Current and emerging therapeutic approaches for T-cell acute lymphoblastic leukaemia

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Summary

T-cell ALL (T-ALL) is an aggressive malignancy of T-cell progenitors. Although survival outcomes in T-ALL have greatly improved over the past 50 years, relapsed and refractory cases remain extremely challenging to treat and those who cannot tolerate intensive treatment continue to have poor outcomes. Furthermore, T-ALL has proven a more challenging immunotherapeutic target than B-ALL. In this review we explore our expanding knowledge of the basic biology of T-ALL and how this is paving the way for repurposing established treatments and the development of novel therapeutic approaches.

Keywords: leukaemia, novel treatments.

The need for new therapies in T-ALL was once driven by the inferior survival outcomes seen in T-ALL compared to B-ALL. Improvements in chemotherapy usage with treatment intensification and minimal residual disease (MRD) monitoring have made a major impact on this disparity.1 The introduction of treatment escalation based on MRD has meant that despite a three-fold higher rate of positive MRD at end of induction (EOI) in T-ALL versus B-ALL,2 both subtypes now have equivalent survival outcomes in children.3 This is particularly relevant to early T-cell precursor T-ALL (ETP-ALL), historically associated with a very high-risk of treatment failure,4 but now with excellent outcomes on MRD risk-directed protocols.5-7 In adults, survival in T-ALL now surpasses B-ALL on some protocols.8 Focus has also been directed to appropriate de-escalation of treatment in those with low risk MRD.9

Long term survival outcomes approach 50% in adults able to tolerate intensive treatment and exceed 90% in childhood ALL,9-12 a remarkable prognosis that may reflect the superior tolerance of children to chemotherapy and a difference in the genetics of childhood leukaemia.13,14 The vast majority of children that remain in remission 2 years from diagnosis will be cured, with rare cases of late relapse (>5 years) likely representing a clonally unrelated secondary T-ALL.15 However relapsed T-ALL is highly aggressive and often resistant to glucocorticoids and chemotherapy, with survival of around 50% in children and less in adults, with the worst outcome in those with the shortest duration of remission.16-19 For those adults who relapse, allogeneic transplant offers the best chance of cure, with recently reported survival outcomes of 40%.19 Despite modern treatment protocols, durable responses for adults unable to proceed to transplant are unlikely, with a median survival of only 8 months.19 In children, allogeneic bone marrow transplantation is generally reserved for those with high-risk relapsed disease and remains one of the few curative options for these patients.20

Genetic markers can identify good prognostic subgroups with potential for treatment de-escalation. Patients with both NOTCH1 and FBXW7 mutations, or two NOTCH1 mutations, have been shown to have an excellent outcome, with 100% 5 years survival in this patient subgroup treated on the paediatric UKALL2003 trial,21 and improved survival seen in adults.22,23 However not all findings are as clear cut, for example PTEN aberrations have added additional prognostic value on some trials,24-26 but not others.27 Given the rarity of the disease, most studies are underpowered to detect small but meaningful differences in outcome among genetic subgroups. As an example, outcome analyses based solely on NOTCH pathway mutations alone are confounded by the high frequency of these mutations in TLX+ cases (approx. 90%) and relative rarity in ETP-ALL (approx. 20%).28

The poor outcomes seen in relapsed/refractory (R/R) T-ALL highlight a pressing need for novel treatments. Improved understanding of the genetics of both normal and aberrant T-cell differentiation is offering new therapeutic avenues. T-ALL is a genetically heterogeneous disease, which can be sub-classified based on first-hit class-defining lesions that commonly affect master regulatory transcription factors

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(e.g. TAL1, LMO1/2, TLX1/3, HOXA). Transcription factors have proven extremely difficult to target pharmacologically, thus the focus of drug development has been on second hit mutations in key signalling pathways. Herein we review some current areas of active translational research in this field.

Nelarabine

Nelarabine is the soluble prodrug of ara-G, which is selectively cytotoxic to T leukaemic cells, likely due to their low endogenous SAMHD1 levels.29 Initial early phase trials of nelarabine showed its efficacy as a single agent in paediatric relapsed/refractory T-ALL, with neurotoxicity the most common dose-limiting toxicity.30,31 In adults, an alternate day dosing schedule limited neurotoxicity whilst maintaining an over all response rate (ORR) of 31%.32 Subsequent data showed the efficacy of Nelarabine in combination with chemotherapy, with NEC (Nelarabine, Etoposide and Cyclophosphamide), with an impressive complete remission (CR) of 71% in relapsed patients.33

More recent data supporting the upfront use of Nelarabine in children and young adults has led some to consider it as standard of care in paediatric T-ALL.34,35 Data for its upfront use in adults is awaited from the UKALL14 trial.

NOTCH inhibitors

NOTCH receptors are part of a conserved protein family that can act both as oncogenes or tumour suppressors, depending on the cellular context.36 NOTCH1 is important for thymocyte development, committing common lymphoid progenitors to a T-cell fate. Activating mutations of NOTCH1 have been found in almost two-thirds of paediatric and adult T-ALL cases.37,38

As one of the most frequently mutated genes in T-ALL, NOTCH1 has generated considerable interest as a therapeutic target. Gamma secretase inhibitors (GSI), originally developed for Alzheimer’s disease, act by preventing the cleavage and activation of the intracellular NOTCH1 fragment (Fig 1A). Their early promise has since been hampered by marked gastrointestinal (GI) toxicity.39 NOTCH1 is an important regulator of intestinal goblet cells and NOTCH1 inhibition by GSIs causes goblet cell accumulation via upregulation of the transcription factor KLF4, resulting in significant diarrhoea (Fig 1B).40 However, the use of a pulsed treatment schedule41 and the addition of glucocorticoids have reduced this toxicity,42 with one reported case of a CR in a patient treated with a GSI and dexamethasone.43 Not only do glucocorticoids improve the side effect profile of GSIs, but also the two treatments work synergistically to induce apoptosis of T-ALL cells, possibly due to increased expression of the glucocorticoid receptor NR3C1 in the presence of the combination.42 However, a recently reported phase 1 trial using a novel inhibitor of the NOTCH ICD in combination with dexamethasone continued to show dose limiting GI toxicity and limited clinical efficacy.44 A more recent approach targeting PSEN1 aims to reduce the systemic toxicity associated with GSIs.45 This sub-unit of the gamma-secretase complex is more highly expressed in leukaemic cells than normal developing T cells and its inhibition has been well tolerated in animal studies.

The NOTCH transcriptional co-activator MAML1 is also a potential target. Stapled z-helical peptides derived from MAML1 (SAHM) compete with MAML1 and inhibit NOTCH1-driven transcription. Mice treated with SAHM show a decrease in leukaemic cell burden and corresponding reduction in NOTCH1 target gene expression, including MYC.46 Additionally their side effect profile seems tolerable in animal models, without the GI toxicity associated with GSIs.

Another method of modulating NOTCH involves the use of the proteasome inhibitor bortezomib, a standard of care treatment in myeloma. Bortezomib has activity in relapsed/refractory T-ALL, potentially by inhibiting transcriptional expression of NOTCH1.47 In a small cohort of children with relapsed/refractory ALL, bortezomib appeared to have particular efficacy in T-ALL with complete response rates of over 70% when used in conjunction with chemotherapy.48

There are other considerations that have clinical relevance for NOTCH inhibition. Firstly, NOTCH1 mutations are often late secondary subclonal events,14,49 meaning NOTCH inhibitors are highly likely to select for NOTCH1 wild-type cells. Secondly, resistance mechanisms can emerge whereby cells are able to maintain MYC levels in the absence of NOTCH signalling, for example through loss of FBXW7 or use of an alternative MYC enhancer.50,51 In the former study, enhancer usage switches from the NOTCH-MYC enhancer to a BRD4 regulated MYC enhancer, providing a rationale for combining NOTCH and BRD4 inhibitors.50

PI3K inhibitors

The PI3K-mTOR pathway plays a key role in both normal T cell and malignant cell development. Phosphoinositide 3 kinases (PI3K) are a family of lipid kinases that act as second messengers and are broadly divided into three classes, which share a common core PI3K motif (Fig 2). Almost half of T-ALL cases have aberrant PI3K activation occurring through deletion or mutation or PTEN, activating mutations of PIK3R1 (typically N564D), PIK3CD (typically E1021K), or loss of function mutations of USP7.28 These mutations are particularly enriched in the TAL1 subgroup,28 with these two oncogenic pathways shown to synergise in mouse models, possibly related to the ability of AKT to phosphorylate and modulate TAL1 activity.52 This raises the possibility that the TAL1 subgroup could be particularly susceptible to PI3K pathway modulation, although this awaits further study.

Mutations in the PI3K pathway also correlate with response to chemotherapy; homozygous deletions of PTEN appear to confer a higher risk of early treatment failure53 and mutations in PTEN are associated with primary glucocorticoid
This is likely driven by AKT1-mediated phosphorylation of the glucocorticoid receptor, leading to impaired nuclear localisation; T-ALL mouse models treated with glucocorticoids and an AKT inhibitor, showed an augmented anti-leukaemic response, compared to mice treated with either agent alone. Another synergistic approach looked to target both PI3K and NOTCH when it was found that T-ALL cells evade cell death when treated with PI3K/mTOR inhibition by upregulating NOTCH target genes such as MYC.

The mTOR inhibitor everolimus acts downstream of AKT and in addition to chemotherapy, gave a 50% response rate in a small cohort of heavily pre-treated R/R T-ALL patients; it is currently being evaluated in a phase I trial with NEC (NCT03228104).
During normal lymphopoiesis, lymphocytes with hyperactive responses undergo negative selection through over-activation of PI3K signalling.58,59 Thus, one concept gaining traction is that TCR stimulation, such as through an activating CD3-targeting monoclonal antibody, rather than inhibition of PI3K signalling may be an exploitable route towards initiating apoptosis in ALL cells.60 A potential concern of this approach would be cytokine release syndrome, as occurred previously in solid organ transplant studies using OKT3.61

**IL7R-JAK-STAT inhibition**

The IL7R-JAK-STAT pathway is responsible for transducing cytokine signalling in the thymus and is required for normal T-cell development. This pathway is frequently aberrantly activated in the TLX1/3+ T-ALL subgroup, but very rarely in TAL1+ T-ALL. Activating mutations occur at multiple levels in the pathway, with recurrent mutations described in STAT5B, IL7R, JAK1 and JAK3.62-66 Interestingly, JAK1 mutations appear to be more common in adult than paediatric T-ALL, and have been associated with reduced overall survival and a high relapse rate.67 Mice transplanted with progenitor cells harbouring the most commonly identified JAK3 mutation (M511I) develop an immature T-ALL through activating JAK1.68

The majority of activating IL7R mutations described thus far in T-ALL involve the insertion of a cysteine in the transmembrane domain leading to disulphide bonding and ligand-independent IL7R homodimerization and JAK1 phosphorylation (Fig 3).62,64,67 Inhibition of the IL7R-JAK-STAT pathway has shown efficacy using clinically available JAK inhibitors in preclinical models69,70 and phase I/II clinical trials of Ruxolitinib are planned. The disulphide bond and homodimer can also be disrupted by the reducing agent N-acetylcysteine (NAC) at doses readily achievable in patients.71 Given its affordability and widespread use in treating patients with paracetamol overdose, such an approach would be particularly attractive in healthcare systems where targeted agents are considered prohibitively expensive.

An additional aspect of JAK inhibition is the possibility of restoring glucocorticoid sensitivity.72,73 This approach is particularly intriguing in ETP-ALL, since ETP-ALL tends to be less sensitive to steroids than other T-ALL subtypes and STAT5 is commonly activated even in the absence of clearly identified upstream mutations.69,74

The long-term efficacy of JAK inhibition in T-ALL is unclear. Treatment does not eradicate leukaemic cells in vitro, leading to rapid relapse upon drug withdrawal, and combination therapy is likely to be required.72 Moreover, due to the specific site of JAK inhibitor binding, it is possible that T-ALL cells will acquire resistance mutations, for example in the ATP-binding pocket of the kinase domain, or with mutations activating other JAK family members.75 Most clinical JAK inhibitors act to competitively inhibit the ATP-binding pocket of the active JAK2 (Type 1), however there is growing interest in Type 2 JAK inhibitors which bind the ATP pocket of the inactive JAK, as well as a less-conserved nearby allosteric pocket.76 This approach may provide a route to avoid resistance. Another strategy is to target further downstream of the IL7R-JAK pathway, such as the kinase PIM1. PIM1 appears to be upregulated in response to chemotherapy and steroids in a (CD127+77 subset of T-ALL.
cells and PLM1 inhibition may be an alternative or adjunct to JAK inhibition. Such inhibitors are already in clinical trials for myelofibrosis.

**Targeting anti-apoptotic machinery**

B Cell lymphoma 2 (BCL2) is an anti-apoptotic protein that was first discovered from cloning the t(14;18) translocation in a case of B cell lymphoma. BCL2 forms part of a protein family that share the BCL2 homology (BH) domain. BH3 proteins, such as BAX and BAK, are pro-apoptotic proteins that are sequestered by BCL2, BCL-XL and MCL1. More mature T-ALL cell lines have been shown to be dependent on BCL-XL, whereas ETP-ALL cells show a greater dependency on BCL2 (Fig 4). Accordingly, ETP-ALL models have shown particular sensitivity to Venetoclax over Navitoclax, with reversal of this pattern in more mature T-ALL cells. There is considerable clinical experience with Venetoclax in the treatment of CLL and more recently in AML, where the drug is generally well-tolerated. There is accumulating evidence for its use in T-ALL; a recent retrospective report of the use of Venetoclax with chemotherapy in R/R T-ALL showed that 6/13 patients achieved a morphological remission. We suggest that Venetoclax should be a priority for incorporation in upcoming clinical trials, for instance in patients with high risk MRD at end of induction, or those with relapsed/refractory disease.

Navitoclax binds preferentially to BCL-XL, with less potent activity against BCL-2. Its initial promise in CLL was limited by thrombocytopenia, as platelet survival requires normal function of BCL-XL. Despite this, it warrants testing in relapsed/refractory cortical and post-cortical T-ALL, where intrinsic mitochondrial chemoresistance is often the major barrier to achieving remission, although thrombocytopenia will need to be cautiously managed. Recent early phase trials of combination Venetoclax and Navitoclax are promising: In a small, heavily pre-treated patient cohort 50% of the T-ALL patients achieved CR/CRi. Direct MCL1 inhibitors such as S63845 have shown pre-clinical efficacy in T-ALL and are in early phase trials. Alternative strategies that downregulate MCL1 expression, such as the CDK9 inhibitor AZD4573, also offer exciting new therapeutic opportunities across diverse haematological cancers.

Several mechanisms of acquired resistance to Venetoclax have been described, including mutations of the drug binding site, deletions/mutations of BIM/BAX, and upregulation of BCL-XL and MCL1. There is thus considerable interest in using Venetoclax in combination with Navitoclax, or MCL1 inhibitors, though how such combinations will be tolerated in terms of toxicity will need to be carefully assessed.

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Fig 3. Schematic of anti-apoptotic dependencies in T-ALL according to level of differentiation arrest. ETP-ALL arrested early in T-cell development are highly dependent on BCL2, with increasing dependency on BCL-XL in T-ALLs that arrest. Adapted from Chonghaile et al. (reference 79) in the cortical/post-cortical stages. This corresponds to vulnerability to Venetoclax and Navitoclax respectively. ETP – early T-cell progenitor; ISP immature single progenitor; EDP early double positive; DP double positive; SP single positive T-cell.
Tyrosine kinase inhibitors

Aberrant tyrosine kinase activation in T-ALL occurs when chromosomal translocations involving the ABL1 oncogene result in ligand independent activation of the ABL1 kinase. However, unlike chronic myeloid leukaemia (CML), the Philadelphia chromosome has only occasionally been reported in T-ALL. Instead the ABL1 oncogene is fused with other partners, typically NUP214 leading to constitutively active kinases. These fusion products are still amenable to inhibition with tyrosine kinase inhibitors (TKIs), a class of drug that has transformed the outcomes of CML and Ph+ALL. NUP214-ABL1 T-ALL cells, found in the TLX1/3 subgroups, respond to treatment with Imatinib, Nilotinib and Dasatinib. These results have been recapitulated in vivo in a NUP214-ABL1 T-ALL xenograft when imatinib treatment resulted in a reduction of leukaemic cell burden, where the addition of Venetoclax further improved the response.

Whilst ABL translocations only account for approximately 5% of T-ALL cases, functional drug testing has unexpectedly revealed that up to 30% of T-ALL cases are susceptible to the ABL/SRC family kinase inhibitor Dasatinib. No correlation was noted between responsiveness and established genetic lesions, including ABL translocations, leading the authors to propose that Dasatinib was targeting SRC, rather than ABL. This hypothesis was consistent with the lack of activity shown by other canonical ABL family kinase inhibitors, such as Imatinib, that do not affect SRC signalling. The in vitro activity of Dasatinib was confirmed by in vivo testing and supported by case reports of T-ALL responders to Dasatinib. These findings are strengthened by those of another group who used in silico drug screening to identify up-regulation of the SRC family kinase LCK as a possible therapeutic target in T-ALL and demonstrated preclinical efficacy of Dasatinib. Recently published data suggests synergy between Dasatinib and dexamethasone mediated via LCK. Overall, it is likely that TKIs will have significant clinical activity in T-ALL, but their clinical use will remain limited until the identification of validated biomarkers.

Cyclin dependent kinase inhibitors

Cyclin dependent kinases (CDKs) are a large family of kinases with diverse roles, including acting as transcriptional co-factors and controlling cell cycle progression. Clinically meaningful inhibition of CDKs has proved technically challenging due to their integral role in normal cell survival and difficulties in targeting specific kinase isoforms. However, a
series of more specific small molecule inhibitors have recently emerged.

D cyclins are key cell cycle regulators that bind and activate CDK4 and 6, leading to activation of the E2F transcription factors that facilitate cell-cycle progression. Cyclin D3 is dysregulated in T-ALL and has been shown to be integral to NOTCH driven leukaemogenesis; Moreover, in a mouse model of T-ALL driven by activating mutations of NOTCH1, conditional ablation of cyclin D3 resulted in marked disease regression, findings that were phenocopied by exposure to the Cyclin D-CDK4/6 kinase inhibitor. Efficacy of CDK4/6 inhibition has also been demonstrated in NOTCH1 wildtype T-ALL cell lines where an in vivo model showed synergism with steroids and mTOR inhibitors.

Several CDK4/6 inhibitors are now in clinical trials and the challenge remains to identify targetable interdependencies between specific CDK isoforms and different mutational drivers in order to synergise their anti-leukaemic properties. In this regard, it will be important to assess whether the recently discovered recurrent mutations of CCND3 are a biomarker for sensitivity to CDK4/6 inhibition.

CDK7 is a key constituent of the cyclin-activating kinase (CAK) complex, which acts to modulate the cell cycle by interacting with the general transcription factor, TFIIH. The CAK complex promotes transcription by activating RNA polymerase II (RNAPII) via CDK7-dependent phosphorylation. The novel agent THZ1 can specifically and irreversibly inhibit CDK7 by covalently binding to an amino acid located outside its kinase domain, resulting in cell death in T-ALL cell lines (Fig 5). Interestingly, the activity of this agent may be associated with its preferential inhibition of super-enhancer driven oncogenes, such as the non-coding mutations that drive TAL1, providing a potential in vivo therapeutic window.

CDK9 regulates transcriptional elongation and is of interest as an anti-cancer target in a range of malignancies, particularly those dependent on MCL1 (Fig 5). Targeted inhibition had initially been challenging due to homology of its ATP-binding site with other CDKs, until the development of the specific inhibitor AZD4573. An alternative PROteolysis Targeting Chimeras (PROTAC) approach was recently developed where THAL-SNS-032 targeted CDK9 protein for proteasomal degradation, inducing apoptosis in T-ALL cell lines.

Drugs in development

In this section we briefly explore further potential therapies that may emerge from encouraging preclinical data.

The Hedgehog pathway is an evolutionarily conserved signalling cascade, however its aberrant activation also drives tumour growth and chemoresistance. This pathway is activated in up to a fifth of T-ALL and is associated with induction of chemotherapy resistance. Inhibition of GLI1, a Hedgehog pathway transcription factor, resulted in improved survival in T-ALL PDX models and could offer a novel target for high-risk T-ALL.

Fig 5. CDK7 and CDK9 regulate oncogenes and are therapeutic targets in T-ALL. Green box: super-enhancers driving oncogenes can be destabilised by inhibition of chromatin interacting proteins such as BRD4. Yellow box: The initiation of RNA polymerase II (RNAPII) mediated oncogene transcription is facilitated by phosphorylation of the C terminal tail on serine 5 and 7 by CDK7 as part of the TFIIH complex, which occurs both at enhancers and gene start sites. CDK7 can be inhibited by small molecules such as THZ1. Red box: Transcriptional elongation by RNAPII is facilitated by phosphorylation of RNAPII on serine 2 by CDK9 as part of the PTEFb complex. CDK9 can be targeted for degradation by the novel agent THAL-SNS-032 or inhibited by CDK9 inhibitors such as AZD4573. CDK9 inhibition has been associated with marked downregulation of MCL1 expression.
Histone deacetylases (HDAC) play a role in the regulation of chromatin structure and the HDAC inhibitors (HDACi) Panobinostat, Vorinostat and Romidepsin are already in clinical use in myeloma and lymphoma. The broad-acting HDACi panobinostat shows particular efficacy in vitro T-ALL models with improved survival seen in combination with chemotherapy.108 Interestingly, there is a suggestion that the efficacy of panobinostat over other HDACi relates to its epigenetic inhibition of the oncogene MYC.105

Exportin 1 (XPO1) (also known as CRM1) is a nuclear-cytoplasmic exporter protein involved in the transport of several proteins involved in cell cycle regulation. It is the only transporter of key tumour suppressors including TP53 and is upregulated in several malignancies, including T-ALL. Selinexor, a selective inhibitor of nuclear export (SINE) compound, is a small molecule XPO1 antagonist and is FDA-approved for myeloma. It has shown preclinical efficacy in T-ALL, although toxicity reported from clinical trials may prove problematic.106

Heat shock proteins act as molecular chaperones for a variety of proteins with key roles in oncogenesis, including the JAK pathway and another, closely related kinase pathway, TYK2. TYK2 acts to upregulate BCL2 via STAT1107 and its inhibition by the drug Luminespib/AUY922 triggers apoptosis in T-ALL in vitro. It appears that this effect is mediated via a reduction in TYK2 and subsequent downregulation of BCL2.108 Early clinical trials of Luminespib in a range of malignancies have been undertaken and this could yet be an additional therapy in T-ALL.

Approximately 75% of proteins currently have no targetable domain. PROteolysis Targeting Chimeras (PROTACs) circumvents this problem by degrading proteins instead of inhibiting them, thus broadening the number of potential targets. This approach may be particularly relevant to diseases such as T-ALL where the majority of driver translocations involve oncogenic transcription factors.

Another technique looks to change the method of drug delivery, using nanoparticles, enabling optimized drug dosing and potentially combining chemotherapy with a targeted ligand. This remains a broadly experimental area of leukaemic treatment, however the success of CPX-351 (a liposomal formulation of cytarabine and daunorubicin) in AML demonstrates the potential of this expanding therapeutic area.

**Immunotherapy**

Immunotherapies are a group of therapeutics that harness the immune system to specifically attack malignant cells. Broadly they can be divided into treatments that: (i) amplify a natural anti-tumour immune response (e.g. immune checkpoint blockade); or (ii) synthetic immunotherapies (e.g. monoclonal antibodies or chimeric antigen receptor T or NK cells). There have been limited studies of immune checkpoint blockade in ALL and the relatively low mutational burden seen, particularly in paediatric ALL may limit the expression of tumour specific neo-antigens, on which this strategy is thought to rely. By contrast, synthetic immunotherapies are having a major impact in the treatment of B-ALL with recent clinical advances including the use of Rituximab, Inotuzumab, Blinatumomab and durable responses to anti-CD19 chimeric antigen receptor (CAR)-T cell therapy.109

All of these successful synthetic immunotherapies rely on the presence of an antigen that is strongly expressed on all leukaemic blasts, but with limited expression on normal tissues. In the case of B cells, strong expression of specific B cell markers such as CD19, CD20 and CD22 have provided good immune targets. Furthermore, whilst normal B cells are also attacked, B cell aplasia is clinically manageable and therefore precision targeting of leukaemic B cells is not an absolute requirement. By contrast, immunotargeting T-ALL has a number of technical hurdles Fig 6. Firstly, antigen expression is variable at different stages of T cell differentiation, and thus there is not a single antigenic target that is likely to be applicable to all T cell malignancies. Secondly, immune destruction of normal T cells would result in a life-threatening immune deficiency disorder. Thirdly, harvesting of autologous T cells for CAR T cell generation risks contamination by T-ALL blasts. Lastly, CAR T cells expressing the same antigen they are targeting would lead to fratricide and T-cell exhaustion. There is therefore limited clinical experience of synthetic immunotherapies in T-ALL, but a number of promising approaches are emerging.

**Monoclonal antibodies**

One of the most promising candidates in the short term is the anti-CD30 drug immunoconjugate Brentuximab Vedotin, which is licensed for use in classical Hodgkin lymphoma and anaplastic large cell lymphoma. Preclinical studies have shown that CD30 is expressed in 13/34 of T-ALL cases tested by flow cytometry, but pre-clinical evidence for functional efficacy is lacking.120 As some T-ALL cases, typically of the ETP subgroup, express CD33, there is also the potential to use Gemtuzumab Ozogamicin in such cases, but clinical data for this approach in T-ALL has not been reported. Another potential surface target in T-ALL is the activation marker CD38, which is highly expressed on T-ALL blasts and targetable by the monoclonal antibody daratumumab. Pre-clinical efficacy of Daratumumab in T-ALL has translated into clinical responses in four post-allogeneic relapsed/refractory cases who achieved MRD negativity, an encouraging set of results for such aggressive disease111-113 and the Delphinus phase II clinical trial is currently recruiting in the UK (NCT03384654).

The early lymphoid cytokine receptor and T-ALL oncogene IL7R is a potentially attractive immunotherapy target. Recent work has preclinically explored a novel human monoclonal antibody targeting IL7R, showing evidence that binding inhibits signalling, functionally sensitises cells to glucocorticoids and elicits natural killer (NK) cell mediated cellular cytotoxicity in vitro, and potentially in vivo.
Furthermore, the antibody is internalised on binding, giving a potential utility in chemo-immunotherapy.\textsuperscript{114} Whilst providing a platform for a number of promising approaches, the potential for on- and off-target toxicities remain to be seen.

**Chimeric antigen receptor cells**

A number of CAR T or NK cell approaches have shown benefit in pre-clinical studies, but there is no single antigenic target that is expressed in all T-ALL cases. An anti-CD4 CAR T cell has shown activity against a CD4\textsuperscript{+} T-ALL cell line in a xenograft model and this approach circumvents CAR T fratricide by sparing cytotoxic CD8\textsuperscript{+} effector cells.\textsuperscript{115} However, only a minority of T-ALL cases express CD4 and prolonged CD4 aplasia is likely to lead to life-threatening infection.

An anti-CD3 CAR-NK cell line has shown activity against a T-ALL cell line in a xenograft model.\textsuperscript{116} The use of a CD3
| Trial number   | Countries recruiting                  | Disease status         | Phase | Treatment                                                                 | Age            | Current status          | Est. closing date |
|---------------|--------------------------------------|------------------------|-------|---------------------------------------------------------------------------|----------------|-------------------------|------------------|
| NCT03328104   | USA                                  | Relapsed/refractory    | I     | Everolimus + nelarabine (+etoposide and cyclophosphamide)                 | 2–29 years     | Recruiting              | Oct 2020         |
| NCT00501826   | USA                                  | Relapsed/refractory    | II    | Nelarabine + Hyper CVAD + Pegaspargase + venetoclax                       | Child/Adult     | Recruiting              | Oct 2020         |
| NCT02763384   | USA                                  | Relapsed/refractory    | II    | Nelarabine + BL8040 (CXC4 antagonist)                                     | >18 years       | Recruiting              | May 2021         |
| NCT02619630   | France                               | New diagnosis          | II    | Nelarabine (+cyclophosphamide + etoposide)                                | 18–59yrs        | Recruiting              | Dec 2020         |
| NCT02881086   | Germany                              | New diagnosis          | III   | Nelarabine (+PEG asparaginase/dex/cyclophosphamide/methotrexate/ cytarabine/vindesine/adriamycin/prednisolone) | 18–55           | Recruiting              | July 2021        |
| NCT01620216   | USA                                  | Relapsed/refractory inc AML | II    | (Dasatinib v ponatinib v sorafenib v nilotinib v sunitinib)               | >18 years       | Unknown                 | March 2019       |
| NCT03613428   | China                                | Relapsed/refractory ETP-ALL | I/II  | Ruxolitinib (+vincristine, prednisolone, L-asparaginase)                  | 13–75 years     | Not yet recruiting      | Dec 2020         |
| NCT03515200   | USA                                  | Relapsed/refractory    | I     | Palbociclib (CDK4/6 inhibitor)+ dexamethasone, bortezomib, dasatinib, doxorubicin | <22 years       | Terminated              | July 2020        |
| NCT03263637   | Germany/Netherlands/UK               | Relapsed/refractory    | I     | AZD4573 (CDK9 inhibitor)                                                  | 18–130 years    | Active, not recruiting  | Dec 2021         |
| NCT03181126   | USA/Australia                        | Relapsed/refractory    | I     | Navotoclax + venetoclax + chemo                                          | 4–16 years      | Active, not recruiting  | Nov 2020         |
| NCT03236857   | USA/Australia/Canada/France/Germany/Netherlands/Switzerland/UK | Relapsed/refractory    | I     | Venetoclax (+/−chemo)                                                     | <25 years       | Recruiting              | Apr 2022         |
| NCT03808610   | USA                                  | Relapsed/refractory    | II    | Venetoclax + low dose chemotherapy                                        | >18 years       | Recruiting              | Dec 2023         |
| NCT04128501   | USA                                  | Morphological remission post allo | II    | Ven/Aza maintenance                                                        | 18–75 years     | Recruiting              | Oct 2022         |
| NCT03504644   | USA                                  | Relapsed/refractory    | 1B/II | Venetoclax + liposomal vincristine                                        | >18 years       | Recruiting              | Apr 2021         |
| NCT03573525   | USA                                  | Relapsed/refractory    | II    | Liposomal daunorubicin & cytarabine (CPX 351)                            | >18 years       | Recruiting              | Oct 2021         |
| NCT03081910   | USA                                  | Relapsed/refractory    | I     | CD5CAR/28zeta CAR T cells                                                 | <75 years       | Recruiting              | July 2021        |
| NCT02742727   | China                                | CD7<sup>+</sup> Relapsed/refractory | I/II  | anti CD7 CAR pNK cells                                                    | >18 years       | Unknown                 | March 2017       |
| Trial number | Countries recruiting | Disease status | Phase | Treatment | Age | Current status | Est. closing date |
|--------------|----------------------|----------------|-------|-----------|-----|----------------|------------------|
| NCT04033302  | China                | Unknown        | I     | Anti CD7 CAR T cells | 6 months-75 years | Recruiting       | July 2021        |
| NCT03690011  | USA                  | Relapsed/refractory | I     | CD7CAR/28eta CAR T cells | <75 years | Not yet recruiting | May 2023        |
| NCT04004637  | China                | Relapsed/refractory | I     | CD7 CAR T cells       | 7–70 years | Recruiting       | June 2021        |
| NCT03384654  | USA/Germany/Belgium/ France/Israel/Italy/Netherlands/ Spain/Sweden/UK | Relapsed/refractory | II    | Daratumumab + vinc/dox/pred/v daratumumab PEG asparaginase/ cyclophosphamide/ cytarabine/6MP/mtx | 1–30 years | Recruiting       | Dec 2021        |
| NCT02553460  | USA/Canada           | New diagnosis   | I/II  | Bortezomib & vorinostat + standard care | <1 year | Recruiting       | July 2021        |
| NCT02112916  | USA/Australia/Canada/ New Zealand | New diagnosis | III   | Bortezomib + cyclophosphamide/ cytarabine/daunorubicin/ dexamethasone/doxorubicin/ etoposide/ifosfamide/leucovorin/ 6MP/PEGasparaginase/ methotrexate/thioguanine/vincristine | 2–30 years | Active, not recruiting | March 2020 |
| NCT02795520  | USA                  | Relapsed/refractory ALL/ AML/CMU/Advanced MDS | I/II  | OTS167IV (MEK inhibitor) | >18 years | Recruiting       | Dec 2021        |
| NCT02484430  | USA                  | Relapsed/refractory | II    | Sapanisertib (mTOR inhibitor) | >18 years | Active, not recruiting | May 2021        |
| NCT02293109  | USA                  | New diagnosis   | I     | Carfilzomib + hyper CVAD | 18–64 years | Active, not recruiting | Nov 2020        |
| NCT03110354  | USA                  | Relapsed/refractory | I     | DS-3201b (EZH1/2 inhibitor) | >18 years | Recruiting       | May 2021        |
| NCT02890758  | USA                  | Relapsed/refractory | I     | NK cells + ALT803 (IL15) | >18 years | Recruiting       | Feb 2021        |
| NCT02392572  | USA                  | Relapsed/refractory | I/II  | ONC201 (Apoptosis initiator) | >18 years | Active, not recruiting | Nov 2022        |
| NCT02663518  | USA/Canada           | Relapsed/refractory | I     | TTI-621 (SIRPaFc blocker) | >18 years | Recruiting       | June 2021        |
| NCT04446130  | China                | Newly diagnosed ETP-ALL | III   | Decitabine (+HAAG chemotherapy) | 15–60 years | Recruiting       | Jan 2023        |
| NCT03555238  | China                | Newly diagnosed ETP-ALL | I/II  | Chidamide (HDACi) + chemotherapy | 14–55 years | Recruiting       | May 2020        |
| NCT03860844  | Denmark/Finland/Norway/ Netherlands/Sweden | Relapsed/refractory inc AML | II    | Isatuximab + standard chemotherapy | 28 days–18 years | Recruiting       | Aug 2021        |
negative NK effector cell avoids any problem with CAR fratricide. However, surface CD3 is a relatively mature T cell marker that would not be expressed in all T-ALL cases and this approach would again result in fatal T cell aplasia, albeit depending on the persistence of NK activity. Similarly an anti-CD5 CAR NK cell has been reported to show activity in a xenograft model.\textsuperscript{117}

Intriguing results have been presented from an anti-CD5 CAR T cell.\textsuperscript{118} CD5 is expressed on most normal T cells and IgM secreting innate B1 B cells. In response to the anti-CD5 CAR T cell both normal and malignant T cells down-regulate CD5, but normal T cells additionally up-regulate anti-apoptotic proteins including BCL2 and PI-9 protecting themselves from cell death. The result is ongoing anti-tumour activity, with minimal CAR T fratricide. Importantly, these results show that CAR T efficacy is not just limited to choice of antigenic target, but also the susceptibility of the target cells to the effector mechanisms of the CAR T cells. A potential problem with this approach, however, is that chronic exposure to the CD5 antigen may result in CAR T exhaustion.

Recent positive pre-clinical results have been achieved targeting the CD1a antigen expressed in cortical T-ALL.\textsuperscript{119} However, CD1a\textsuperscript{+} T-ALL has a good prognosis, meaning relapsed/refractory CD1a\textsuperscript{+} T-ALL is rare.\textsuperscript{120}

One approach to avoid CAR T cell fratricide is to delete the targeted antigen in the CAR T cells during CAR T cell production. This has been successfully achieved in an anti-CD7 CAR T cell that has its own CD7 loci disrupted by CRISPR/Cas9 genome editing.\textsuperscript{121} Importantly, these CAR T cells demonstrated both effective anti-tumour responses, but also the ability to respond to viral peptides indicating that some broader cellular immunity may be re-established from the CAR T population itself. Another recently reported strategy targeting CD7 involves a CD7 expression blocker which results in the intracellular retention of CD7. Eight patients treated with this approach had limited cytokine release syndrome (CRS) (including no reported neurotoxicity) and 50% remain cytokine release syndrome (CRS) MRD negative (Zhang M et al, ASH 05/12/20).

An elegant approach has sought to exploit the mutual exclusive expression of TRBC1 and TRBC2 at the TCR\(\beta\) b-constant region, a process with similarities to B cell kappa/lambda restriction.\textsuperscript{122} TRBC1 and TRBC2 differ by just four amino acids. Normal T cells express one or other, but not both, while clonal disorders such as T-ALL will express only one of the antigens Fig 6. Generating a CAR T cell against TRBC1 circumvents both CAR T cell fratricide and T cell aplasia (killing only 50% of polyclonal T-cells), while showing activity against TRBC1+ T-ALL cell lines in a xenograft model. Early phase clinical trials of this CAR T cell in T cell lymphoma are currently recruiting. It should be noted that high-risk ETP-ALL cases that have arrested prior to TCR\(\beta\) rearrangement will not express either isoform, with only approximately 25% of T-ALL cases amenable to TRBC1 directed therapy.\textsuperscript{122}

### Considerations in multiply relapsed and refractory patients ineligible for clinical trials

If possible, all relapsed/refractory patients should be entered onto clinical trials (Table I). For refractory patients fit for intensive therapy, our standard practice has been to attempt re-induction with either Fludarabine-Cytarabine-Idarubicin (FLA-Ida) or a Nelarabine containing regimen. However, for patients ineligible for clinical trials who are fit for active treatment, we would recommend considering the following possibilities, although this assumes the ability to access the various

| Drug             | Adjuvant treatment                                                                 | Appropriate patient cohort                                                                 |
|------------------|------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| **Nelarabine**   | Can be used as single agent                                                        | Caution in patients with neuropathy                                                        |
|                  | Combination with etoposide & cyclophosphamide (NEC protocol)                      | ETP-ALL                                                                                     |
| **Venetoclax**   | Vincristine/steroids/daunorubicin                                                   | Non-ETP-ALL                                                                                 |
|                  | azacytidine/decitabine                                                            | Monitor for thrombocytopenia                                                                |
| **Navitoclax**   | Vincristine/steroids/daunorubicin                                                   | Caution in patients with neuropathy                                                        |
|                  | Azacytidine/decitabine in combination with venetoclax                               | Patients fit for re-induction                                                              |
| **Bortezomib**   | Vincristine/steroids/daunorubicin/asparginase                                       | FLT3 mutated (approx. 5% T-ALL)                                                            |
| **FLT3 inhibitor** | Can be used as single agent                                                       | Associated with ETP-ALL and CD117\textsuperscript{a}                                       |
| **Ruxolitinib (or other JAKI)** | Dexamethasone (pre-clinical data suggesting synergy)                  | IL7R or JAK activating mutations                                                            |
| **Dasatinib**    | Either as single agent or with chemotherapy combination                            | No current validated biomarkers for patient selection. LCK phosphorylation or in vitro drug |
|                  |                                                                                    | sensitivity testing may be of value                                                        |
| **Daratumumab**  | Vincristine/steroids/daunorubicin/asparginase                                       | Appropriate antigen expression. Clinical use currently unproven for brentuximab and       |

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\(\textsuperscript{a}\) In the context of FLT3 inhibitor use, sensitivity testing may be of value
agents on compassionate access schemes or through local/personal funding streams, accepting use of many of these agents is outside licence, and usage should be assessed by a specialist team on a case-by-case basis (Table II). Genetic and drug profiling is likely to assist the best treatment approach.

Conclusion

Instigating novel therapies for T-ALL is challenging due to the rarity of the disease, genetic heterogeneity and the toxicity associated with ablating the T cell repertoire. Immunotherapy has an increasing role in haematological cancers, with CAR T cell trials beginning in T-lineage malignancies. However, CAR T therapy requires expensive infrastructural support, both in terms of production and delivery, and is unlikely to be available outside of select institutes in high-income countries.

Advances in our understanding of the genetics and epigenetics of this disease have contributed to the novel uses of previously well-described therapies, as well as the advent of new drugs. Targeting synthetic lethal pathways, such as recently described for CHK1 inhibition in EZH2 deficient T-ALL, offers the opportunity to spare normal tissues and reduce toxicity.123 Personalised medicine has already been demonstrated to identify new therapeutic targets in cancer, including T-ALL, and we strongly believe that this approach will transform the outlook for this disease.124 We propose a combination of genomics and drug profiling may enable the most appropriate treatment to be selected. However, such an approach is not without its difficulties, given the cost and requirement for rapid results, especially in a disease as aggressive as T-ALL. The paucity of validated biomarkers means there is no quick answer for the best treatment for an individual and for the moment such approaches are only feasibly delivered by large research centres. Lastly, given the rarity of the disease, multinational collaborative trials will be required to make meaningful progress in the relapsed/refractory setting.

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Author Contributions

RP, SER, NF and MM wrote the manuscript and generated the figures. RP and MM had final responsibility to submit for publication.

Conflict of interest

The authors declare no competing interests.

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