[13] Bone marrow MRD at the end of consolidation (EOC), also called IB by some cooperative groups, is the best predictor of outcomes in T-ALL. Multiple studies have shown that patients who are MRD-positive (≥0.1%) at end of consolidation have very poor prognosis, requiring hematopoietic stem cell transplantation (HSCT) for cure. Only a small minority of patients have persistent

disease at EOC[13, 20] and end of induction (EOI) MRD remains the major factor used in risk stratification. While most patients who are MRD <0.01% at EOI have favorable prognosis, a significant percentage of relapses do occur in these patients. More recently, methodologies with increased sensitivity in measuring MRD are being used in the clinic, such as next-generation sequencing. This will likely increase detection of residual disease and therefore increase the fraction of patients who have detectable disease at EOC. Whether this information is useful for prognostication will need to be determined in future trials.

For T-LBL, response to therapy in the form of EOI MRD has been found to be prognostic as well, using a cutoff of 0.1%. It is interesting to note that while EOI MRD is prognostic and therefore useful in risk stratification in T-LBL, changes in disease as seen by imaging in response to therapy is not as predictive of outcome.[18]

2.3 Immunophenotypic factors

The European Group for the Immunologic Classification of Leukemia (EGIL) divided T-ALL into groups based on the corresponding stage of thymocyte differentiation as defined by various cytoplasmic and surface markers:

- pro-T ($CD1a^-$, $CD2^+$, cytoplasmic (c)$CD3^+$, surface (s)$CD3^-$, $CD5^-$, $CD7^+$, $CD34^+$)
- pre-T ($CD1a^-$, $CD2^+$, $cCD3^+$, $sCD3^-$, $CD5^+$, $CD7^+$, $CD34^-$)
- cortical T ($CD1a^+$, $CD2^+$, $cCD3^+$, $sCD3^{+/-}$, $CD5^+$, $CD7^+$, $CD34^-$)

- mature T ($CD1a^-$, $CD2^+$, $cCD3^+$, $sCD3^+$, $CD5^+$, $CD7^+$, $CD34^-$)

Older trials found pro- and pre-T-ALL were associated with inferior outcomes and cortical T-ALL with more favorable outcomes, however the EGIL immunologic classification is has not been found to be independently prognostic with modern response-based risk stratification and treatment.[12]

Early T-cell precursor T-ALL (ETP-ALL) was first described in 2009 based on immunophenotypic similarities with normal early T-cell precursors, defined as expressing CD2, cCD3, and CD7, absent CD1a and CD8, and absent or dim CD5 expression. Early studies found that patients with ETP-ALL have inferior outcome. However, recent trials found that patients with ETP immunophenotype have similar outcome to non-ETP T-ALL and near-ETP ALL. Near ETP describes cases that would otherwise have the immunophenotype of ETP if not for higher CD5 expression (CD5 on >75% of blasts).[21] While outcomes are similar comparing those with ETP, near ETP and non-ETP immunophenotypes, the type of events differ. ETP and near ETP are more likely to have slower response to therapy or refractory disease than non-ETP, likely from intrinsic corticosteroid resistance; however, that is balanced by fewer relapses.[2, 13]

2.4 Genetic factors

T-ALL/LBL is subdivided by the WHO into two groups: ETP and not otherwise specified (NOS), i.e. non-ETP.[5] The International Consensus Classification (ICC) recognizes

three groups, T-ALL NOS, ETP NOS, and ETP *BCL11B*-activated, and recently added 8 provisional entities: *TAL1/2*-rearranged, *TLX1*-rearranged, *TLX3*-rearranged, *HOXA*, *LMO1/2*-rearranged, *NKX2*-rearranged, *SPI1*-rearranged, and *BHLH*-other.[21] These classifications are based on relatively small studies and are underpowered for prognostication.[6] There is currently no consensus in defining subtypes, likely due to the prevalence of non-coding alterations that drive T-ALL/LBL oncogenesis.[22]

One T-ALL risk assignment strategy originally published by the Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL) described better outcomes in cases of *NOTCH1* and/or *FBXW7* mutation without *N/K-RAS* mutation and/or *PTEN* mutation/deletion.[23] This scheme was validated in a set of pediatric patients, however it was not upheld when other cooperative groups tested it in their populations.[24] GRAALL more recently used a hybrid capture-based NGS approach in adults, and validated this approach in pediatric patients, defining low risk disease as having *NOTCH1* and/or *FBXW7* mutations, *PHF6* mutations, or *EP300* mutations without *N/K-RAS, PI3K* pathway (*PTEN, PIK3CA, PIK3R1*), *TP53, DNMT2A, IDH1/2,* or *IKZF1* alterations. When combined with WBC count at diagnosis and EOI MRD, this method identified a favorable risk group with a cumulative relapse incidence of 12% at 5 years, compared to an adverse risk group with 51% cumulative relapse incidence at 5 years.[25] This NSG-based stratification needs to be validated by other groups.

A recent landmark study by Pölönen and colleagues performed whole genome, exome, and transcriptome sequencing on more than 1300 T-ALL cases to better understand T-

ALL biology and determine predictors of relapse and treatment failure. This study identified genetic drivers in 95% of cases, defining 15 subtypes of T-ALL, some of which were novel. One of the new proposed subtypes was termed ‘ETP-like’ is enriched in, but not limited to, the ETP and near-ETP immunophenotypes and defined by a distinct gene expression profile.[6] Patients with ETP-like disease were found to have inferior outcomes regardless of immunophenotype. In contrast, patients with the ETP immunophenotype only had inferior outcomes if they were in the ETP-like genomic subtype. Pölönen and colleagues demonstrated that in ~60% of cases the primary leukemic driver involved non-coding regions of the genome, underscoring the need for whole genome sequencing for characterization of T-ALL. Interestingly, specific types of genetic alterations, rather than solely the gene, were found to have specific outcome associations. For example, intragenic loss and intronic single nucleotide variants (SNV) in *NOTCH1* were associated with poor outcome whereas other types of *NOTCH1* alterations were associated with favorable outcomes. *LMO2* intergenic loss, *NOTCH1* intragenic deletions, *TCR::MYC,* and *PTEN* deletions, among others, were specifically associated with relapse (Fig 1a).

Within the ETP-like subtype, cases were divided into further genetic subgroups based on specific driver mutations, for example ETP-like KMT2A and ETP-like MLLT10 groups, each with prognostic value. Individually, the poorest survival outcomes were seen with SPI1 and LMO2 γδ-like subtypes, and ETP-like KMT2A, and ETP-like MLLT10 subgroups (Fig. 1b). Subtype superseded specific driver mutation. For example unlike

genetic subgroups within the ETP-like group, the non-ETP-like KMT2A and the non-ETP-like MLLT-10 subgroups had favorable prognosis.

Two separate multivariate models were constructed that incorporated subtype, gene alterations, and pathway dysregulation information to stratify patients into risk groups. The highest risk for relapse was seen with features including ETP-like KMT2A, SPI1, and LMO2 γδ -like subtypes (5-year EFS<60%) whereas ETP-like ZFP36L2, TAL1 DP-like RPL10, TLX1, KMT2A (non-ETP-like), and HOXA9 TCR among others had favorable outcomes (5-year EFS>98%) (Fig. 1c).[6]

3. Established therapies for relapsed disease

Re-induction therapy at relapse typically consists of multi-agent chemotherapy to achieve complete response (CR), in addition to chemotherapy administered directly to the CNS to eliminate CNS disease or prevent spread. The goal of treatment at relapse is to achieve a second remission, ideally without detectable MRD, to bridge to HSCT which is the only proven curative treatment for relapsed T-ALL and T-LBL, regardless of relapse site or timing.[26] Outcomes of HSCT for relapsed T-ALL are improved when total body irradiation (TBI) is included in conditioning,[27] and are superior for patients with isolated extramedullary relapse.[28]

There are multiple reinduction regimens that have been used in r/r T-ALL/T-LBL. Most commonly these include a four-drug re-induction (vincristine, corticosteroid, asparaginase, anthracycline) with or without a newer agent. Four multi-agent regimens often used in pediatrics are shown in Table 2. As response rates are similar overall for each of the reinduction regimens based on early phase trials, the choice of is often based on access to agents, prior toxicities or reactions to medications, CNS status at relapse, timing of relapse, and recent chemotherapy exposure. Importantly, each of these re-induction regimens were tested in relatively small non-randomized early phase trials in different eras with different standard therapy for newly diagnosed patients, making comparison between regimens challenging.

UKALLR3 is an intensive dexamethasone-based regimen containing mitoxantrone, established as an effective approach for r/r pediatric ALL with studies reporting 85% complete response (CR), 62% MRD-negative rate, 3-year progression-free survival (PFS) and OS of 65% and 69%, however ~90% of patients had B-ALL.[29, 30] A more recent study evaluated the use of UKALLR3 in a larger number of pediatric patients with r/r T-ALL and reported 29% and 33% 10-year EFS and OS.[31] Multiple studies have shown the UKALLR3 regimen has significant toxicity with high-rates of treatment-related mortality. Thus, despite the high response rates (at least for B-ALL[32, 33]), it is used less commonly.[34] ALL-REZ BFM 2002 is an alternate regimen of intensive multi-agent chemotherapy that includes dexamethasone but varied significantly from UKALLR3 in dose, schedule, and type of chemotherapeutics. ALL-REZ BFM 2002 was compared to UKALLR3 in relapsed ALL in a randomized international multi-center trial[33] and

achieved similar outcomes to UKALLR3 with 27% and 30% 10-year EFS and OS for patients with r/rT-ALL.[31] Combining outcomes for patients with r/r T-ALL treated on UKALLR3 and AALL-REZ BFM 2002, 64% of 115 patients had CR after the first block of treatment.[31] Data comparing outcomes between UKALLR3 and ALL-REZ BFM 2002 limited to patients with T-ALL are currently unpublished.

An alternate regimen, using a less-intensive prednisone-based 4 drug regimen plus the proteasome inhibitor bortezomib, was studied on COG AALL07P1. AALL07P1 included 32 patients with pediatric T-ALL/LBL, achieving CR 68% (15/22), and MRD <0.01% in 20% of T-ALL cases at end of block 1. Sub-analysis of 20 evaluable patients with T-ALL stratified by MRD status at the end of block 1 demonstrated 3 year survival as 75% EFS and 67% OS for patients who were MRD-negative (N=4), and 43% EFS and 44% OS for those MRD-positive (N=16).[35]

The DELPHINUS trial (NCT03384654) investigated the combination of the anti-CD38 monoclonal antibody daratumumab with a multi-agent chemotherapy backbone. This backbone was similar to AALL07P1 except for fewer doses of pegaspargase and it did not include bortezomib. Response rates for 29 children and young adults with r/r T-ALL were promising with an ORR 83% (CR plus CR with incomplete hematologic recovery (CRi)) after 2 blocks of therapy and a 41% MRD-negativity rate. 72% of patients were successfully bridged to HSCT. Overall survival was reported as 33% EFS and 39% OS for patients with T-ALL[36] (Table 2).

The purine nucleoside antimetabolite nelarabine was initially studied as monotherapy for r/r disease and demonstrated CR 48% and ORR 55% (N=33) in children with first relapse of T-ALL.[37] Nelarabine can cause neurotoxicity that can be permanent. The NECTAR regimen combining nelarabine with cyclophosphamide and etoposide was recently published, reporting CR 25% and ORR 33% (N=12) with 42% of patients going to HSCT for T-ALL[38] (Table 2). Of note, these trials were in different eras and therapy for newly diagnosed patients has intensified, potentially explaining the inferior results of NECTAR as compared with nelarabine monotherapy. Access to nelarabine is limited outside of North America.

4. Precision Therapies

Inhibition of molecular pathways responsible for oncogenesis has proven effective for some forms of ALL. The prototypical example is the use of tyrosine kinase inhibitors, such as imatinib, dasatinib, and ponatinib, for *BCR::ABL1* (Philadelphia chromosome positive (Ph+)) ALL which, when combined with cytotoxic chemotherapy, has significantly improved overall outcomes compared to chemotherapy alone.[39, 40] Several approaches with preclinical or clinical experience in T-ALL/LBL are summarized in Table 3, with notable agents detailed below.

4.1 BCL-2 and BCL-X_L

The B-cell lymphoma-2 (BCL-2) family of proteins regulate apoptosis through both pro- and anti-apoptotic activity. Several subtypes of ALL have been found to be dependent on anti-apoptotic BCL-2 family proteins, particularly BCL-2, BCL-X_L, and MCL-1, for survival and therefore sensitive to blockade by BH3-mimetic drugs. Profiling of T-ALL cell lines and primary samples found that ETP-ALL has specific dependence on BCL-2, while non-ETP-ALL was more dependent on BCL-X_L.[41]

Venetoclax, a BCL-2 inhibitor, has shown efficacy in r/r ETP-ALL when combined with various combinations of chemotherapy.[42] Navitoclax, a dual BCL-2/BCL-X_L inhibitor, was recently studied in combination with venetoclax and multi-agent chemotherapy in adults and children with r/r ALL in a phase I clinical trial and reported combined outcomes for pediatric patients (B- and T-ALL, N=12) receiving a range of cycles of therapy with 75% CR, 50% MRD-negative rate and 12 month OS 61%. For all patients with T-ALL (all ages, N=19) receiving a range of therapy cycles, CR was 53%, 32% were MRD-negative, and 12-month OS was 30%.[43] These results led to additional trials combining venetoclax with navitoclax for r/r T-ALL/LBL, however these trials were stopped because navitoclax is no longer available even in an investigational setting. Inhibition of BCL-2 +/- BCL-X_L is an area of intense interest, and several other inhibitors are in development with the potential for utility in r/r T-ALL/LBL (Table 3).

4.2 CDK4/6

Cyclin-dependent kinases are involved in cell cycle progression, transcription, and DNA repair, and are attractive therapeutic targets in hematologic malignancies where they

are frequently overexpressed.[44] In T-ALL, >70% of cases have genetic alterations leading to CDKN2A/B loss, which often leads to concomitant upregulation of CDK4/6.[14] CDK4 and CDK6 complex with D-type cyclins, which function to phosphorylate Rb releasing it from repressive Rb-E2F complexes and promoting G1-S cell cycle progression.[45] CDK6 has been found to be required for both Notch1- and AKT-driven disease.[46, 47]

The CDK4/6 inhibitors palbociclib and ribociclib have shown promising results in preclinical models of T-ALL.[45, 46] Ribociclib synergized with glucocorticoids and mTOR inhibitors in mice, which prompted the launch of a phase 1 clinical trial treating pediatric patients with ribociclib, everolimus, and dexamethasone (NCT03740334).[48] A separate phase 1 pediatric ALL trial, COG AINV18P1 (NCT03792256), treated children with r/r T- and B-ALL with palbociclib added to a prednisone-based four drug reinduction; palbociclib was well-tolerated and 2 of 4 patients with multiply-relapsed T-ALL achieved CR.[49]

4.3 Jak/Stat

Aberrant Jak/Stat signaling is common in T-ALL, particularly in ETP and ETP-like ALL, often through activating mutations in *JAK1, JAK3, STAT5,* and *IL7Ra*, *JAK2* fusions, or *PTPN2* deletions.[12, 50] The Jak1/2 inhibitor ruxolitinib was found to be effective independent of Jak/Stat pathway mutations in preclinical ETP models.[51] *IL7R* mutations are associated with glucocorticoid resistance and ruxolitinib has been shown to reverse

glucocorticoid resistance and synergize with glucocorticoid treatment in preclinical models.[52]

Case reports have demonstrated clinical response to Jak inhibitors in r/r T-ALL/LBL harboring *JAK3* mutations.[53] Additionally, BH3 profiling and transcriptome sequencing of over 100 pediatric T-ALL cases identified *JAK3* activating mutations as being strongly associated with mitochondrial apoptosis resistance.[54] Prospective trials are needed to ascertain the efficacy of Jak inhibition, either as monotherapy or as part of a multi-agent regimen.

4.4 LCK

Lymphocyte-specific protein tyrosine kinase (LCK) is an SRC-family tyrosine kinase and member of the pre-TCR pathway required for normal T-cell development.[55] Dasatinib, an ABL- and SRC-family kinase inhibitor, has been shown to be effective for T-ALL driven by *ABL1* fusion in case reports.[56]

More recently, studies conducted *ex vivo* drug profiling and found 30-44% of T-ALL cases tested were exquisitely sensitive to dasatinib through high expression of LCK.[57, 58] Recent work suggests that LCK activation is more common in more mature-lineage T-ALL.[6] Clinical trials and preclinical studies using alternate LCK inhibitors are underway.[59, 60]

4.5 Notch

The Notch signaling pathway plays a critical role in T-cell differentiation and proliferation and activating Notch pathway mutations are among the most frequent abnormality in T-ALL.[6, 14] Notch signaling is dependent on γ-secretase which cleaves the intracellular domain of the Notch receptor allowing translocation to the nucleus where it activates transcription of several key genes. Several γ-secretase inhibitors (GSI) have been investigated in T-ALL, mostly with limited clinical success (Table 3) and dose-limiting toxicities.[61] Alternate Notch-targeting approaches have been developed and tested in preclinical models, including selective inhibition of PSEN-1 and sarco/endoplasmic reticulum calcium ATPase (SERCA) channel inhibition.[62]

4.6 Other

Other promising small molecule inhibitors include PDGFRA inhibitors including avapritinib,[63] exportin-1 inhibitors,[64] and OBI-3424 which inhibits AKR1C3. AKR1C3 is highly expressed in several cancers, including T-ALL.[65] Currently, OBI-3424 is being investigated in the phase 2 trial, S1905 (NCT04315324).

5. Immunotherapy for relapsed T-ALL/LBL

Immunotherapy has revolutionized the treatment of several types of cancers, including B-cell malignancies. Unfortunately, translation of immunotherapies for T-ALL/LBL have lagged behind those of B-ALL, based on multiple concerns including toxicity through elimination of healthy T-cells and fratricide. Nevertheless, multiple trials using immunotherapy for T-ALL are on-going and early results are promising.

5.1 Antibody-based therapies

Antibody-based therapies target membrane-bound or extracellular molecules and can be modified to traffic a bound cytotoxic agent specifically to target cells (antibody-drug conjugate, ADC), or to link endogenous cytotoxic cells to target cells as in the case of bi-specific T-cell engaging antibodies. Antibody-based approaches have been reviewed in detail[66-68] and will be summarized here (Table 4).

5.1.1 CD38

Daratumumab is a monoclonal antibody against CD38, a type II transmembrane glycoprotein present on thymocytes, activated T cells, and differentiated B cells. CD38 is highly expressed on most cases of T-ALL and its expression persists at relapse (see

[67]). Preclinical studies using daratumumab in T-ALL have shown promising activity as monotherapy or in combination with chemotherapy, leading to clinical trials such as the aforementioned DELPHINUS trial (Table 2).

Despite expression on activated T cells, daratumumab has not been shown to cause T-cell aplasia. The most common side effect is infusion reaction;[36] CD38 is present on mast cells and risk of infusion reactions is high with the first dose but markedly lower with subsequent doses as mast cells do not regenerate quickly. CD38 is expressed at low levels by red blood cells (RBC) and daratumumab can persist on RBCs for several months, which can complicate RBC cross-matching but has not been found to cause hemolysis. Special techniques are used to safely transfuse patients recently with daratumumab.[69] Daratumumab is also being investigated as monotherapy to prevent relapse after HSCT for r/r T-ALL/LBL in an ongoing clinical trial (NCT04972942).

A separate CD38-targeting antibody, isatuximab, has been developed which binds a different epitope on CD38 from daratumumab. Two clinical trials investigating isatuximab for hematologic malignancies including T-ALL, NCT02999633 and NCT03860844, were terminated early.

Bispecific antibodies link endogenous cytotoxic T-cells to target cells with a single molecule, inducing destruction of the target cell. Blinatumomab, a CD19/CD3-bispecific is highly effective in relapsed B-ALL.[70] The bispecific CD38-/CD3-binding antibody XmAb18968 showed promise in a small study of four adult patients with r/r T-ALL (see

[67]) and has been expanded into a larger trial (NCT05038644). Additional CD38/CD3 bispecific antibodies are being investigated, including ISB1342.[71]

5.1.2 CD52

CD52 it is expressed on T-cells across stages of differentiation, as well as other lymphocytes including B- and NK-cells. The anti-CD52 antibody alemtuzumab was studied as monotherapy in an early phase trial in children with r/r T-ALL (ADVL0222) but stopped early for excessive toxicity (see [66]).

5.1.3 CD25

CD25, the alpha subunit of the receptor for IL-2 (IL2RA), is highly expressed on T-cell malignancies including T-ALL. Published clinical experience with basiliximab, a monoclonal antibody against CD25, is limited to the case of a child with refractory T-ALL complicated by a paraneoplastic rash in whom remission was induced with the administration of basiliximab (see [67]).

5.1.4 Additional antibody targets

CD47 is highly expressed in ~50% of T-ALL/LBL and CD47 functions as an immune checkpoint, acting as a ligand for signal regulatory protein alpha (SIRPα) to prevent phagocytosis.[72] Encouraging results combining CD47 blockade with daratumumab were seen in T-ALL preclinical patient-derived xenograft (PDX) models (see [67]).

Several additional targets have been investigated that show promise in preclinical models. The alpha subunit of the IL-7 receptor (IL7RA), also termed CD127, is expressed on 60-80% of T-cell subtypes and has been shown to increase with exposure to multiagent chemotherapy. Anti-IL7R antibodies have shown activity in preclinical models (see [67]). Notch1, CD3, and CD99 are also being explored as targets for antibody therapy for T-ALL (Table 4).

5.2 Cellular therapies

CAR-T therapy has revolutionized the treatment of B-cell malignancies including B-ALL, achieving impressive outcomes especially in the r/r setting, with some studies showing remission rates >90% and very high rates of MRD negativity among highly refractory patients.[73] CAR-T for T-cell disease has a number of challenges (reviewed in [74]). These include fratricide, which can occur when the target antigen is present on both malignant cells and CAR-T cells. Techniques to prevent fratricide focused on masking or deletion of the target antigen from CAR-T have shown promise. Product contamination with

malignant cells is a theoretical concern, however, T-ALL blasts do not survive *ex vivo* and have not been seen in products after development. Targeting antigens present on healthy T-cells risks T-cell aplasia with risk of severe infections. The aggressive nature of T-ALL/LBL embodies challenges to CAR-T in that the disease is often rapidly progressive, limiting the time available for CAR-T manufacturing, and the intense regiments used to treat T-ALL/LBL limit the number of healthy endogenous T-cells available for manufacturing. To avoid these issues, genetically modified “off-the-shelf” allogeneic CAR-T cell products have been developed that are currently in clinical trials (Table 5).

NK cells functionalized with CAR (CAR-NK) may be attractive for T-ALL therapy as they may facilitate easier fratricide avoidance (limited expression of T-cell markers), and may be more readily used as allogeneic “off-the-shelf” products.[75]

5.2.1 Cell therapy targets

CD7 is expressed highly on the majority of cases of T-ALL. Evaluation of 49 T-ALL cases detected CD7 expression on blasts in 94%; similar expression has been seen after chemotherapy and at relapse (see [76]). CD7 is also expressed on most healthy T-cells and NK cells. Several CAR-T products targeting CD7 have been developed (Table 5). One product, which survives fratricide without external manipulation (“natural selection”), has been used in a relatively large number of patients and achieved CR at one month in >90% of patients and 2-year PFS >50% with a tolerable toxicity profile

(N=60 adult and pediatric patients).[77] The same CAR-T product was effective when followed by HSCT for pediatric patients with r/r T-ALL/LBL, with a 2-year leukemia-free survival (LFS) of 87.5% (N=10).[78] A separate CD7-targeted CAR-T has CD7 downregulated by a protein expression blocker (PEBL) and was studied in a cohort of 17 patients with r/r T-ALL (13 pediatric), achieving 94% MRD-negative CR within one month, with mild toxicities. Over a median follow-up of 15 months, there were two cases of relapse (one CD7-negative), 24-month OS was estimated at 55% with cumulative incidence of relapse and remission failure of 27%.[79] T-cell depletion is a common finding with CD7 CAR-T, but fortunately T-cell aplasia is not seen. Rather, polyclonal CD7-negative T-cells have been found to expand while CD7 CAR-T persist and have at least some anti-infectious activity.[79]

Allogeneic products have been developed which utilize T-cells for CAR-T from healthy donors, including one that derives CAR-T from HLA-matched donors and incorporates PEBL to avoid fratricide. Impressive results were seen in the 20 pediatric and adult patients with relapsed T-ALL who received this CAR-T, with 85% CR and 95% ORR by day 30. At a median follow-up of 27 months, six patients had relapse with four being CD7-negative, 2y-PFS 37% and 2y-OS 42%. All patients had mild cytokine release syndrome (CRS) and cytopenias with eventual expansion of CD7-negative T- and NK-cells, and 80% developed any grade GVHD (5% grade ≥3). It is notable that 6 of the 12 patients who did not have consolidating HSCT after CAR-T had infection, 5 of whom had severe (grade ≥3) infection, while one patient of the 7 who did have HSCT had severe infection,[80] raising concern for the efficacy of CD7-negative immune cells that

expand in the setting of CAR-T persistence. HSCT may be needed following CD7 CAR-T even in the absence of universal T-cell aplasia.

Universal donor ("off the shelf") CAR-T products targeting CD7 that do not require HLS-matched donors, making them more clinically available, have also been developed. One product uses cytosine editing to silence expression of CD7, TRAC to prevent GVHD, CD52 to prevent elimination by lymphodepleting serotherapy, and PD-1 to avoid CAR-T silencing, and demonstrated efficacy in murine models of pediatric T-ALL.[81] Another product uses CRISPR/Cas9 to knockout CD7 and TRAC, and in a pilot study 58% of adults with r/r T-ALL/LBL reported 58% CR (6 of 7 CR being MRD-negative) and overall mild toxicity with no GVHD or prolonged T-cell aplasia.[82]

CD5 is another attractive target as it is expressed on as many as 80% of T-ALL/LBL cases.[76] Clinical studies of CD5-targeted CAR-T are limited. An autologous CD5-targeting CAR-T was tested in patients with r/r T-ALL or T-cell non-Hodgkin's lymphoma (T-NHL) and achieved CR in 1 of 4 patients with r/r T-ALL and 2 of 5 with T-NHL (see [76]). An allogeneic product modified to have a molecular "off" switch for safety caused severe skin toxicity in all three patients treated, necessitating induction of off signaling in each case.[83]

Several other promising surface markers have been identified and targeted by cellular therapy products in preclinical studies, including CD1a, CD2, CD3, CD4, and CD38

(Table 5). CAR-NK products targeting various T-cell markers have shown promise in preclinical models as well (Table 6).

6. Conclusion

Relapsed/refractory T-ALL and T-LBL pose major therapeutic challenges. Several therapies have been developed for T-ALL/LBL, and while many have improved outcomes or show promise in the preclinical setting, major clinical breakthroughs have yet to be seen. Fortunately, many of these novel therapies continue to hold promise, and there are a number of clinical trials currently underway (Table 7).

Recent insights into T-ALL biology have led to improved risk-stratification and the identification of novel targets for small molecule inhibitors. While in its infancy in T-ALL/LBL, several immunotherapies have demonstrated striking efficacy in early phase trials. Of critical concern is the target used for immunotherapy: on-target off-tumor cell killing can cause highly harmful T-cell aplasia or a GVHD-like toxicity. In the setting of treatment with immunotherapies, including CAR-T, whether subsequent HSCT remains required for cure in r/r T-ALL is unknown. Nevertheless, most current early phase immunotherapy trials in r/r T-ALL recommend or require consolidation with HSCT. Additionally, while viral infections have been reported following CAR-T, unmitigated T-cell aplasia has not been seen due to expansion of rare populations. Several promising therapeutics have been highlighted in this review, however there is a notable paucity of randomized-controlled trials comparing regimens. Nevertheless, with the improved

ability to risk stratify patients, the recent identification of novel targets for small molecule inhibitors and the ability to safely and effectively translate immunotherapies, it is possible we are entering a new era where almost all children with T-ALL/T-LBL are cured.

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Tables

Table 1. Central nervous system disease (CNS) classification*

CNS Disease	Blasts (Y/N)	RBC (1/μL)	WBC (1/μL)	$\frac{\text{CSF WBC}}{CSF\ RBC} > 2\frac{Blood\ WBC}{Blood\ RBC}$ (Y/N/NA)
1	N	any	any	NA
2a	Y	< 10	< 5	NA
2b	Y	≥ 10	< 5	NA
2c	Y	≥ 10	≥ 5	N
3a	Y	< 10	≥ 5	NA
3b	Y	≥ 10	≥ 5	Y
3c	Symptomatic CNS involvement			

CNS, central nervous system; RBC, red blood cell; WBC white blood cell; Y, yes; N, no; NA, not applicable

*Some cooperative groups combine patients with traumatic lumbar puncture into a single group, e.g. the approximate equivalent of CNS 2b, 2c and 3b.

Table 2. Established multi-agent regimens for relapsed/refractory T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma (T-ALL/LBL)

Regimen	Re-induction (block 1)	Outcomes in r/r T-ALL/LBL	Comments	Ref
UKALL R3 (mitoxantrone arm [29])	Vincristine, dexamethasone, asparaginase, mitoxantrone	10y EFS 29%, 10y OS 33% (N=21)	Associated with high rates of infections, for example 40% of on AALL1331 had grade ≥3 infection due to UKALL R3 reinduction [Hogan Blood 2018], other studies reporting rates as high as 90% [30]	31
ALL-REZ BFM 2002	Dexamethasone, vincristine, methotrexate, asparaginase, cytarabine	10y EFS 27%, 10y OS 30% (N=67)	Increased cumulative dosing of chemotherapy compared to UKALL R3.	31
AALL07P1	Bortezomib plus VPLD	T-ALL (N=22): CR 68%, MRD-negative rate 20%, 3y EFS/OS 75%/67% when MRD-neg after block 1 (N=4), 3y EFS/OS 43%/44% when MRD-pos after block 1 (N=16). T-LBL (N=10): CR 30%, ORR 70%	Bortezomib overall well-tolerated with most common toxicity being hematologic (55%). Grade 3 neuropathy in 3%, no grade >3 neuropathy. Overall infection grade ≥3 rate 21-29% (N=146)	35
DELPHINUS	Dara plus VPLD	T-ALL (N=29): CR+CRi 83% after block 2, MRD-negative rate 41%, HSCT rate 72%, 2y EFS/OS 33%/39%. T-LBL (N=10): CR 40%, ORR (CR+PR) 50% after block 2, MRD-negative rate 50%, HSCT rate 30%, 2y EFS/OS 20%.	Grade ≥3 infection in 44% of pts with T-ALL/LBL. No TEAE related to dara. Dara IRR occurred in 72% of pts with T-ALL/LBL, primarily abdominal pain, vomiting, pyrexia, cough. 2 of 28 IRR were greater than grade 2. Dara exposure was associated with decreased CD38 expression on blasts.	36
NECTAR	Nelarabine, cyclophosphamide, etoposide	T-ALL (N=12): CR 25%, ORR 33%, HSCT rate 42%; T-LBL (N=9): CR 33%, ORR 44%, HSCT rate 56%	Grade ≥3 neurotoxicity in 13% (N=23)	38

Abbreviations: r/r T-ALL/LBL, relapsed/refractory T-cell acute lymphoblastic leukemia / lymphoblastic lymphoma; EFS, event free survival; OS, overall survival; VPLD, vincristine, prednisone, asparaginase, doxorubicin; MRD, minimal residual disease; MRD-neg, MRD-negative; MRD-pos, MRD-positive; PR, partial response; CRi, complete response with incomplete count recovery; Dara, daratumumab; YA, young adult; TEAE, treatment-ending adverse event; IRR, infusion-related reaction.

Table 3. Precision therapies for relapsed/refractory T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma (T-ALL/LBL)

Target	Therapeutic agent	Details on therapy or therapeutic agent	Clinical and preclinical outcomes	Ref
	Venetoclax + Navitoclax	Combination with VAD	Pediatric r/r ALL (~50% T-cell disease): CR 75%, ORR 83%, MRD-negative 50%, 12mo-OS 61% (N=12). T-ALL/LBL all ages: CR/ORR 57%, MRD-negative 33%, 12mo-OS 30%(ALL) 67%(LBL) (N=21). Common study drug-related TEAE were neutropenia and thrombocytopenia	43
	Venetoclax + Navitoclax	Combination with VAD at investigator's discretion	Pediatric r/r ALL/LBL (~50% T-cell disease): CR 71%, ORR 86% (N=7). T-ALL/LBL all ages: CR/ORR 44%, MRD-negative 31%, 22% went to HSCT. Common study drug-related TEAE were hematologic	84
	Venetoclax	Combination with multimodal cytotoxic chemo	Retrospective analysis of pediatric r/rT-ALL/LBL at single institution: CR 75%, median PFS 4mo, OS 7.7mo (N=8). Common study drug-related TEAE were neutropenia, thrombocytopenia, elevated bilirubin, and sepsis	85
	LP-118	Next-gen dual BCL-2/BCL-X_L inhibitor	Preclinical - effective against primary CLL cells resistant to venetoclax. Currently in phase 1 trial for hematologic malignancies (NCT04771572)	86
BCL-2 / BCL-X_L	Lisaftoclax (APG-2575)	Next-gen BCL-2 inhibitor	Preclinical - overcomes resistance to venetoclax. Recently studied in phase 1/2 trial for hematologic malignancies (not including T-ALL) (NCT04494503)	See 87
	Palcitoclax (APG-1252)	Next-gen dual BCL-2/BCL-X_L inhibitor	Preclinical - avoids hematologic toxicity (thrombocytopenia) seen with navitoclax, effective in model of peripheral T-cell lymphoma	See 87
	Sonrotoclax (BGB-11417)	Next-gen BCL-2 inhibitor	Preclinical - increased potency overcomes resistance to venetoclax, effective in models of venetoclax-resistant hematologic malignancies	88
	DT2216	BCL-X_L PROTAC	Preclinical - increased potency in cancer cells plus less-toxic to platelets, effective in xenograft models. Recently studied in phase 1 trial for r/r malignancies (NCT04886622)	89
	S65487	Next-gen BCL-2 inhibitor	Preclinical - effective in models of venetoclax-resistant hematologic malignancy. Currently under investigation for hematologic malignancies (NCT03755154, NCT04742101)	90
	AZD0466	Dual BCL-2/BCL-X_L inhibitor with improved biodistribution	Preclinical - activity against venetoclax-resistant disease. Recently in phase 1/2 trial for hematologic malignancies (NCT04865419)	See 87
CDK4/6	Palbociclib	+ 4-drug reinduction	Pediatric T-ALL/LBL: CR 50% (N=4)	49

	Ribociclib	+ everolimus / dexamethasone	Preclinical - T-ALL cell lines and orthotopic mouse models are sensitive to ribociclib, and there is synergy in combination with corticosteroids and/or mTOR inhibition. In phase I trial for r/r ALL (NCT03740334)	[48]
Jak/Stat	Ruxolitinib		Effective in PDX models of ETP-ALL. Induced CR in a patient with extramedullary relapse of T-ALL (*JAK3*-mutated) for 5 months. Effective for glucocorticoid-resistant T-ALL in preclinical models.	[51, 53]
	Tofacitinib + Ruxolitinib	Broader Jak inhibition	Induced remission for 10 mo in a patient with relapsed T-PLL (*JAK3*-mutated)	[91]
LCK	Dasatinib		Extensive preclinical data demonstrating efficacy for T-ALL. Single case reports of time-limited efficacy as monotherapy on the basis of ex vivo drug profiling, on basis of *ABL1* fusion, or *ABL1* amplification, or *PDGRFβ* fusion coupled with ex vivo drug profiling	[56, 58, 59, 92]
	PROTAC-mediated LCK inhibition		Preclinical - improved efficacy in mouse models	[60]
Gamma secretase	CD-103 (limantrafin)	pan-transcriptional Notch inhibitor	Outcomes for T-cell disease not reported	See [61]
	LY3039478 (crenigacestat)	pan-transcriptional Notch inhibitor	Limited efficacy for adults with r/r T-ALL/LBL	
	MK-0752	pan-transcriptional Notch inhibitor	Limited efficacy for adults/peds with r/r T-ALL	
	PF-03084014 (Nirogacestat)	reversible Notch inhibitor	8 adults with T-ALL treated, 1 had CR that lasted for 3 months	
	MRK-560	PSEN-1 selective	Preclinical - effective and lower toxicity in preclinical models, exhibits synergy with dexamethasone and XPO1 inhibitor	
SERCA	Thapsigarin	Prevents trafficking of gamma secretase to cell surface	Expected to have significant cardiotoxicity, but can be conjugated to folate for T-ALL targeting	See [62]
	CAD204520	Preferentially targets mutated Notch1 protein	Less cardiotoxicity in murine model	
PDGFR	Avapritinib	monotherapy	Given to a child with *PDGFRA* fusion and separate activating mutation p.D842N, causing > 50% reduction in marrow burden and tumor lysis syndrome, although CR was not reached.	[63]

	CP-673451		Preclinical - effective in PDX of T-LBL harboring *MYH9::PDGFRB* fusion	[93]
Extropin 1	KPT-330 (selinexor)	first-generation XPO1 inhibitor	Efficacy in preclinical leukemia models	See [64]
	KPT-8602 (eltanexor)	second-generation XPO1 inhibitor	Efficacy in preclinical T-ALL model	

r/r T-ALL/LBL, relapsed/refractory T-cell acute lymphoblastic leukemia / lymphoblastic lymphoma; VAD vincristine, asparaginase, dexamethasone; CR, complete response; ORR, overall response rate; MRD, minimal residual disease; HSCT, hematopoietic stem cell transplant; mo, month; TEAE, treatment-ending adverse event; PFS progression free survival; CLL, chronic lymphoblastic leukemia; PROTAC, proteolysis-targeting chimera; PDX, patient-derived xenograft; ETP, early T-cell progenitor; T-PLL, T-cell prolymphocytic leukemia

Table 4. Antibody-based therapies

Target	Agent	Therapy details	Clinical outcomes	Ref
CD38	Daratumumab	DELPHINUS (Dara + VPLD)	r/r pediatric T-ALL (N=29): CR+CRi 83% after block 2, MRD-negative rate 41%, HSCT rate 72%, 2y EFS/OS 33%/39%. r/r pediatric T-LBL (N=10): CR 40%, ORR (CR+PR) 50% after block 2, MRD-negative rate 50%, HSCT rate 30%, 2y EFS/OS 20%.	36
		Dara + ven + CAGE	r/r adult T-ALL/LBL: CR 48%, ORR 57%, 95% went to HSCT, median PFS and OS not reached (median f/u 7.4 mo) (N=21). ETP-ALL: CR 71% (N=7)	94
		Dara + ven + bortezomib	r/r T-ALL (age 20-30): 100% CR (N=3)	See 68
	XmAb18968	Bispecific T-cell engaging antibody	r/r adult ETP-ALL: 67% CR after 1 cycle (N=3)	See 67
	ISB 1342	Bispecific T-cell engaging antibody	Preclinical - superior efficacy against multiple myeloma cells compared to dara, preclinical data in T-ALL needed	71
	CD38-077	Antibody-drug conjugate	Preclinical - effective in models of Burkitt lymphoma and multiple myeloma, preclinical data in T-ALL needed	See 68
CD52	Alemtuzumab	single agent	1/13 pts with r/r ALL had CR, trial closed to toxicity	See 66
		Alemtuzumab added to post-remission therapy	r/r adult T-ALL (N=5), overall effect was to reduce MRD but was associated with viral infections.	
CD25	Basiliximab	monotherapy	Induced remission in a child with T-ALL and severe dermatologic paraneoplastic syndrome	See 66
IL-17RA	Lusvertikimab (OSE-127)	combination with chemo	Preclinical - MRD negativity in >50% of mouse PDX models	See 68
	A7R-ADC-SN-38	Antibody-drug conjugate	Preclinical - effective against IL7RA-expressing B-ALL cell line in mouse model	See 22
CD3	Teplizumab		Preclinical - effective in preclinical model when paired with etanercept or birinapant	95
CD47	Hu5F9-IgG2o	Combination with dara	Preclinical - improved survival in mouse PDX models with modest or no response to dara alone	See 67

Notch1	MAb604.107	Higher affinity for T-ALL-related Notch1 mutation than wildtype	Preclinical - reduced T-ALL cell proliferation and CD34/CD44-high cells specifically	[96]
CD99	10A1 HCscFv	Quadrivalent antibody	Preclinical - induced apoptosis of T-ALL blasts but not healthy PBMC in vitro	[97]

Dara, daratumumab; VPLD, vincristine, prednisone, asparaginase, doxorubicin; ven, venetoclax; r/r T-ALL/LBL, relapsed/refractory T-cell acute lymphoblastic leukemia / lymphoblastic lymphoma; CR, complete response; CRi, complete response with incomplete count recovery; MRD, minimal residual disease;, EFS, event free survival; OS, overall survival; ORR, overall response rate; PR, partial response; HSCT, hematopoietic stem cell transplant; ven, venetoclax; CAGE, cytarabine, alcarubicin, granulocyte colony-stimulating factor, etoposide; PFS, progression free survival; ETP, early T-cell progenitor; PDX, patient-derived xenograft; B-ALL, B-cell ALL; PBMC, peripheral blood mononuclear cells.

Table 5. CAR-T products

Target	Cell source	Pre-infusion manupulation / fratricide avoidance	Outcomes	Toxicities	Ref
CD7	Auto	In vitro "natural selection" via fratricide	60 pts (19 <=14 years): 94.4% CR at one month, 2y OS 63.5%, 2y PFS 53.7%. Separately study: 10 pts <=14 years with r/r T-ALL/LBL treated with CAR-T followed by HSCT had LFS and OS 87.5% at 2y	CRS in 91.7% of pts (11.7% grade ≥3), ICANS in 5% (1.7% grade ≥3), infection in 37% (17% grade ≥3) including 8% viral reactivation.	77, 78
	Auto	CD7 kd with PEBL	17 pts (13 pediatric): 94% MRD-negative CR at one month, 2y OS 55%, 53% went to HSCT and all in remission at time of report	CRS in 76% of pts (0% grade ≥3), ICANS in 12% (all grade 1), Infection in 47% (29% grade ≥3). No persistent T-cell aplasia (CD7-negative T-cell expansion)	79
	Auto	CD7 nanobody expression	8 adults: 88% CR (7/8), 100% of pts with BM involvement (6/6) had MRD-negative CR, 4/7 relapsed within 12mo	88% (7/8) with any CRS, 1/7 with CRS grade ≥3, no ICANS. No persistent T-cell aplasia (CD7-negative T-cell expansion)	See 76
	Auto	CD7 kd with IntraBlock PEBL	5 adults: 2/5 had CR and 1/5 had PR. 3/3 pts with objective response relapsed with CD7+ T-ALL	CRS in 60% (grade ≥3 in 20%), none had ICANS. 2 of 5 developed IEC-HS. 2/5 had viral reactivation	See 77
	Allo	CD7 kd with IntraBlock PEBL, derived from matched donors	20 adults: 85% CR, 95% ORR by 1 mo, 35% went to HSCT, 37% PFS and 42% OS at 2y.	100% of pts had CRS, 2/20 grade ≥3, 3/20 had ICANS (all grade 1). 30% had infection (25% grade ≥3), 80% had GVHD (5% grade ≥3). 100% had cytopenia, ALC returned to normal in ~50% with CD7- T-cells	80
	Allo	CD7 kd with IntraBlock PEBL, derived from matched donors	2 pediatric pts, CAR-T followed by haplo-HSCT (same donor): 100% CR and no relapse at time of report (>2y)	CRS grade 2 in 100%, one had GVHD skin and liver. One pt had HHV6 reactivation prior to HSCT, then EBV and CMV reactivations after HSCT with PTLD and CMV retinitis	98

Allo	CRISPR ko of CD7 and TRAC (GVHD prevention)	12 adults: 58% CR, 50% MRD-negative CR, 17% to HSCT	CRS in 78% (5% grade ≥3), ICANS in 5% (grade 1), no GVHD prolonged T-cell aplasia	[82]
Allo	CRISPR ko of CD7 for fratricide, TRAC, IL7Ra induction (survival)	12 adults: 92% CR at 1 month, one went to HSCT, 4 pts had relapse within 5-10mo. Two pts were still in CR at time of report, one at almost 1y the other at 3y	83% with any CRS, 67% with CRS grade 3. No ICANS. Endogenous T-cells recovered over time but remained low at 6mo	[99]
Allo	CRISPR ko of CD7, TCR, HLA-II (GVHD prevention); E-cadherin induction to prevent NK-cell attack	11 adults with T-ALL/LBL and 1 with AML (CD7+): 64% CR,82% ORR, 27% went to HSCT. 57% (4/7) pts with initial CR remained in CR at median f/u of 10.5mo	CMV and/or EBV reactivation seen in several pts, including one pt developed EBV-associated DLBCL after CR and died	See [76]
Allo	Base-editing to inactivate CD7, TRBC (GVHD prevention), CD52 (avoid serotherapy)	Three children with relapsed T-ALL: 67% CR followed by HSCT and continued in remission at time of report	3/3 any CRS, 1/3 with grade ≥3 CRS; 1/3 with ICANS (grade 1), 2/3 had CMV reactivations and one died of fungal infection.	See [76]
Auto	Ex vivo selection of naturally-occurring CD7-neg T cells using magnetic beads	Preclinical - CD7-neg CAR-T retained potent antitumor activity and outperform CAR-T made from bulk (predominantly CD7+) T-cells		See [76]
Auto	CD7 CAR construct insertion at CD7 locus, disrupting CD7 expression	Preclinical - effective in mouse xenograft model of T-ALL		See [76]

	Allo	Cytosine base editing to ko expression of CD7, CD52, TRAC, PD1 (avoid CAR-T silencing)	Preclinical - effective in mouse PDX models of T-ALL		[81]
	Allo	CRISPR ko of CD7 and TRAC	Preclnical - effective in mouse xenograft models of T-ALL		See [76]
CD5	Auto	None	9 pts (8 adults, 1 YA) with r/r T-ALL/NHL: 33% CR, 44% ORR	33% (3/9) any CRS, none grade >=3. 11% (1/9) with ICANS (grade 2). 22% (2/9) with cytopenias > 35d. 1 pt had CMV and BK virus reactivation.	See [66]
	Allo	CRISPR ko of CD5, tEGFR "off" switch	3 adults with r/r T-NHL. 33% CR, 100% ORR	100% CRS (grade 1) followed by severe rash refractory to CS necessitating centuximab for "off" switch	[83]
CD3		TALEN-mediated disruption of TCR	Preclinical - effective in mouse PDX model of T-ALL		See [100]
CD4		none	Preclinical - effective against mouse xenograft model of T-NHL		See [66]
CD38		none	Preclinical - effective against mouse PDX models of T-ALL, AML, MM		[101]
CD1a		none	Preclinical - effective against mouse PDX model of cortical T-ALL		See [66]
CD2	Allo	CRISPR ko of CD2 and TRAC	Preclinical - effective against mouse PDX models of T-ALL and CTCL		[102]
CCR9		none	Preclinical - effective against mouse PDX models of T-ALL		See [67]
TRBC 1/2		none	Preclinical - effective against mouse xenograft model of T-ALL		See [66]

CAR-T, chimeric antigen receptor T-cell; Auto, autologous; allo, allogeneic; Pt, patient; CR, complete response; y, year; mo, month; OS, overall survival; PFS, progression free survival; r/r T-ALL/LBL, relapsed/refractory T-cell acute lymphoblastic leukemia / lymphoblastic lymphoma; HSCT, hematopoietic stem cell transplant; LFS, leukemia free survival; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; IEC-HS, immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome; PEBL, protein expression blocker; kd, knockdown; ko, knockout; GVHD, graft versus host disease; ALC, absolute lymphocyte count; HHV6, human herpesvirus 6; EBV,

Epstein-Barr virus; CMV, cytomegalovirus; PTLD, post-transplant lymphoproliferative disorder; CRISPR, clustered regularly interspersed short palindromic repeats; YA, young adult; AML, acute myeloid leukemia; DLBCL, diffuse large B-cell lymphoma; PDX, patient-derived xenograft, TALEN, TAL effector nuclease; T-NHL, T-cell non-Hodgkin's lymphoma; MM, multiple myeloma, CTCL, cutaneous T-cell lymphoma;

Table 6. CAR-NK products

Target	Preclinical efficacy	Ref
CD7	Effective against mouse PDX models of T-ALL	See [66]
CD4	Effective against mouse xenograft model of T-NHL	See [67]
CD5	Effective against mouse xenograft models of T-ALL	
CD3	Effective against mouse xenograft model of T-ALL	

PDX, patient-derived xenograft; T-ALL, T-cell acute lymphoblastic leukemia; T-NHL, T-cell non-Hodgkin's lymphoma

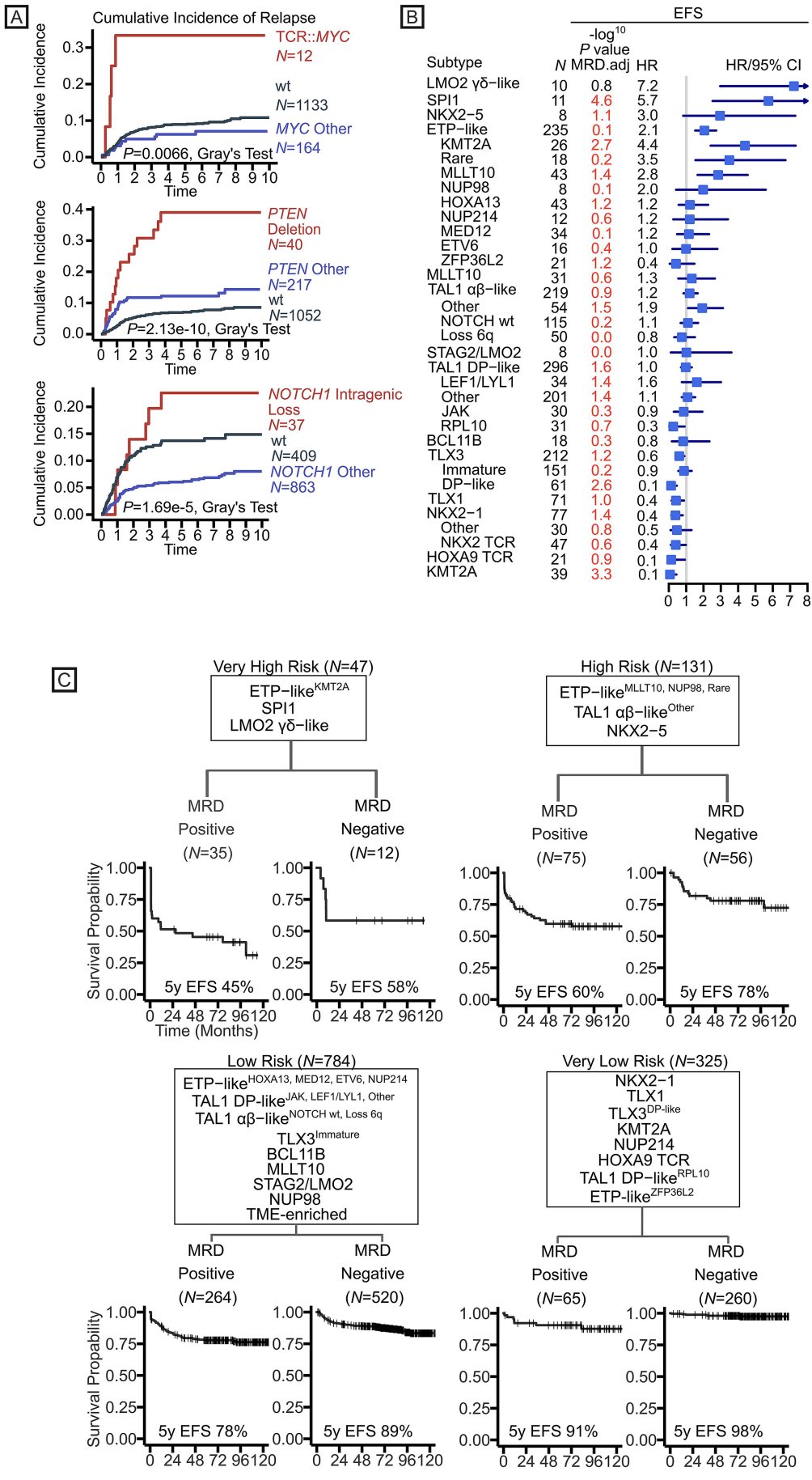
Table 7. Open clinical trials for pediatric T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma (T-ALL/LBL)

Trial number	Therapy details
NCT03081910	Phase 1. Autologous CD5-targeted CAR-T for T-cell malignancies including r/r T-ALL/LBL (USA)
NCT03117751	Phase 2/3. Precision medicine strategies (TKI, Jak inhibitors, bortezomib, nelarabine) for T-ALL/LBL (USA)
NCT03132454	Phase 1. Palbociclib with sorafenib, decitabine, or dexamethasone for r/r leukemia (USA)
NCT03236857	Phase 1. Venetoclax monotherapy for r/r malignancies, completed (USA)
NCT03384654	Phase 2. Dara + standard chemo for pediatric and YA with r/r B- or T-ALL/LBL (USA & Europe)
NCT03690011	Phase 1. Autologous CD7-targeted CAR-T (non-edited) for T-cell malignancies including r/r T-ALL/LBL (USA)
NCT03740334	Phase 1. Ribociclib with everolimus and dexamethasone for r/r ALL (USA)
NCT03829540	Phase 1. Autologous CD4-targeted CAR-T for r/r T-ALL/LBL (USA)
NCT04029688	Phase 1/2. Idasanutlin as single agent or with chemotherapy or venetoclax for pediatric and YA with acute leukemias or solid tumors (International)
NCT04599556	Phase 1. Autologous CD7-targeted CAR-T for CD7+ hematological malignancies including r/r T-ALL/LBL (China)
NCT04840875	Phase 1. Autologous CD7-targeted CAR-T for VHR T-ALL/LBL (China)
NCT04860817	Phase 1. Autologous CD7-targeted CAR-T, CD7 blockade with ER anchor for r/r T-ALL/LBL(China)
NCT04972942	Phase 1. Dara after TBI-conditioned allogeneic HSCT for VHR T-ALL/LBL (USA)
NCT04996160	Phase 1. Palbociclib with dexamethasone, bortezomib, doxorubicin for r/r ALL (USA)
NCT05043571	Phase 1. Autologous CD7-targeted CAR-T, CD7 blockade with PEBL for r/r T-ALL (Singapore)
NCT05291055	Phase 1. Autologous CD7-targeted CAR-T for CD7+ hematological malignancies including r/r T-ALL/LBL (China)
NCT05397184	Phase 1. Allogeneic base-edited CD7-targeted CAR-T for r/r T-ALL (UK)
NCT05032599 NCT05487495	Phase 1. HSCT donor-derived CD5-targeted CAR-T for r/r T-ALL/LBL (China)
NCT05658640	Phase 1/2. Trametinib with dexamethasone cyclophosphamide, and cytarabine for r/r ALL/LBL with RAS/RAF/MAPK alterations. This sub-protocol is part of a larger master protocol HEM-iSMART using detailed molecular characterization to allocate patients to different targeted therapies. (Netherlands)
NCT05885464	Phase 1/2. Allogeneic quadruple base-edited CD7-targeted CAR-T (BEAM-201) for r/r T-ALL/LBL(USA)
NCT06064903	Phase 1/2. Autologous CD7-targeted CAR-T, CD7 expression blockade with PEBL for r/r T-ALL/LBL(Italy)
NCT06326463	Phase 1. Autologous CD70-targeted CAR-T for hematologic malignancies including CD70+ r/r T-ALL/LBL (USA)

CAR-T, chimeric antigen receptor T-cell; r/r, relapsed/refractory; T-ALL/LBL, T-cell acute lymphoblastic leukemia / lymphoblastic lymphoma; USA, United Sates of America; TKI, tyrosine kinase inhibitor; Dara, daratumumab; YA, young adult; VHR, very high risk; ER, endoplasmic reticulum; TBI, total body irradiation; HSCT, hematopoietic stem cell transplantation; PEBL, protein expression blocker

Figures

Figure 1. Whole genome, exome, and transcriptome sequencing of more than 1300 cases of T-ALL has redefined the genetic landscape of T-ALL and created a new understanding of risk for relapse. (A) Cumulative incidence of relapse varies significantly depending on specific type of genomic alteration in the same gene (time in months). (B) Forest plot of T-cell acute lymphoblastic leukemia (T-ALL) comparing clinical outcome by event-free survival (EFS) for each of 15 subtypes compared to the whole, with significant associations (P<0.1) in red. (C) 15 T-ALL subtypes categorized into four risk groups, and subdivided by end of induction measurable residual disease (EOI MRD) (negative = MRD<0.1%) to stratify patients into outcome groups ranging from 5-year EFS 45 to 98%. HR, hazard ratio; OR, odds ratio. Adapted from Pölönen et al., Nature 2024.[6]


A
Cumulative Incidence of Relapse
Cumulative Incidence
TCR::MYC N=12
wt N=1133
MYC Other N=164
P=0.0066, Gray's Test
Time
PTEN Deletion N=40
PTEN Other N=217
wt N=1052
P=2.13e-10, Gray's Test
NOTCH1 Intragenic Loss N=37
wt N=409
NOTCH1 Other N=863
P=1.69e-5, Gray's Test
B
EFS
-log10 P value
Subtype N MRD.adj HR HR/95% CI
LMO2 γδ−like 10 0.8 7.2
SPI1 11 4.6 5.7
NKX2−5 8 1.1 3.0
ETP−like 235 0.1 2.1
KMT2A 26 2.7 4.4
Rare 18 0.2 3.5
MLLT10 43 1.4 2.8
NUP98 8 0.1 2.0
HOXA13 43 1.2 1.2
NUP214 12 0.6 1.2
MED12 34 0.1 1.2
ETV6 16 0.4 1.0
ZFP36L2 21 1.2 0.4
MLLT10 31 0.6 1.3
TAL1 αβ−like 219 0.9 1.2
Other 54 1.5 1.9
NOTCH wt 115 0.2 1.1
Loss 6q 50 0.0 0.8
STAG2/LMO2 8 0.0 1.0
TAL1 DP−like 296 1.6 1.0
LEF1/LYL1 34 1.4 1.6
Other 201 1.4 1.1
JAK 30 0.3 0.9
RPL10 31 0.7 0.3
BCL11B 18 0.3 0.8
TLX3 212 1.2 0.6
Immature 151 0.2 0.9
DP−like 61 2.6 0.1
TLX1 71 1.0 0.4
NKX2−1 77 1.4 0.4
Other 30 0.8 0.5
NKX2 TCR 47 0.6 0.4
HOXA9 TCR 21 0.9 0.1
KMT2A 39 3.3 0.1
C
Very High Risk (N=47)
ETP−like KMT2A
SPI1
LMO2 γδ−like
MRD Positive (N=35)
MRD Negative (N=12)
Survival Propability
5y EFS 45%
5y EFS 58%
Time (Months)
High Risk (N=131)
ETP−like MLLT10, NUP98, Rare
TAL1 αβ−like Other
NKX2−5
MRD Positive (N=75)
MRD Negative (N=56)
5y EFS 60%
5y EFS 78%
Low Risk (N=784)
ETP−like HOXA13, MED12, ETV6, NUP214
TAL1 DP−like JAK, LEF1/LYL1, Other
TAL1 αβ−like NOTCH wt, Loss 6q
TLX3 Immature
BCL11B
MLLT10
STAG2/LMO2
NUP98
TME-enriched
MRD Positive (N=264)
MRD Negative (N=520)
5y EFS 78%
5y EFS 89%
Very Low Risk (N=325)
NKX2−1
TLX1
TLX3 DP-like
KMT2A
NUP214
HOXA9 TCR
TAL1 DP−like RPL10
ETP-like ZFP36L2
MRD Positive (N=65)
MRD Negative (N=260)
5y EFS 91%
5y EFS 98%