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Adoptive Cell Therapy for Acute Myeloid Leukemia and T-cell Acute Lymphoblastic Leukemia

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Abstract

Refractory and relapsed acute myeloid leukemia (AML) and T-lineage leukemia (T-ALL) have poor prognosis and limited therapeutic options. Adoptive cellular immunotherapies are emerging as an effective treatment for patients with chemotherapy refractory hematological malignancies. Indeed the use of unselected donor lymphocyte infusions (DLIs) have demonstrated successes in treating patients with AML and T-ALL post-allogeneic. The development of *ex vivo* manipulation techniques such as genetic modification or selection and expansion of individual cellular components has permitted the clinical translation of a wide range of promising cellular therapies for AML and T-ALL. Here, we will review clinical studies to date using adoptive cell therapy approaches and outline the major challenges limiting the development of safe and effective cell therapies for both types of acute leukemia.

Keywords

Acute myeloid leukemia; T- Acute lymphoblastic leukemia; Adoptive cell therapy

A. Introduction:

Cell therapy for Myeloid and T cell leukemia is more challenging than equivalent therapy for B cell malignancies. Targeting lineage-restricted antigens such as CD19 on B cells will ablate both malignant B cells and their normal equivalents, but the consequent impairment of humoral immunity may be compensated for by infusion of intravenous immunoglobulin. By contrast, targeting lineage restricted antigens on normal and malignant myeloid and T cells will cause more profound failure of innate and adaptive immunity. For that reason, until very recently the focus of adoptive cell therapy to treat myeloid and T-lymphoid malignancy has been on adoptive therapy in the context of allogeneic hematopoietic stem cell transplantation (HSCT). Investigators either exploited the alloreactivity of donor

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lymphocytes to eradicate residual (normal and malignant) host myeloid and lymphoid cells or used donor lymphocytes and HSCs to rescue the patients from the consequences of lineage ablation by the initial anti-leukemic therapy.

The cells used for adoptive transfer initially consisted of bulk donor lymphocyte infusions (DLIs), containing a panoply of immune effectors including; conventional α/β -T cell receptor (TCR) T cells; unconventional T cells, expressing γ/δ TCRs or invariant chain-TCRs (natural killer T cells – NKT); and NK cells. More recently, investigators have focused on more defined subsets, targeting myeloid and T cell malignancies through endogenous or transgenic T cell receptors, through chimeric antigen receptors or through endogenous or transgenic NK cell receptors.

Adoptive cellular therapy may use cells from three sources; the patient (autologous); the transplant donor (following HSCT); or banked (off the shelf) cells from a third party. Each source has advantages and disadvantages (Table 1) but to date most clinical experience has been gained from the adoptive transfer of autologous or HSC donor-derived cells.

In this article, we review progress to date and describe some of the modifications that we hope will make adoptive cell therapy of myeloid- and T-cell leukemia safer and more effective.

B. Myeloid Leukemia

B1. Donor Lymphocyte Infusions (DLIs):

DLIs were the first adoptive cellular therapy to establish the presence of a potent graft-versus-leukemia effect (GVL) in myeloid leukemia. Indeed, by the late 1990's, DLI infusions alone were producing durable response rates of 60 – 80% in patients with chronic myeloid leukemia (CML) who had relapsed following HSCT¹. These benefits led to the use of DLI to prevent or treat relapse in patients with a broad spectrum of hematological malignancies post-HSCT. Acute myeloid leukemia (AML) is currently the commonest indication for an HSCT and for patients who relapse after the procedure, the combination of DLI with chemotherapy is consistently superior to chemotherapy alone (complete remission, CR: 37% to 41% and 2-year overall survival-OS: 20 to 30% versus CR: 10% to 16%, 2-year OS: 9 to 20%)². Thus, most centers now use DLIs as standard of care for the treatment of relapse post-HSCT.

Unfortunately, the anti-leukemic effects of unmodified DLI are unpredictable, and due to their broad alloreactivity there is a major risk of inducing severe or even fatal graft versus host disease (GvHD) in the recipient. Initial studies focused on separating GVHD from the graft- versus-leukemia (GVL) effect, by manipulating DLI's to deplete alloreactive T cell subsets (eg. T_{naive} CD45RA+ or α/β -T cells), sometimes with the transfer of a genetic safety switch to control any GvHD from residual alloreactivity^{3–5}. These approaches have shown safety benefits in numerous early and late phase clinical studies, but so far are proving too complex for universal adoption. More recently, the focus has shifted to addressing the variable potency of DLIs, by manipulating individual effector cell populations to generate cellular products with enhanced and consistent anti-leukemic activity.

B2. T cells:

Conventional T cells (Tcon) target specific antigens through a polymorphic TCR that recognizes processed (peptide) antigens in physical association with major histocompatibility (MHC) molecules (Fig 1B). In the context of AML, Tcon play a major role in mediating both GVHD as well as GVL post-HSCT by responding to mismatched MHCs or aberrant expression cancer-testis antigens etc., eg: Wilm's tumor antigen-1 (WT1)^{6,7}. Clinical trials have adoptively transferred Tcon cells for AML by selectively expanding leukemia-associated antigen (LAA)- specific T cells or by transducing a polyclonal population of T cells with LAA-specific receptors. Investigators have expressed transgenic LAA specific native T cell receptors (TCRs), which are restricted to specific HLA-types, or chimeric antigen receptors (CARs), which are HLA unrestricted, since they recognize their targets independently of MHC molecules

B2.1. Primed unmodified TCR-T cells: In this approach, T cells that possess native TCR specificity for target LAAs are expanded *ex vivo* and then infused into patients.

B2.1.1. Donor-derived products: Kim et al, reported a single patient treated with donor-derived WT1-specific T cells to treat AML relapse post-HSCT⁸. Donor cells were enriched for WT1-reactivity in cultures with dendritic cells (DCs) transfected to express WT1. The patient entered a CR that persisted for over 3 years before relapsing. The group recently published a larger series of 8 patients with relapsed or refractory (r/r) AML who received HSCT followed by infusions of donor-derived WT1-specific T cells. While 3 patients relapsed within 1 year post-infusion, five remain in long-term CR (>8 years)⁹.

Using a different expansion strategy, Chapuis et al, isolated and expanded CD8+ T cell clones with native TCR-specificity for an HLA-A*0201 restricted WT-1 peptide and gave these WT1-reactive clones to 8 HLA-A*0201-positive patients with AML post-HSCT. The infusions were safe and all six patients in CR post-HSCT remained in CR for >2 years post-infusion. One patient with measurable residual disease (MRD) entered CR while another patient with frank relapse had a transient decline in blasts, but eventually progressed coincident with the loss of circulating WT1-reactive T cells¹⁰.

Our group is simultaneously targeting multiple LAAs: WT1, PRAME, Survivin and NYESO1 using a single T cell product, thereby minimizing the risk for antigen-loss immune escape. We have reported outcomes in 20 patients who received donor-derived “multiLAA” T-cell products following HSCT to treat AML or MDS. Thirteen patients were infused while in CR, while 7 had relapsed. Nine of 13 infused while in CR remain alive and in CR (8 to 30 months, post-infusion) and 2 out of 7 with active AML had an objective response: 1 CR and 1 PR¹¹.

B2.2. Transgenic TCR-T cells: Transducing T cells with LAA-specific α/β TCRs could overcome the variability in LAA-directed specificity of antigen-primed but otherwise non-modified donor-T cells. However, transgenic TCRs can pair with the native TCR chains within individual T cells generating novel TCR specificities and the potential for serious off-target effects. So, strategies to limit cross-linking with native TCRs by introducing TCR-

silencing RNA or by selectively transducing cells with defined native TCR specificity such as virus-specific T cells have both been tested in AML patients.

B2.2.1. Autologous products: Tawara et al. tested a transgenic TCR specific for an HLA-A*24:02 restricted epitope of WT1 that also contains a small interfering RNA for endogenous TCR to limit mispairing¹². Of 8 patients treated, 4 had no response while 4 had a transient decline in marrow blasts. There were no toxicities. Interestingly, 4 of the 5 patients who had long term persisting transgenic T cells (> 2 months) remained alive 1-year post-infusion while those with poor persistence died rapidly from relapse.

A transgenic TCR-T cell product specific for a second LAA (PRAME) has entered clinical testing (NCT02743611). The transgenic T cells also express an inducible caspase 9 (iC9) as a suicide switch that can be activated by administration of a small dimerizer molecule (rimiducid) in the event of untoward toxicities. Patients who are HLA-A*0201 positive and have PRAME-positive r/r AML are being accrued to this study.

B2.2.2. Donor-derived products: Investigators from Fred Hutchinson Cancer Center have treated AML patients with CMV and/or EBV-specific donor-T cells transduced with a TCR specific for an HLA-A*0201 restricted WT1 peptide. Results from this trial have so far been presented in abstract form, and report 22 patients with AML, 11 in frank relapse and 11 others in CR who have been treated post-HSCT¹³. All patients had cytokine release syndrome (CRS) - now a recognized complication of genetically-modified T cell products- but this was limited to grade II. None of the patients with frank relapse had a clinical response, but all 11 patients infused while in CR remain alive and in a long-term remission (>1 year) post-infusion.

In another study from the same center, donor T cells are transduced with a TCR specific for HA1 when it is expressed in the context of HLA-A*0201 (NCT03326921). HA1 is a minor histocompatibility antigen (mHA), and giving T cells expressing this receptor to patients after HSCT can exploit any difference in mHA expression between donor and recipient pairs. Targeting mHAs carries the risk of inducing non-hematopoietic toxicities such as the serious pulmonary toxicities seen in 3 subjects after infusions of mHA-primed, unmodified donor T cells in patients with B-ALL¹⁴. HA-1 expression, however, is restricted to the hematopoietic system, so HA1-directed donor T cells could safely and specifically eradicate recipients' hematopoietic cells and blasts¹⁵. One patient with active disease had a complete response to this therapy but other outcomes from this trial have yet to be reported.

B2.3. Chimeric antigen receptor (CAR)-T cells: T lymphocytes can also be redirected to (unprocessed) surface expressed LAAs by genetically introducing a chimeric antigen receptor (CAR). In this approach, transduced T cells possess a single chain variable fragment (sc Fv) for a surface LAAs such as CD33, which is coupled with the CD3 signaling domain of the T cell receptor complex (first generation CAR) along with one or more costimulatory domains such as CD28 (second generation CAR and beyond). CAR-T cells thus activate on engaging cognate LAAs, bypassing the requirement for MHC presentation (Figure 1A). Identifying suitable CAR-target antigens in AML has been more challenging than for B cell malignancies because the antigen expression pattern of AML

cells overlaps considerably with that of HSCs. Indeed myelosuppression is an expected “on-target, off-tumor” effect of available AML-directed CAR-T cells in the clinic. Nevertheless, several groups have attempted targeting a variety of AML/HSC antigens such as CD123 while also exploring innovative strategies to minimize the severity or duration of myelosuppression. More recent AML-CAR trials have incorporated pre-infusion cytoreductive chemotherapy because of the superior efficacy observed once CD19-CAR T cell therapy was given after such treatment. Table 3 provides a selected list of CAR T cell products currently in the clinic, together with their construct designs, vector choice, cytoreductive regimens and clinical outcomes.

B2.3.1. Autologous CAR-T cells: Targeting CD33 with the FDA approved monoclonal antibody gemtuzumab can effectively treat AML. Therefore, multiple centers have developed CD33 directed CAR-T cells for clinical testing. Nonetheless concerns remain about prolonged myelosuppression or even myeloablation, as CD33 is also expressed by normal HSCs. In preclinical work, investigators at University of Pennsylvania have devised a strategy to delete the expression of CD33 from patient-derived normal HSCs with the intent of transferring CD33 negative HSCs to rescue hematopoiesis in patients who have prolonged myelosuppression after CD33-CAR T cells¹⁶. In an alternative strategy, MD Anderson Cancer Center investigators have introduced an epidermal growth factor receptor tag (EGFRt) into their CD33-CAR that can be targeted by cetuximab to eliminate CAR-T cells in the event of severe or prolonged toxicities (NCT03126864).

So far clinical data from just one study of CD33-CAR T cell transfer has been published. In a report from China, a patient with relapsed AML and over 30% marrow blasts received an autologous, second generation CAR-T cell product¹⁷. The patient had a transient clinical response by week 3 (<5% marrow blasts), but relapsed with >90% blasts, dying just 6 weeks later, so that the effects of the CAR-T cells on long term myeloablation could not be ascertained. The relapsing blasts in the patient remained CD33 positive and could be killed in *in vitro* co-cultures with the patients CD33-CAR T cells indicating that mechanisms other than antigen loss explained this patients’ failure to respond.

CD123, like CD33, is a pan myeloid progenitor antigen that is highly expressed on AML cells and HSCs. To date, abstracts from two centers report clinical outcomes using CD123 CAR-T cells. At the City of Hope Medical Center, 11 patients with r/r AML were treated with second generation CD123 CAR-T cells equipped with an EGFRt “suicide system” in the event of prolonged HSC ablation¹⁸. As expected, all patients became pancytopenic post-infusion but recovered spontaneously 12 weeks later without cetuximab treatment. Additionally, 3 patients developed grade III cytokine release syndrome (CRS) without neurotoxicity. Two of 11 patients achieved a complete remission (CR) by week 4 and subsequently underwent an allogeneic HSCT. This study continues to accrue patients.

Instead of using integrating retroviral vectors to introduce CARs, a group from the University of Pennsylvania used a short-lived “bio-degradable” mRNA CAR to limit the duration of transgene expression and correspondingly shorted the duration of myelosuppression. In their clinical trial, all 5 treated patients experienced grade II CRS but none of the patients had detectable CAR+T cells within the first week. Unfortunately, none

of the patients had a clinical response leading to the termination of this study¹⁹. These investigators have now initiated a clinical trial with a lentivirus transduced CD123 CAR-T cell product in which patients are required to have an allogeneic HSC rescue option prior to treatment (NCT03766126).

AML cells like solid tumors can aberrantly glycosylate or express carbohydrate antigens like Lewis-Y (LeY) and MUC-1 on their surface which uncovers opportunities to selectively target malignant cells without potential on-target, off-tumor toxicities to normal myeloid cells. Indeed, both LeY and MUC-1 CAR-T cell products from multiple centers are in clinical testing. Thus far, one clinical trial has published results from a study in which 4 patients with LeY positive r/r AML were treated with LeY CAR-T cells after fludarabine cytoablation²⁰. None of the patients experienced grade III infusion-related toxicities but none had a durable response. Notably, the infused CAR-T cells were labeled with Indium and could be seen trafficking to the sites of disease by SPECT imaging. The leukemic cells at progression remained LeY+ despite persisting LeY-CAR T cells. So, the same group is now exploring the efficacy of “optimized” (optimization processes not released) LeY-CAR T cells for the treatment of patients with LeY+ solid tumors (NCT03851146).

Similar to aberrant carbohydrate antigens, ligands for the NK cell receptor NKG2D are selectively upregulated on cancer cells in response to DNA-damage, making them an attractive CAR-target. A group from Dana Farber Cancer Institute have now tested an NKG2D ligand (NKG2D-L)-specific CAR in 7 patients with NKG2D-L positive r/r AML among other cancer types. No serious infusion-related toxicities were noted but again none of the patients had a clinical response²¹. The authors reasoned that the low infused cell dose (max. 3×10^7 cells) without antecedent cytoreductive chemotherapy might have contributed to the limited efficacy. Thus, in the commercial development of this product, patients receive chemotherapy followed by high dose NKG2DL-CAR T cells (10^9 cells/dose). Encouragingly, the first patient to be treated with this combination in the phase II trial entered a CR, which allowed them to proceed to a second allogeneic HSCT²².

Beyond the approaches discussed above, CAR-T cell products specific for a range of other AML expressed targets such as CLL-1, FLT-3, Folate receptor β , CD7, CD38 etc. are in early phase clinical testing. Multi-antigen specific CAR-products are of particular interest to minimize the risk for antigen negative immune escape. An abstract from China reported outcomes in 2 patients with AML treated with a compound CAR product that was specific for CLL-1 as well as CD33 followed by a reduced intensity haploidentical donor- HSCT (Liu et al. unpublished, European Hematology Association 2018 abstract#149). Both patients had transient CRS while one developed neurotoxicity. Importantly both patients developed pancytopenia which was rescued by HSCT. Encouragingly, both patients entered a CR after the combination of dual-targeted CAR T cell and HSCT.

B2.3.2. Universal CAR-T cells: The high cost and lengthy and complex logistics required to manufacture gene modified autologous T cells is increasing interest in developing banked or ‘off-the-shelf’ T cells. For these to be successful, two problems must be overcome; GvHD from alloreactive T lymphocytes in the banked cells; and rejection of the banked cells (host versus graft disease) by recipient effector cells. Multiple approaches

have been used to prevent GvHD, including the deletion of TCRs from banked T cells by gene-editing using TALENs or CRIPR/Cas9. Prevention of rejection is a more complex problem and while several organizations are developing and testing universal CD123- and CD33-CAR-T cells for AML, results are not yet available.

B3. NK cells:

Unlike T cells, NK cells recognize their targets through a group of activating or inhibitory receptors (eg: NKG2D or killer immunoglobulin-like receptors- KIR respectively) (Figure 1C). Normal activation of NK cells depends on the balance between activating and inhibitory ligands on target cells. While many AML cells upregulate NK activating ligands such as MIC-A on AML cells, they can also remain resistant to autologous NK killing if they retain intact inhibitory ligands such as HLA-B and C (KIR-ligands). AML cells that both upregulate NKG2D-Ls and also have mismatches at specific HLA-B or C with NK cells would be susceptible to NK mediated lysis. This phenomenon is exploited after HSCT where patients with AML who receive donor grafts mismatched at KIR-ligands have superior relapse-free survival compared to those who received KIR-matched products²³. These observations have led to numerous clinical trials using adoptively transferred NK cells sourced from HSC donors and also from third-party or haploidentical donors as cellular therapy for patients with AML pre- or post-HSCT (Table 4).

B3.1. NK cells from the HSC-Donor: Passweg et al. transferred KIR-mismatched HSC-donor NK cells to five AML patients post-HSCT who had developed mixed chimerism (4) or graft failure (1)²⁴. None of the patients developed GVHD post-infusion, supporting the safety of transferred allogeneic NK cells, and 2 of 5 had improved donor chimerism (rising from 89% to 100% in one and 26% to 60% in another) which was sustained for over 6 months. The same group then administered purified donor-NK cells to 8 AML patients in CR post-HSCT to prevent relapse²⁵. They reported 2 cases of grade II GvHD including a death from GVHD. At 6 years follow-up, 4 of 8 (50%) patients remain free of disease.

To extend the benefits of NK cellular therapy to patients with refractory AML who would not otherwise qualify for an allogeneic HSCT, a few groups have combined infusions of donor-derived NK cells and an allogeneic HSCT. In one report, Twenty-nine patients with AML underwent a haplo-HSCT followed by multiple haplo-NK cell infusions²⁶. One year post-transplant, 11 of 29 patients (38%) relapsed compared to 75% in a historical cohort of patients from the same South Korean center. Notably, no between-group differences in rates of GVHD or treatment-related mortality was observed.

B3.2. NK Cells from a Haploidentical donor: Establishing the safety of allogeneic NK infusions has allowed investigators to test haploidentical NK cells, as a treatment for AML, regardless of whether the patient has undergone HSCT. In a study conducted by Lee et al, 15 patients with refractory AML were treated with haplo-NK cells 8 days prior to an allogeneic HSCT²⁷. This strategy resulted in 7 of 15 patients remaining disease-free one-year post-HSCT suggesting donor-NK infusions may have AML-directed activity both pre- and post-transplant. Further, two separate reports from Rubnitz et al. confirmed that it was safe to give haplo-NK cells to patients who had not undergone an HSCT or whose HSCT

came from an unrelated donor. In their first report, all 10 treated patients who were in CR at the time of infusion remained in CR 3 years later²⁸. They next reported a 50% CR rate in 12 patients with chemo- and HSCT-refractory AML after clofarabine cyto-reduction and NK cell infusions²⁹. They observed no GvHD in either study. Similarly, among older individuals with refractory AML unable to undergo an allogeneic HSCT, Curti et al. administered haplo-NK cells as the only consolidative treatment in 6 patients and as a treatment for ongoing r/r AML in 7 others. While 3 of 6 in the adjuvant cohort eventually relapsed, they observed 3 CRs among the seven patients with r/r AML post-NK infusion³⁰.

Until recently, NK cells for adoptive transfer were isolated by magnetic bead selection for CD3-CD56+ cells in donor blood. A group from Washington University devised an *ex vivo* cytokine stimulation culture to generate activated NK cells that could have superior killing properties. In their *in vitro* studies, they demonstrated that “pre-activated cytokine-induced memory like NK cells” (CIK) had greater potency against AML than bulk NK cells. In their proof-of-principle clinical trial, 4 of 9 AML patients with HSCT-refractory AML achieved a CR (45% CR rate) after Haplo-CIK infusions without inducing GVHD³¹.

B3.3. Universal NK cells: As NK infusions have proven safe to the degree of haploidentical matching, Boyiadzis et al. adoptively transferred an immortal, patient-derived NK cell lymphoma cell line- NK92 to 8 patients with AML³². The cells were irradiated prior to transfer to minimize the risk for NK92 lymphoma engraftment but retained transient killing activity. Though there were no treatment-related toxicities observed, the infused product had minimal *in vivo* expansion and no demonstrable clinical efficacy.

C. T-cell ALL

C1. DLIs, TCR- and NK-based therapies for T-ALL

Progress in developing adoptive cell therapies for T-cell acute lymphoblastic leukemia (T-ALL) has been modest at best. The relative rarity of T-ALL compared to B-ALL has led to few reports in post-HSCT DLI or NK trials for hematological malignancies. From this limited published literature, it appears that in contrast to AML, in which non-targeted adoptive cell therapies with DLI or NK cells can produce significant benefit, similar approaches in T-ALL had only modest effects. While in rare instances patients with refractory or relapsed T-ALL responded to DLI (alone or in combination with chemotherapy)^{33,34}, in most patients the efficacy of such non-specific cell therapy approaches was limited, highlighting the need for more effective targeted cell therapy options.

Finally, no clinical trials have reported outcomes using native or transgenic TCRs for patients with T-ALL. Thus we limit our discussion of targeted cellular therapies for T-ALL to the use of CAR-T cells.

C2. CAR-T cells for T-ALL

As for AML, extending the potency of CAR T cells to T-ALL is associated with unique challenges, some of which arise from the shared expression of most targetable antigens between normal and malignant T cells³⁵. First, expression of a CAR specific to a common T-

cell antigen can result in continuous CAR stimulation and fratricide, potentially limiting the ex vivo expansion and therapeutic potency of the resulting product. Secondly, CAR-T cell cytotoxicity against normal lymphocytes and their progenitors may suppress systemic T-cell function and induce temporary or prolonged immunodeficiency, similar to that observed in patients after HSCT. Thirdly, there is a risk of genetically modifying circulating leukemic blasts, which are abundant in many T-ALL patients with chemorefractory disease, facilitating the emergence of treatment-resistant tumor clones that downregulate target CAR antigen³⁶.

We first reported a CAR with high anti-tumor activity in preclinical models of T-ALL in 2015. This CAR targeted CD5, a pan-T cell antigen commonly expressed in T-ALL^{35,37} and included the CD28 costimulatory endodomain^{38,39}. Expressing the CAR in normal T cells promoted rapid loss of CD5 on their cell surface, resulting in resistance to fratricide and normal expansion of CD5 CAR T cells. The expanded cells were highly cytotoxic to CD5+ malignant T cell lines and primary blasts, both in vitro and in mouse models of disseminated T-ALL xenografts. At the same time, CD5 CAR T cells showed limited activity against normal CD5+ T cells, possibly due to intrinsic mechanisms of resistance to self-inflicted cytotoxicity in normal mature T cells^{37,38}. These CD5 CAR T cells are currently being evaluated in a Phase I study of patients with T-ALL at Baylor College of Medicine (NCT03081910).

CD7 is another attractive target antigen for T-ALL, as it is expressed at a high level in leukemic cells while its normal expression is limited to T- and NK-cells and their immediate progenitors^{35,37}. However, we found that expression of a CD7-specific CAR on normal T cells promoted widespread fratricide and precluded their expansion⁴⁰. Genomic disruption of the CD7 gene using CRISPR/Cas9 mitigated this fratricide^{40,41}, as did trapping the CD7 protein inside the cell by anchoring a CD7-specific scFv in the endoplasmic reticulum⁴². Both of these methods prevent surface expression of CD7 and enable the expansion of CD7 CAR T cells with high cytotoxicity against CD7+ T-ALL blasts in multiple preclinical models of disseminated leukemia. A second generation CD7 CAR expressed on CRISPR-edited CD7 knockout T cells is being evaluated at Baylor College of Medicine (NCT03690011).

Although CD5 and CD7 are commonly expressed in T-ALL, other target antigens with more restricted expression have also been explored. About a third of T-ALL express surface CD3 and other components of TCR that can be targeted via specific CARs^{35,37}. Indeed, T cells expressing a CD3-specific CAR potently eliminated CD3+ T-cell malignancies in vitro and in vivo⁴³. Similar to CD7, expression of CD3 has to be disrupted in T cells in order to prevent fratricide of CD3 CAR T cells. TCR+ leukemic T-cell blasts can be more selectively targeted by a TRBC1-specific CAR, which recognizes a TCR β chain variant expressed in ~35% of normal T cells and a similar proportion of CD3+ T-ALL⁴⁴. A multi-center clinical study is currently underway in the UK to evaluate the safety and efficacy of TRBC1 CAR T cells in patients with T-cell malignancies including T-ALL (NCT03590574). One important advantage of this approach is that most peripheral T cells are TRBC1-negative and thus will be spared by the CAR T cells.

CD30 is expressed in a subset (17–37%) of T-ALL, although in most patients only on a subset of the leukemic cells⁴⁵. CD30 CAR T cells have recently demonstrated remarkable efficacy in patients with Hodgkin's lymphoma and peripheral T-cell lymphoma^{46,47} so it may also be effective against T-ALL patients with CD30+ disease, despite limited expression.

As described for AML above, developing safe and effective cell therapies for T-ALL requires predicting and managing potential off-tumor toxicities. Toxicities directed against normal T cells and their progenitors are especially critical when targeting pan-T cell markers like CD5 and CD7, as CARs specific to more restricted T-cell antigens such as TRBC1 would spare large subsets of normal T cells. One approach limiting the extent and duration of off-tumor toxicities is to engineer CAR T cells for transient expansion and persistence. For example, integration of a CD28 costimulation in a CAR often promotes robust anti-tumor activity with short-term persistence of functional CAR T cells, whereas 4–1BB costimulation favors the formation of long-lived CAR T cells with continuous on-target cytotoxicity^{48,49}. Additionally, suicide switches like iC9 or surface epitopes for cytotoxic antibodies can be incorporated in CAR T cells to enable their control post-infusion⁵⁰. An example of this approach is the ongoing clinical study of TRBC1 CAR T cells expressing a synthetic safety switch RQR8⁵¹ (NCT03590574).

Further, as for the treatment of AML, even CAR T cells that are fully ablative of normal T lymphocytes can be used as a bridge to transplant, by eliminating T-ALL blasts and thereby making it feasible to proceed to a potentially curative HSCT. Stem cell transplant terminates the activity of CAR T cells, resetting normal hematopoiesis and eventually replenishing the peripheral T-cell pool. To reduce just such a risk of prolonged T-cell aplasia, both CD5- and CD7-directed “first-in-man” clinical studies at Baylor College of Medicine are designed as a bridge to HSCT, (NCT03081910, NCT03690011). An alternative approach is to engineer fratricide-resistant T cells (e.g., CD7-edited) with intact TCR function that could repopulate the host and provide temporary host protection against most prevalent environmental and endogenous pathogens in the event of CAR-induced aplasia⁴⁰. The depth and duration of therapy-induced T-cell aplasia has to be evaluated in clinical studies before selecting the optimal strategy to counteract potential immune deficiencies.

Finally, the risk of genetic modification of malignant blasts can be reduced by enriching the starting cell population for normal T cells (e.g., positive CD3+ enrichment to deplete CD3– T-ALL blasts) or eliminated altogether by manufacturing T cells from a healthy donor. As discussed in section B2.3.2, the risk of GVHD in the latter case can be significantly decreased by removing surface-expressed TCRs^{41,43} or by using virus-specific T cells from a closely HLA-matched donor. The latter option is particularly attractive, as the infused CAR-T cell product would exert both anti-tumor activity (mediated by the CAR) and antiviral function (mediated by the TCR) in the event of a prolonged T-cell aplasia.

Ongoing and upcoming clinical studies directed at various T-ALL antigens will reveal safety and efficacy of current therapeutic strategies and inform the design of the next-generation targeted cell therapies for this disease.

D. Summary and Future Prospects,

Adoptively transferred T cells have demonstrated safety and clinical activity toward AML and other hematological malignancies in early phase clinical trials. Ongoing studies are exploring strategies to enhance the effects of adoptively transferred cells by optimizing selection and culture conditions. Success in the treatment of AML is also reported with NK cells and studies that genetically modify them by adding CARs or cytokine genes/receptors are in progress. There is also increasing interest in the use of NK cells and ‘unconventional’ T cells, including $\gamma\delta$ T cells and invariant chain NKT cells. These cells may have biological properties that are complementary to conventional T cells and be particularly well suited to banked use for AML. Although treatment of T-ALL is at a much earlier stage, many of the initial roadblocks to cell therapy have been passed and several clinical studies using T cell directed CARs are in progress.

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Figure 1. Adoptive cellular therapy strategies:

A: chimeric antigen receptor (CAR)-T cells targeting surface cancer-antigens, **B:** Transgenic or native TCRs specific for leukemia antigens expressed through MHC molecules and **C:** NK cells interacting with leukemia cells through activating and inhibitory receptors.

Table 1:

Contrasting properties of different types of cellular therapy products

Properties	Autologous	Donor-derived	Third-party
Inducing GVHD	No risk	Possible	Modifications required to mitigate GVHD potential
Risk for allorejection (<i>in vivo</i> persistence)	None (long-lived)	Same as autologous in post-HSCT setting	High risk (short-lived)
Quality of starting material/Manufacture success	Poor	Good	Good
Manufacture time needed before treating patients	Yes	Yes	No
HSC donor required	No	Yes	No

Table 2:

Selected published or ongoing TCR based cellular therapy trials for AML

Published or presented	Target	Transgenic?	Prior chemo	Cell dose	Patients	Outcomes	grade III AEs
1. HSC Donor- products							
2010 ⁸ & 2018 ⁹	WT1	Unmodified	None	Up to 10 ⁸	8 In CR post-HSCT	3 relapse by year-1, 5/8 long term CR (>8 years)	None
2013 ¹⁰	WT1 (HLA-A02 peptide)	Unmodified	None	Up to 10 ⁹	6 in CR; 2 in relapse	1 of 2 CRs in relapse group, 3 of 6 eventually relapse in CR group	None
2016 ¹³	WT1 (HLA-A02 peptide)	Yes, lenti-v transduction	Cy/Flu and post-IL2	Up to 10 ¹⁰	11 in CR 11 in relapse	11 of 11 remain in CR (1-yr) No durable responses in relapse arm	None
2019 ¹¹	WT1, PRAME, NYESO1, Survivin	Unmodified	None	Up to 2×10 ⁷ /m ²	13 in CR 7 in relapse	9 of 13 remain in CR (1-yr), 2 of 7 in relapse responded	Transient LFT rise in 1
NCT03326921 (U of Washington)	HA-1 (HLA-A2 peptide)	Yes, lenti-v has iCaspase switch	Not available	Not available	Up to 24 in relapse	Not available	Note available
2. Auto products							
2017 ¹²	WT1 (HLA-A24 peptide)	Yes, retro-v transduction with si-RNA for native TCR	None	Up to 10 ⁹	8 in relapse	Transient responses only	None
NCT02743611 (Many)	PRAME (HLA-A2 peptide)	Yes, has iCaspase switch	Not released	Up to 5×10 ⁶ /kg	116 in relapse	Not released	Not released

Abbreviations: lenti-v: lentivirus, Cy/Flu: cytoreductive chemotherapy with cyclophosphamide and fludarabine, Post IL-2: Interleukin-2 injections post-cellular therapy, LFTs: liver function tests

Table 3:

Selected published or ongoing CAR based cellular therapy trials for AML

Published or presented	Target	Construct, vector	Prior chemo	Cell dose	Patients	Outcomes	grade III AEs
<i>1. Auto products</i>							
2014 ¹⁷	CD33	4-1BBz-GFP, lenti-v	None	Up to 4.25×10 ⁸ CAR+	1 in relapse	Initial CR (2 wk) then relapse	1 CRS
2013 ²⁰	Lewis-Y	28z, retro-v	None	up to 10 ⁹	4 in relapse	Transient responses in 2	None
2018 ¹⁹	CD123	4-1BBz, electroporated	No or Cy only	up to 6 doses of 4×10 ⁶	5 in relapse	No responses	None
2018 ¹⁸	CD123	28z-tEGFR, lenti-v	Flu/Cy	up to 2×10 ⁸	9 in relapse	2 CRs	1 CRS
2018 ²¹	NKG2DL	DAP10, retro-v	None	up to 3×10 ⁷ total	9 in relapse	No responses	None
NCT03126864 (MDACC)	CD33	Not available, lenti-v	Cy/Flu	Not available	Not available	Not available	Not available
NCT02799680 (PLA China)	CD33	Not available	Cy/Flu	Not available	Not available	Not available	Not available
NCT03631576 (Fujian Medical U, China)	CD123/CLL-1	Not available	Not available	Unknown	Up to 20 in relapse	Not available	Not available
NCT03222674 (Multiple sites in China)	MUC-1, CD38, CD33, CD123, CD56, CLL1	Not available, lenti-v	Not available	Not available	Up to 10 in relapse	Not available	Not available
<i>2. Universal products</i>							
NCT03190278 (Multiple sites in USA)	CD123	Not available, lenti-v, Universal, (TCR deleted by TALEN)	Cy/Flu	Not available	Not available	Not available	Not available

Abbreviations: GFP: green fluorescent protein, tEGFR: Epidermal growth factor receptor tag, TALEN: transcription activator like effector nucleases

Table 4.

Selected published or ongoing NK based cellular therapy trials for AML

Published or presented	HSCT Source	Prior chemo	Cell dose	Patients	Outcomes	grade III AEs
1. HSC-donor products						
2004 ²⁴	HaploHSCT	None	Up to 9.3×10 ⁶ /kg	4 mixed chimera, 1 graft failure	2 of 5 improved chimerisms	None
2013 ²⁵	HaploHSCT	None,	Up to 12.1×10 ⁶ /kg	8 in CR postHSCT	4 of 8 eventually relapsed	1 death from GVHD
2014 ²⁶	HaploHSCT	HSCT conditioning	Up to 2×10 ⁸ /kg	29 in active relapse	11 of 29 (38%) in relapsed by 1-year postHSCT	17% grade II aGVHD 24% grade II cGVHD
NCT03068819 (Wash U)	Any HSCT	FLAG regimen	Up to 10×10 ⁶ /kg “pre-activated NKs” + DLIs	Up to 24 in relapse postHSCT	Not available	Not available
NCT03300492 (U of Basel)	HaploHSCT	Unknown	Up to 10 ⁹ /kg	Up to 10 in CR postHSCT	Not available	Not available
NCT01823198 (MDACC)	Any HSCT	None	Up to 10 ⁸ /kg	Up to 72	Not available	Not available
2. Haplo-NK cells, without haplo-HSCT						
2010 ²⁸	No HSCT	Cy/Flu	Up to 29×10 ⁶ /kg	10 in CR1	10 of 10 remain in CR (3 years post-infusion)	1 febrile neutropenia
2015 ²⁹	No or any prior HSCT	Clofara/Etop/Cy	Up to 18.6×10 ⁶ /kg	12 in relapse	6 entered CR	None
2016 ²⁷	MRD or MUD	Bu/Flu- HSCT conditioning	Up to 5×10 ⁶ /kg	15 in relapse	7 of 15 entered CR for >1 year	2 grade III aGVHD and 7 extensive cGVHD
2011 ³⁰	No HSCT	Cy/Flu	Up to 2.7×10 ⁶ /kg	Active disease cohort: 7 Adjuvant cohort: 6	Active: 3 of 7 entered transient CR (< 9 mo) Adjuvant: 3 of 6 relapsed by year-3	None
2016 ³¹	No HSCT	Cy/Flu	Up to 10×10 ⁶ /kg “pre-activated” NKs	9 in relapse	4 of 9 entered CR	None
NCT03081780 (U of Minnesota)	No or any prior HSCT	Cy/Flu	Not available	Up to 20 in relapse	Not available	Not available
NCT02809092 (Brazil)	No or any prior HSCT	FLAG regimen	Not available	Up to 30 in relapse	Not available	Not available
NCT01787474 (MDACC)	No or any prior HSCT	FLAG regimen	Not available	Up to 44 in relapse	Not available	Not available
NCT02229266 (multiple German centers)	No or any prior HSCT	Cy/Flu	Not available	Up to 56 in relapse	Not available	Not available
3. Universal NK cells						
2017 ³²	No HSCT	None, irradiated NK92 cell line	Up to 3×10 ⁹ /m ²	7 in relapse	No responses	None

Published or presented	HSCT Source	Prior chemo	Cell dose	Patients	Outcomes	grade III AEs
NCT02890758 (Case Cancer Center)	No or any prior HSCT	Cy/Flu (+ALT803 post-infusion)	Up to 10 ⁹ /kg	Up to 54 in relapse	Not available	Not available

Abbreviations: Clofara/etopo/Cy: clofarabine, etoposide and cyclophosphamide chemotherapy regimens, FLAG: Fludarabine, cytarabine and granulocyte-colony stimulating factor, Bu/Flu: Busulfan and fludarabine chemotherapy regimen, MRD: Matched related donor, MUD: Matched unrelated donor.

Table 5:

Ongoing T-ALL CAR-T cell trials (Autologous products)

NCT	Target	Construct, vector	Prior chemo	Cell dose	Patients	Outcomes	grade III AEs
NCT03081910 (Baylor Coll of Med)	CD5	28z, retro-v	Cy/Flu	Up to $10^8/\text{m}^2$	Active CD5+ T-ALL	Not available	Not available
NCT03690011 (Baylor Coll of Med)	CD7	28z, retro-v	Cy/Flu	Up to $10^8/\text{m}^2$	Active CD7+ T-ALL	Not available	Not available
NCT03590574 (Univ Coll of London and others)	TRBC1	Not available	Cy/Flu	Up to $2.25 \times 10^8/\text{m}^2$	Active TRBC1+ T-ALL	Not available	Not available

Abbreviations: TRBC1: T cell receptor Beta Constant-1, retro-v: retrovirus.