Gamma delta T cells in acute myeloid leukemia: biology and emerging therapeutic strategies

Adishwar Rao, Akriti Agrawal, Gautam Borthakur, Venkata Lokesh Battula, Abhishek Maiti

ABSTRACT

γδ T cells play an important role in disease control in acute myeloid leukemia (AML) and have become an emerging area of therapeutic interest. These cells represent a minor population of T lymphocytes with intrinsic abilities to recognize antigens in a major histocompatibility complex-independent manner and functionally straddle the innate and adaptive immunity interface. AML shows high expression of phosphoantigens and UL-16 binding proteins that activate the Vδ2 and Vδ1 subtypes of γδ T cells, respectively, leading to γδ T cell-mediated cytotoxicity. Insights from murine models and clinical data in humans show improved overall survival, leukemia-free survival, reduced risk of relapse, enhanced graft-versus-leukemia effect, and decreased graft-versus-host disease in patients with AML who have higher reconstitution of γδ T cells following allogeneic hematopoietic stem cell transplantation. Clinical trials leveraging γδ T cell biology have used unmodified and modified allogeneic cells as well as bispecific engagers and monoclonal antibodies. In this review, we discuss γδ T cells’ biology, roles in cancer and AML, and mechanisms of immune escape and antileukemia effect; we also discuss recent clinical advances related to γδ T cells in the field of AML therapeutics.

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous hematological malignancy that is characterized by differentiation arrest and clonal proliferation of malignant myeloid progenitor cells. Adverse-risk and relapsed/refractory (R/R) AML remain highly challenging to treat and to target in the process of drug development. While conventional intensive chemotherapy remains the mainstay for younger and fit patients, it may not be suitable for the majority of patients, particularly patients over the age of 60 years and those who are unfit for intensive therapy. Venetoclax-based lower-intensity regimens have improved outcomes and are the current standard for older patients. However, cures are elusive, and the 3-year overall survival (OS) remains poor, at 25%, with dismal outcomes after the failure of frontline therapy. Long-standing knowledge about the graft-vs-leukemia effect following allogeneic stem cell transplantation and transformative advances with αβ chimeric antigen receptor (CAR)-T cell therapies in lymphoma and myeloma have fueled the search for effective cellular therapies for AML. However, these therapies have only been modestly successful, with none receiving regulatory approval so far. Toxicities noted with CAR-T cell therapeutics, including cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), have led to increased interest in alternative strategies with a lower risk of such toxicities, including natural killer (NK) cell-based therapies.

NK cell-based and γδ T cell-based strategies have emerged as a promising new approach to counter AML because of these cells’ unique biology, lower secretion of cytokines that are implicated in CRS and ICANS, and low risk of graft-vs-host disease (GvHD). γδ T cells are a subset of T lymphocytes that express the γδ T-cell receptor (TCR) instead of the αβ TCR, which is expressed by the αβ T cells that form the majority of T lymphocytes in humans. In this review, we discuss γδ T cells’ biology, roles in cancer and AML, and mechanisms of immune escape and antileukemia effect; we also discuss recent clinical advances using γδ T cells in the field of AML therapeutics.

Development of γδ T cells

γδ T cells develop in the thymus during fetal maturation. The double-negative thymocytes (CD4− and CD8−), through multiple developmental stages and gene rearrangements, result in the emergence of two primary T cell lineages, αβ and γδ T cells. According to the Signal-Strength Model, the intensity of the TCR signal to the double-negative thymocytes, which is dependent on the extracellular signal-regulated kinase pathway, determines the commitment of these cells to the αβ or γδ T cell lineages, with a strong signal, among
other factors, favoring the γδ T cell lineage. The double-positive thymocytes (CD4γδ, CD8γδ) give rise to multiple cell lineages, including cells that express the Vα14+ invariant αβ TCR, which act as progenitors of invariant NK T cells. The key differences among the αβ T cell, γδ T cell, invariant NK T cell, and NK cell lineages are summarized in table 1.

| Table 1 | Key differences between αβ T cells, γδ T cells, iNKT cells, and NK cells |
|---------|-----------------|---------|---------|---------|
| Properties | γδ T cells | αβ T cells | NK cells | iNKT cells |
| Surface receptor variety | γδ TCR | αβ TCR | AR, IR | invariant TCR |
| Frequency in peripheral blood (% of total lymphocytes) | 0.3–3.35 | 63–66 | 5–15 | 0.01–0.36 |
| Distribution | Epithelial layers, blood | Lymphoid organs, tissues | Blood, lymphoid organs, tissues | Thymus, liver |
| Type of immunity | Innate+adaptive | Adaptive | Innate | Innate |
| Require antigen presentation | No | Yes | No | No |
| Repertoire diversity | Limited | Almost unlimited | Limited | Limited |
| Effector cell generation time | 1–2 days | 7 days | 3 days | – |
| Predominant cytometric phenotype | CD4+, CD8− | CD4+, CD8+ | CD56, CD16, NKp46 | CD4+, CD8+, CD24+, CD44+ |
| GvHD risk | Minimal | Yes | No | Minimal |
| Risk of CRS/ICANS | Low | Yes | No | Low |

AR, activating receptor; CRS, cytokine release syndrome; GvHD, graft-versus-host disease; ICANS, immune effector cell-associated neurotoxicity syndrome; IR, inhibitory receptor; NK, natural killer; TCR, T cell receptor.

γδ T cells are classified on the basis of TCR Vδ expression as Vδ1, Vδ2, Vδ3, and Vδ5 subtypes. Vδ1 and Vδ3 T cells are present in the intestinal mucosa, skin, liver, lungs, reproductive tract, thymus, and spleen, while Vδ2 T cells are predominantly found in the peripheral blood. On the basis of TCR Vγ expression, there are numerous Vγ T cell types, Vγ 2–9 and Vγ 11. γδ T cells can differentiate between healthy and tumor cells through their reliance on certain physiological receptors, such as the γδ TCR and the natural cytotoxicity receptors, including Nkp30 and Nkp44.

Healthy adults have approximately a median of 63 γδ T cells/µL (0.25–539 cells/µL), representing 2.3% (1.2%–15.4%) of all T cells in the peripheral blood. Vδ2 T cells comprise approximately 1%–10% of all T cells in the peripheral blood, with absolute counts of nearly 39 cells/µL (243 cells/µL), and more than 60% of γδ T cells. Of all the Vδ2 T cell subtypes, Vγ9Vδ2 occupies the largest proportion, constituting 50%–95% of Vδ2 T cells. In contrast, Vδ1 T cells comprise only 10%–30% of γδ T cells in the peripheral blood, suggesting absolute counts of nearly a median of 16 cells/µL (2–75 cells/µL). The distribution of γδ T cells in the lymphoid tissue, including the gut and skin-associated lymphoid systems, is similar to that seen in the peripheral blood.

In addition to differences in the TCR representation, γδ T cells are known to differ from αβ T cells in their antigen recognition. Vδ1 T cells recognize tumor cells through their interaction with major histocompatibility complex (MHC)-related molecules, such as MHC class I-related molecule A, MHC class I-related molecule B, and UL16 binding proteins (ULBPs), or via the detection of MHC-related class-1b molecules representing the glycolipids CD1c and CD1d. In contrast, Vδ2 T cells detect phosphoantigens (pAgs) such as isopentenyl pyrophosphate, a derivative of the mevalonate pathway, or those released by pathogens (e.g., [E]-4-hydroxy-3-methyl-but-2-enylpyrophosphate) and are activated by them. Although an in-depth understanding of the mechanism is yet to be developed, Vγ9Vδ2 T cells are specifically thought to be activated through their interaction with pAgs, dependent on the butyrophilin (BTN) family (BTN3A1, BTN3A2, BTN3A3, and BTN2A1) of proteins. Vδ3 T cells, similar to Vδ1 T cells, recognize glycolipids bound to CD1d molecules expressed by antigen-presenting cells.

Functions of γδ T cells
γδ T cells serve as a bridge between the innate and adaptive immune systems. As γδ T cells are essentially T lymphocytes consisting of TCR rearrangements, their ability to induce immunological memory and destroy target cells categorizes them as members of the adaptive immune system. On the other hand, their ability to recognize multiple molecular patterns, resulting in swift responses independent of MHC restriction, and the expression of receptors similar to those expressed by other members of the innate immune system, such as activating NK receptors (NK2D, Nkp30, and Nkp44) and Toll-like receptors, are characteristics that contribute to their categorization as members of the innate immune system. γδ T cells play an important role in the physiologic functioning of the immune system. These cells induce immunity against pathogenic organisms, regulate responses of the immune system, promote tissue healing, and carry out tumor surveillance in the normal human body.
Moreover, γδ T cells, like adaptive T cells, differentiate into various T helper-like cells (Th1-like, Th2-like, and Th17-like cells), leading to the production of a multitude of cytokines via which they can orchestrate the function of other innate and adaptive cells.32 (figure 1). The presence of transformed, infected, or stressed cells could lead to the induction of cell death in the target cells through γδ T cells, mediated by the production of cytotoxic molecules, such as perforins and granzymes, or by activating pathways involved in increasing the expression of tumor necrosis factor-related apoptosis-inducing ligand receptors and death-inducing receptors (CD95).20 γδ T cells also interact with neutrophils, γδ T cells, dendritic cells, and B cells to exert their immune response. These cells are known to draw other immune mediators to the site of infection via the production of interferon-gamma (IFN-γ) and interleukin-17 (IL-17).26

γδ T cells, as tumor-infiltrating lymphocytes, are a marker of significantly favorable cancer prognosis.16 Multiple studies have suggested that they have antitumor activity in numerous solid cancers, such as melanoma, prostate cancer, bladder cancer, pancreatic cancer, colon cancer, and breast cancer and hematological malignancies such as lymphomas, leukemias, and multiple myelomas.20 The proposed mechanisms of antitumor activity include direct cytotoxicity via perforins, granzymes, and tumor necrosis factor-related apoptosis-inducing ligand receptors; Fas ligand; antibody-dependent cellular cytotoxicity; and alteration of the tumor microenvironment dynamics. γδ T cells exert antibody-dependent cellular cytotoxicity, leading to tumor lysis, through the expression of CD16 (Fcγ receptor III), which interacts with the target cell-bound Fc region of antibodies.6 20 This has been capitalized clinically, in conjunction with certain monoclonal antibodies, such as trastuzumab and rituximab, where the stimulation of Vγ9Vδ2 T cells leads to the upregulation of CD16, resulting in antibody-dependent cellular cytotoxicity-mediated tumor lysis.12

Role of γδ T cells in AML
AML blasts express high levels of IL-8, CXC-chemokine ligand-10, or interferon γ-induced protein-10 and CC-motif chemokine ligand-5, also known as RANTES (regulated on activation, normal T cell-expressed and secreted), among other chemokines.17 18 In response to some of these factors, the tumor-reactive Vδ1 cells in the peripheral blood strongly upregulate their expression of CXC chemokine receptor-1 (CXCR1) while also increasing the expression of C-C motif chemokine receptor-2 (CCR2), CXCR3, and, minimally,CCR5, resulting in the production of higher levels of IFN-γ and homing of the Vδ1 cells into the leukemia microenvironment.17 18

In addition, xenotransplantation murine models have demonstrated the homing abilities of the Vγ9Vδ2 cells, from the blood into the bone marrow.18 Vγ9Vδ2 cells respond to some of these chemokines expressed in the leukemia microenvironment by strongly expressing CCR5, along with the expression of CXCR3 and CXCR4 and weak expression of CXCR1.17 In addition to these mechanisms, in vitro studies have also explored the role of the CXCR4-CXC-chemokine ligand-12 pathway in the relocation of γδ T cells into the bone marrow microenvironment in AML.19

γδ T cells use γδ TCR, coreceptors such as NK cell receptors, metabolic antigens such as pAgs, and CD1-dependent mechanisms, among others, to recognize and eliminate leukemia cells.21 PAgS are captured by BTN molecules in normal and leukemia cells. The Vγ9 chain, a component of the Vγ9Vδ2 TCR, is germline-encoded to identify BTN molecules and leads to the activation of Vγ9Vδ2 T cells.22 23 BTN2A1 protein has a pertinent role in the recognition of leukemia blasts, despite being incapable of directly capturing pAg molecules. It indirectly sends activating signals to the Vγ9Vδ2 T cells through the other isoforms of the BTN protein, such as BTN3A1 (CD277) and BTN3A3.24 Moreover, the TCRs of non-Vγ9Vδ2 T cells such as Vδ1 and Vδ3 T cells, while potentially lacking pAg-BTN-Vγ9 axis recognition capabilities, are capable of more robust recognition of antigens such as pathogenic and non-pathogenic lipids when presented by a member of the CD1 protein family, particularly CD1b, CD1c, or CD1d.25 26 31 These mechanisms of antigen recognition, which are otherwise typically associated with NK cells, are a key factor in the interest in developing γδ T cell-based therapies in AML and other hematological malignancies.21 24 31 The process of antigen recognition mediated by Vδ1 γδ T cells has been shown to identify leukemia cells with upregulated expression of ULBP2, ULBP3, and MHC class I-related molecule A (figure 2), leading to higher IFN-γ and tumor necrosis factor-mediated antitumor effects, while Vδ2 γδ T cells can recognize upregulated ULBP1 and ULBP2 expression.21 33 AML blasts express high to very high levels of ULBP1, which is a strong incentive for the development of γδ T cells for AML immunotherapy.34 Molecules such as nectin-2 (CD112) and polio virus receptor (CD155), expressed in leukemia cells, may also act as ligands for NK cell receptors like DNAX accessory molecule-1, which have been observed to play an advantageous role in immunotherapy for AML.21 35-38

Mechanisms of immune escape from γδ T cell-mediated eradication
AML blasts can evolve to select for clones that are capable of maintaining an immunosuppressive and highly tolerogenic milieu to evade γδ T cell-mediated killing.35 AML progenitor cells are known to upregulate regulatory T cells and myeloid-derived suppressor cells (MDSCs) and repressive factors, such as transforming growth factor-beta, adenosine, prostaglandin-E2, and enzymes such as arginase and indoleamine 2,3-dioxygenase.40 Upregulation of immune checkpoints, such as programmed cell death protein-1 (PD-1), T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), and T cell immunoreceptor with Ig and ITIM domains (TIGIT), and epigenetic downregulation of human leukocyte
Figure 1  Effect phenotypes of γδ T cells. Butyrophilin molecules expressed on AML blasts sense phosphoantigens derived from the mevalonate pathway, activate γδ T cells, and promote their differentiation into two major phenotypes—cytotoxic T lymphocytes and T helper-like lymphocytes. The cytotoxic T lymphocyte forms promote leukemic cell killing through cytotoxic cytokines, Fas (CD95), and perforins and granzyme, whereas, the T helper-like phenotypes predominantly activate and recruit other cells of the immune system by promoting the activation of macrophages, NK cells and neutrophils; differentiation of αβ T cells; isotype switching of B lymphocytes; and migration of leucocytes toward the tumor microenvironment. AML, acute myeloid leukemia; BTN, butyrophilin; CD, cluster of differentiation; CTL, cytotoxic T lymphocyte; IFN, interferon; IL, interleukin; NK, natural killer; TCR, T cell receptor; Th, T helper.
antigen class II molecules, has also been implicated in immune escape.\textsuperscript{16,38,41,42} (figure 3). Dendritic cells, macrophages, and certain immature myeloid subsets, such as MDSCs, have inhibitory action on γδ T cells. M2-type macrophages and MDSCs, which have been found to be elevated in patients with AML, modify the leukemia microenvironment by producing factors such as transforming growth factor-beta and arginase.\textsuperscript{40} AML blasts and polymorphonuclear-MDSCs may limit IFN-γ production by γδVβ2 T cells and reduce their antileukemic cytotoxicity in an arginase-I or arginase-II-dependent manner.\textsuperscript{43}

AML blasts express molecules, such as programmed cell death ligand-1 (PD-L1), galectin-9, CD112 and CD155, CD80 and/or CD86, and herpes virus entry mediator, which act as ligands for immune checkpoints expressed by γδ T cells, such as PD-1, TIM-3, TIGIT, cytotoxic T-lymphotoyte antigen 4 and B and T lymphocyte attenuator, respectively.\textsuperscript{44} While PD-1, TIM-3, and TIGIT are significantly expressed in γδ T cells, cytotoxic T-lymphocyte antigen 4 is only rarely expressed.\textsuperscript{45} γδ T cells express PD-1, which combines with programmed cell death ligands (PDLs) to initiate an intracellular signaling cascade that restricts the activation of γδ T and αβ T cells. Interestingly, PDLs belong to the B7 protein family, similar to BTN-3, which activates γδ T cells.\textsuperscript{46} A transient upregulation of PD-1 has been demonstrated on highly active γδ T cells on stimulation by pAgs, possibly to avoid the targeting of healthy tissues.

**Figure 2**  Activation of γδ T cells and crosstalk with other immune cell populations. Activated γδ T cells process tumor antigens and present those to CD4+ and CD8+ αβ T cells. γδ T cells also promote maturation of dendritic cells and activation of NK cells. Additionally, activated γδ T cells mediate leukemia cell-lysis by antibody-dependent cellular cytotoxicity, perforin-granzyme mediated cytosis and Fas-mediated cytotoxicity. ADCC, antibody-dependent cellular cytotoxicity; CD, cluster of differentiation; DNAM, DNAX-activating molecule; MHC, major histocompatibility complex; NK, natural killer; TCR, T cell receptor; TRAIL, Tumor necrosis factor-related apoptosis-inducing ligand; ULBP, UL16 binding protein.

Simultaneous expression of PDLs, like PDL-1, has also been demonstrated on the surface of γδ T cells to potentially prevent fratricide. While this may be a part of the physiological mechanism needed to prevent overactivation of the immune system, AML blasts also express the PD-1 ligands PD-L1 and PD-L2 and use this mechanism to suppress γδ T cells.40

Murine models suggest the presence of a subset of γδ T cells that may demonstrate protumor activity by limiting αβ T cell infiltration into leukemia sites as a consequence of PD-1/PDL-1 signaling.46 Although the influence of PD-1/PDL-1 axis signaling on the cytotoxic potential of γδ T cells is arguably weak in immunocompetent healthy environments, it may become significant in the immunosuppressed leukemia microenvironment.21 46 However, xenograft models indicate that γδ T cells may partially overcome this PD1/PDL-1 axis-mediated immune evasion by AML blasts if activating signals of sufficient intensity are transduced through their γδ TCR.47 Similar to PD-1, TIM-3 and TIGIT are also regulatory receptors expressed on γδ T cells that can be leveraged by AML blasts for immune evasion.21 AML blasts express Gal-9, which acts as a ligand for TIM-3, while CD112 and CD155 are ligands for TIGIT, similar to DNAX accessory molecule-1 (CD226).40 48 AML patients have an increased population of bone marrow-resident Vδ1 T cells that express PD-1, TIM-3, and TIGIT.49 The coexpression of these inhibitory molecules may suggest a state of functional exhaustion in these cells.50 Moreover, in patients with certain subsets of AML, a higher frequency of TIGIT+ CD226− γδ T cells may be associated with an inferior 12-month OS (27% vs 81%, p=0.028).51 Lastly, exposure to certain types of

Figure 3  Factors influencing γδ T cell fitness and exhaustion. Exhaustion of γδ T cells can be induced in by multiple factors in the tumor microenvironment. AML blasts directly induce exhaustion of γδ T cells via PD-1-PD-L1 interaction, Gal-9-TIM-3 interaction, and CD112/CD155-TIGIT interactions. An immunosuppressive milieu can accelerate exhaustion of γδ T cells. Immunosuppressive MDSCs and Tregs, soluble factors, for example, TGF-β, adenosine and PGE2, and enzymes, for example, arginase and IDO, can contribute toward the immunosuppression. Lastly, lymphotoxic chemotherapy, such as fludarabine and cladribine, frequently used in AML lead to depletion and exhaustion of γδ T cells. AML, acute myeloid leukemia; CD, cluster of differentiation; IDO, indoleamine 2,3-dioxygenase; MDSC, myeloid derived suppressor cells; MHC, major histocompatibility complex; PD, programmed cell death protein; PD-L1, programmed cell death protein ligand 1; PGE, prostaglandin E; TCR, T cell receptor; TGF, transforming growth factor; TIGIT, T cell immunoreceptor with Ig and ITIM domains.
lymphotoxic chemotherapy, such as fludarabine and cladribine, may decrease fitness in γδ T cells.

**Impact of γδ T cells on post-transplantation outcomes**

γδ T cells enhance the graft-vs-leukemia effect and reduce the risk of GvHD. Studies in murine models show that in the absence of donor γδ T cells, mice had a significant reduction in body weight and survival.52 Death due to accelerated acute GvHD was evident in cases of γδ T cell-deficient donor grafts, suggesting that donor γδ T cells suppress acute GvHD, potentially through the inhibition of CD4+ T cell activation. Similar results have been obtained in humans in the postallogeneic transplantation setting. γδ T cells reconstitute faster than αβ T cells in the myeloablated marrow after transplantation. One study of patients undergoing allogeneic stem cell transplantation for myeloid and lymphoid leukemias showed significant improvement in OS and relapse-free survival, and a significantly lower risk of acute GvHD in patients with high γδ T cell reconstitution at 56 days after transplantation.53 A significantly lower risk of acute GvHD with death was observed in patients with higher concentrations of total γδ T cells (p=0.02), Vδ1 γδ T cells (p=0.08), and Vδ2 γδ T cells (p=0.02) 28 days after allogeneic stem cell transplantation. Patients with hematological malignancies with γδ T cell concentrations lower than the median had an estimated five times higher risk of death (HR=5.16, p=0.001) and a nearly three times higher risk of relapse (HR=2.7, p=0.007) than did those whose γδ T cell concentrations were higher than the median.53

Among pediatric patients with leukemia undergoing allogeneic stem cell transplantation, those who recovered with a higher number of γδ T cells had a significantly higher event-free survival rate than did those who recovered with a normal or lower number of γδ T cells (91% vs 55%, p=0.04).34 In another study in patients with acute leukemia undergoing stem cell transplantation from partially mismatched-related donors, increased γδ T cells after allogeneic hematopoietic stem cell transplant (allo-HSCT) conferred significantly improved 5-year leukemia free survival (LFS) (34.4% vs 19.1%, p=0.0003) and OS rates (70.8% vs 19.6%, p<0.0001) compared with normal or lower numbers of γδ T cells.55 Importantly, recovery of γδ T cells following allo-HSCT can overcome the negative prognostic impact of persistent measurable residual disease before transplantation. Newly diagnosed patients and those with R/R AML undergoing allo-HSCT

Table 2: Current clinical trials investigating the role of γδ T cells in AML

<table>
<thead>
<tr>
<th>Agent</th>
<th>Description</th>
<th>Ph.</th>
<th>Dose (cells/kg)</th>
<th>Population</th>
<th>Rec.</th>
<th>NCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCB-202-001</td>
<td>Ex vivo expanded allogeneic Vγ9Vδ2 T cells</td>
<td>I</td>
<td>1×10⁶</td>
<td>R/R AML</td>
<td>C</td>
<td>NCT03790072</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1×10⁷</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1×10⁸</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCB-008</td>
<td>Ex vivo expanded allogeneic γδ T cells</td>
<td>II</td>
<td>7×10⁸</td>
<td>R/R AML, MRD+</td>
<td>R</td>
<td>NCT05358808</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7×10⁷</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INB-100</td>
<td>Allogeneic γδ T cells</td>
<td>I</td>
<td>1×10⁶</td>
<td>R/R AML</td>
<td>R</td>
<td>NCT03533816</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3×10⁶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICT01+VEN/AZA</td>
<td>Vγ9Vδ2 T cell activator (anti-BTN3A(CD277)mAb)</td>
<td>II</td>
<td>–</td>
<td>Newly diagnosed AML</td>
<td>R</td>
<td>NCT04243499</td>
</tr>
<tr>
<td>γδ T cell infusion</td>
<td>Artificial APC-expanded donor γδ T cell infusion</td>
<td>I/ib</td>
<td>1×10⁶</td>
<td>R/R AML</td>
<td>R</td>
<td>NCT05015426</td>
</tr>
<tr>
<td>GDX012</td>
<td>Allogeneic γδ1 T cells</td>
<td>I</td>
<td>–</td>
<td>R/R AML</td>
<td>RYR</td>
<td>NCT05886491</td>
</tr>
<tr>
<td>LAVA-051*</td>
<td>CD1d-Vδ2TCR (Vγ9Vδ2 T cell) engager and iNKT activator (bispecific antibody)</td>
<td>I/ll</td>
<td>100µg</td>
<td>R/R CLL, MM, AML</td>
<td>ANR</td>
<td>NCT04887259</td>
</tr>
<tr>
<td>CTM-N2D</td>
<td>iPSC-derived γδ2 T cells (NKG2D CAR)</td>
<td>I</td>
<td>1×10⁷</td>
<td>Multiple cancers</td>
<td>NYR</td>
<td>NCT05302037</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5×10⁸</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3×10⁸</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1×10⁹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADI001</td>
<td>Allogeneic Vδ1 γδ T cells (CD20 CAR)</td>
<td>I</td>
<td>–</td>
<td>R/R B-cell NHL, DLBCL</td>
<td>R</td>
<td>NCT04735471</td>
</tr>
<tr>
<td>ADI925</td>
<td>Vδ1 T cells (chimeric adapter)</td>
<td>–</td>
<td>–</td>
<td>Multiple cancers</td>
<td>–</td>
<td>85</td>
</tr>
</tbody>
</table>

*Development has been discontinued.

AML, acute myeloid leukemia; ANR, active but not recruiting; APC, antigen-presenting cell(s); C, completed recruitment; CAR, chimeric antigen receptor; DLBCL, diffuse large B cell lymphoma; iPSC, induced pluripotent stem cells; mAb, monoclonal antibody; MRD, measurable residual disease; NCT, National Clinical Trial identifier; NHL, non-Hodgkin’s lymphoma; NYR, not yet recruiting; Ph, phase; R, recruiting; Rec, recruitment status; R/R, relapsed/refractory; VEN/AZA, venetoclax/azacytidine.
measurable residual disease-positive remission had higher than the median level of γδ T cells at 30 and 100 days after allo-HSCT and showed a significant improvement in 5-year LFS (HR=0.42, p=0.007 and HR=0.42, p=0.011, respectively) and OS (HR=0.44, p=0.038 and HR=0.33, p=0.009, respectively). Additionally, patients with a lower Vδ2 T cell concentration at 90 days after haploidential hematopoietic cell transplantation had significantly higher non-relapse mortality rates at 2 and 5 years (p=0.017). These data suggest that overall, γδ T cells exert their graft-vs-leukemia effect while mitigating acute GvHD post-allo-HSCT, leading to an improved prognosis in acute leukemia patients.

### γδ T cell-based adoptive cellular therapies

Robust γδ T cell expansion methods are critical to advance adoptive cellular therapies using these cells. Vγ9Vδ2 T cells can be extracted from peripheral blood and expanded ex vivo owing to the distinct characteristics of the γδ TCR. This has been capitalized to develop allogeneic γδ T cell products. While the peripheral blood accommodates approximately 1%–5% of the T cell population in the form of γδ T cells, umbilical cord blood, another potential source of γδ T cells, hosts less than 1%. As a result, the process of expansion of γδ T cells has been studied preferentially for peripheral blood over umbilical cord blood, despite diminished chances of GvHD in the latter. Healthy donor apheresis can be used as an allogeneic source of Vγ9Vδ2 T cells, with isolated lymphocytes subsequently enriched preferentially, ex vivo, using aminobisphosphonates (eg, zoledronic acid or pamidronate) and cytokine supplementation (with agents such as IL-2), followed by depletion of contaminants (eg, αβ T cells), using microbeads. Alternatively, synthetic pAgs, like 2-methyl-3-butenyl-1-pyrophosphate or bromohydrin pyrophosphate, in combination with IL-2, have been used in different cancers for the activation of Vδ2 T cells, including solid cancers and hematological malignancies. To maintain an optimal level of cytotoxicity, these cells require coculture with K562 feeder cells for 10–14 days. Activation-induced cell death is a potential efficacy-limiting phenomenon seen in Vγ9Vδ2 T cells after prolonged exposure to pAgs, resulting in the production of exhausted cells. Recent approaches have addressed the numerical expansion constraints by adding a second phase of expansion of γδ T cells using K562 feeder cell-derived artificial antigen-presenting cells, resulting in average pure-cell expansions as significant as >0.2 million-fold. Adoptive cell transfer has gained more relevance over in vivo activation of γδ T cells because of its functional superiority, which is due to decreased anergy and exhaustion. Allogeneic cell transfers are preferred in AML, despite the chances of allorejection, because of their cytotoxic dominance over their autologous counterparts, which are costly and time-consuming to harvest. Adaptive cells also outrank autologous cells in terms of fitness, exhaustion, and overall activity.

### γδ T cell-based therapies in AML

Adoptive cellular therapy, bispecific engagers, and activating antibodies to harness γδ T cells have been evaluated in AML. TCB-202-001, an allogeneic unmodified ex vivo-expanded Vγ9Vδ2 T cell therapy, was evaluated in a phase I study in patients with R/R AML. Three dose levels of 1×10^6, 1×10^7, and 1×10^8 cells/kg were evaluated using cells from matched or haploidential donors. Patients underwent lymphodepletion with 25 mg/m^2 fludarabine for 5 days and 500 mg/m^2 cyclophosphamide for 2 days. Among two patients who received a low dose of TCB-202-001, 1 achieved a morphological leukemia-free state and one had stable disease. Among three patients at a higher dose level, one achieved complete remission (CR), one reported progressive disease, and one showed a significant blast count reduction on the 14th day. Patients with CR, morphological leukemia-free state, and stable disease received repeat infusions, without lymphodepletion; no toxicities, such as acute GvHD, ICANS, or CRS, were noted. A phase II trial with TCB-008 is currently ongoing in Europe.

Another trial evaluated the feasibility of delivering INB-100, an ex vivo-expanded donor-derived γδ T cell therapy, to reduce the risk of relapse following allo-HSCT for R/R acute leukemias. Patients received reduced-intensity conditioning, followed by allo-HSCT from a haploidential donor and a single infusion of donor-derived γδ T cells within 5 days of neutrophil engraftment. Six patients received treatment at two dose levels of 1×10^6 cells/kg and 3×10^6 cells/kg. All patients had received one prior line of therapy except for one patient with ALL with prior exposure to chemotherapy and CAR-T cell therapy. Four patients developed grade 2 skin GvHD and three patients remained in remission after 1 year of follow-up. Several other clinical trials have evaluated adoptively transferred γδ T cells in solid tumors and multiple myeloma. While the results from these trials were underwhelming, it is noteworthy that they were conducted in an era when there was only a rudimentary understanding of the biology of γδ T cells.

More recently, novel activating antibodies and bispecific engagers have been evaluated to harness γδ T cells in cancer. ICT01 is a first-in-class antibody that binds to BTN3A (CD277) to activate the Vγ9Vδ2 T cell-mediated antitumor response. The ongoing EVICTION trial has shown robust pharmacodynamic effects, with rapid trafficking of activated Vγ9Vδ2 T cells into the tumor microenvironment and sustained partial responses in different solid tumors, including ipilimumab/nivolumab refractory melanoma (NCT04243499). Azacytidine and venetoclax have been shown to potentiate the activity of ICT01, warranting evaluation in AML. LAVA-051 is a humanized bispecific antibody that engages with CD1d and the V82 TCR of the Vγ9Vδ2 T cells to mediate the killing of CD1d-positive tumor cells. Early results from the ongoing dose escalation study demonstrated pharmacodynamic effects in terms of the activation of Vγ9Vδ2 T cells and invariant NK T cells, with no increase in...
CRS-related cytokines, with predominantly grade 1/2 adverse events. The findings of relevant clinical trials have been summarized in table 2.

Future directions

Although the existing information provides a promising insight into a potentially successful immunotherpay for AML, a vast realm remains to be explored. Further clarity may be attained when the allogenic products in phase I/II clinical trials progress to phase III. Therapies may be developed that are modified on the basis of engineered γδ T cells, enabling selective antitumor effects through gating mechanisms. Presently, clinical trials are primarily being conducted in R/R AML patients who may be shifted to diverse cohorts, including patients with early-stage AML. The reconstitution and persistence of γδ T cells after transplantation must be a focus, as should enhancement of the persistence of terminally differentiated memory γδ T cells, owing to their strong cytotoxic potential (online supplemental figure S1). Studies on counteracting immune evasion by AML blasts and the mechanism of invigorating exhausted γδ T cells could play a pivotal role in the therapeutic landscape of AML. In addition, the methods for enhancing the homing mechanism of γδ T cells to the leukemia microenvironment and the exploration of the antileukemic properties of bone marrow-resident Vδ1 T cells could strengthen the immuno-therapies and potentially broaden their application to patients with early-stage AML. γδ T cells have unlocked new doors for AML immunotherapy; however, further research and investigation remain to be conducted before patients can benefit in the form of a large-scale off-the-shelf therapy.

CONCLUSIONS

γδ T cells have emerged as a new modality of adoptive cellular therapy across cancers, particularly for R/R AML. Their unique biological characteristics, including a lack of alloreactivity, a lower probability of CRS and ICANS, and the capacity to influence the host immune microenvironment make them highly attractive candidates for off-the-shelf cellular therapy approaches. Reconstitution of these cells after allo-HSCT may be suggestive of a favorable importance in AML patients. Susceptibility to functional exhaustion and relatively low persistence still remain the Achilles heel of γδ T cells. Novel advances in expansion methods have led to various modified and unmodified γδ T cell therapies to advance into clinical trials.

Acknowledgements

AM is supported by the Leukemia SPORE and the Gateway For Cancer Research. This work was supported in part by the MD Anderson Cancer Center Support grant P30CA016672 from the National Cancer Institute and the Research Project Grant Program (R01CA235622) from the National Institutes of Health. Editing Services were provided by the Research Medical Library, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Contributors

Conception and design: AM, AR and AA. Administrative support: AM. Provision of study materials or patients: not applicable. Collection and assembly of data: AR, AA and AM. Data analysis and interpretation: AR, AA and AM. Manuscript writing: AR, AA and AM. Critical revision for important intellectual content: all authors. Final approval of manuscript: all authors reviewed and approved the final version of the manuscript.

Funding

AM is supported by the Leukemia SPORE, Gateway for Cancer Research. This study was supported in part by the MD Anderson Cancer Center Support Grant CA016672 from the National Cancer Institute and the Research Project Grant Program (R01CA235622) from the National Institutes of Health.

Competing interests

AR and AA: None. AM: Reports research funding to the institution from Chimeric Therapeutics, LinBio Sciences, Celgene, Sanofi-Aventis, CytoMed Therapeutics, BioSight, IGM Biosciences, Electra Therapeutics, Astex Pharmaceuticals. Other: Cero Therapeutics.

Patient consent for publication

Not applicable.

Provenance and peer review

Commissioned; externally peer reviewed.

Supplemental material

This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access

This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iDs

Adishwar Rao http://orcid.org/0000-0003-1744-4217
Akriti Agrawal http://orcid.org/0000-0001-7372-7081
Venkata Lokesh Battula http://orcid.org/0000-0001-5415-9058

REFERENCES


Open access


65 TC Biopharm. Safety and efficacy of ex-vivo expanded allogeneic γδ T-lymphocytes (Ommunieve®) in patients with active relapsed or refractory acute myeloid leukemia (AML) who are not eligible for or do not consent to high dose salvage chemotherapy and/or allogeneic haematopoietic cell transplantation (HCT). A dose escalation, open-label, phase I study. 2021. Available: https://clinicaltrials.gov/ct2/show/NCT03790072


67 INNbio, Inc. INNbio announces new data at ASH showing 100 percent of cohort 1 patients maintained durable complete response in ongoing phase 1 trial of INB-100; n.d.


81 LAVA Therapeutics. LAVA therapeutics announces updated data from the phase 1/2A clinical trial of LAVA-051 at the 64th American society of hematology (ASH) annual meeting and exposition. n.d. Available: https://ir.lavatherapeutics.com/node/7506/pdf

82 CytoMed Therapeutics Pte Ltd. A phase I trial to evaluate allogeneic NKG2Dl-targeting chimeric antigen receptor-grafted γδ T cells (CTM-NZ0) in subjects with advanced solid tumours or haematological malignancies. 2022. Available: https://clinicaltrials.gov/study/NCT05302037

83 Adicet Bio, Inc. Evaluation of non-gene edited allogeneic “off-the-shelf” Vδ1 V8 δ9 CAR T cells targeting CD20 for B cell malignancies. n.d. Available: https://investor.adicetbio.com/static-files/2d972c43-47c7-4e9a-a11c-38f0c27c6552


**Figure S1. Memory phenotypes of γδ T cells.** The different memory states of γδ T cells can be explained on the basis of the expression of cellular markers, such as CD45RA and CD27, and lymph node homing receptors, such as CD62L and CCR7.

<table>
<thead>
<tr>
<th>Memory phenotype</th>
<th>Naive (γδ T&lt;sub&gt;naive&lt;/sub&gt;)</th>
<th>Central memory (γδ T&lt;sub&gt;cm&lt;/sub&gt;)</th>
<th>Effector memory (γδ T&lt;sub&gt;em&lt;/sub&gt;)</th>
<th>Terminally differentiated memory (γδ T&lt;sub&gt;emra&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potential for proliferation</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>(pAg stimulated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effector functions</td>
<td>(Cytokine release)</td>
<td>(Cytokine release)</td>
<td>(Cytokine release)</td>
<td>(Cytokine release)</td>
</tr>
<tr>
<td>(Predominant function)</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

CD, cluster of differentiation, CCR, c-c chemokine receptor, CM, central memory, EM, effector memory, EMRA, terminally differentiated effector memory cells re-expressing CD45RA, pAg, phosphoantigen.