Adoptive cellular therapy in acute myeloid leukemia: Current scope and challenges

Sankalp Arora
Internal Medicine Residency Program, University of Alabama at Birmingham, Birmingham, Alabama, USA

Palash Asawa
Internal Medicine Residency Program, Allegheny Health Network, Pittsburgh, Pennsylvania, USA

Aravind Ramakrishnan
Sarah Cannon Transplant and Cellular Therapy Program at St. David's Austin Medical Center, Austin, Texas, USA

Carlos Bachier
Sarah Cannon Transplant and Cellular Therapy Program at Methodist Hospital, San Antonio, Texas, USA

Navneet S Majhail
Sarah Cannon Transplant and Cellular Therapy Program at TriStar Centennial, Nashville, Tennessee, USA, navneet.majhail@sarahcannon.com

Follow this and additional works at: https://www.hosct.org/hematology-oncology-and-stem-cell-therapy

Part of the Cancer Biology Commons, Hematology Commons, and the Oncology Commons

Recommended Citation
Arora, Sankalp; Asawa, Palash; Ramakrishnan, Aravind; Bachier, Carlos; and Majhail, Navneet S (2022) "Adoptive cellular therapy in acute myeloid leukemia: Current scope and challenges," Hematology/Oncology and Stem Cell Therapy Vol. 15 : Iss. 3 , Article 12.
Available at: https://doi.org/10.56875/2589-0646.1060

This Review Article is brought to you for free and open access by Hematology/Oncology and Stem Cell Therapy. It has been accepted for inclusion in Hematology/Oncology and Stem Cell Therapy by an authorized editor of Hematology/Oncology and Stem Cell Therapy.
Adoptive Cellular Therapy in Acute Myeloid Leukemia: Current Scope and Challenges

Sankalp Arora a, Palash Asawa b, Aravind Ramakrishnan c, Carlos Bachier d, Navneet S. Majhail e,*

a Internal Medicine Residency Program, University of Alabama at Birmingham, Birmingham, AL, USA
b Internal Medicine Residency Program, Allegheny Health Network, Pittsburgh, PA, USA
c Sarah Cannon Transplant and Cellular Therapy Program at St. David’s Austin Medical Center, Austin, TX, USA
d Sarah Cannon Transplant and Cellular Therapy Program at Methodist Hospital, San Antonio, TX, USA
e Sarah Cannon Transplant and Cellular Therapy Program at TriStar Centennial, Nashville, TN, USA

Abstract

Adoptive cellular therapies have revolutionized the management of hematologic malignancies, particularly lymphoma and multiple myeloma. These therapies targeting disease-specific antigens, such as CD19 in lymphoma and B cell maturation antigen in multiple myeloma, are efficacious and well-tolerated compared with conventional chemotherapies. Unfortunately, their potential remains unrealized in acute myeloid leukemia (AML). This is because most targetable antigens on AML cells are also expressed on healthy myeloid hematopoietic stem cells (HSC). Therefore, targeting them results in severe myeloablative effects and pancytopenia. Several strategies have been devised to overcome this barrier, including identifying AML-specific antigens, limiting CAR-T cell persistence to prevent prolonged myeloablation, and creating AML-specific antigens through manipulating HSCs prior to allogenic transplant. In this review, we discuss these strategies and the ongoing clinical trials on adoptive cellular therapies in AML, limiting our focus to chimeric antigen receptor-T cells (CAR-T) and chimeric antigen receptor-natural killer cells (CAR-NK).

Keywords: Acute myeloid leukemia, Chimeric antigen receptor, Cellular therapy, Adoptive therapy, CAR-T cells, CAR-NK cells

1. Introduction

Adoptive cellular therapy, particularly chimeric antigen receptor-T cells (CAR-T), has revolutionized the management of hematologic malignancies, particularly lymphoma, acute lymphoblastic leukemia (ALL), and multiple myeloma (MM) [1,2]. Currently, six CAR-T products are approved by the United States Food and Drug Administration (FDA). These include CD-19-directed axicabtagene ciloleucel, tisagenlecleucel, and lisocabtagene maraleucel for relapsed/refractory MM [7,8]. The success and promise of CAR-T cell therapy have not been realized in acute myeloid leukemia (AML), and currently, there are no FDA-approved products for the disease.

Apart from CAR-T, adoptive cellular therapy has expanded over the years to include several other constructs that are currently being evaluated in hematologic and solid malignancies (for example, chimeric antigen receptor-natural killer cells [CAR-NK], tumor-infiltrating lymphocyte therapy [TIL], tumor-activated T cells and T cell receptor [TCR]) [9]. CAR-NK cells may have potential advantages over CAR-T cell therapy, including reduced toxicity, reduced incidence of cytokine release syndrome (CRS), neurotoxicity, low risk of graft-versus-host disease in the allogenic setting, and possible “off-the-shelf” use [10]. While there are no FDA-
approved CAR-NK therapies, several products are being investigated in hematologic malignancies, including AML [11].

We aim to review the current scope of adoptive cellular therapy in AML, including the challenges in their development and clinical trials evaluating their use. For the scope of this article, we will be limiting our focus to CAR-T and CAR-NK cell therapies.

2. Approaches to adoptive T cell therapy in AML

In the context of AML, an ideal target for adoptive cell therapy is expressed on the blast cell surface, including leukemia stem cells, and is absent from normal hematopoietic cells. It has been challenging to identify such targets in AML since the commonly expressed antigens are also present on normal hematopoietic stem cells (HSCs), including CD33, CD34, and CD123 [12]. Hence, CAR-T/NK cells targeting these antigens are unable to differentiate between cancer and normal cells. Studies have shown prolonged severe myelosuppression and transfusion dependence with their use. [13–15], resulting in potentially fatal bleeding, neutropenia, and opportunistic infections. Several approaches to address these challenges are undergoing investigation.

2.1. Identifying appropriate AML-specific antigens

When compared with solid tumors, AML has one of the lowest mutational burdens. Hence, it has lesser neoantigens that could act as targets for adoptive T cell therapy. In addition, given the heterogeneous nature of the disease, there is no single protein target expressed by all AML subtypes [16]. Several methods have attempted to identify AML-specific antigens and neoantigens to overcome the challenges associated with prolonged myeloablation. Currently, several AML-specific targets are being utilized in clinical trials (Table 1).

2.1.1. Cell-surface targets

CD7, a transmembrane protein expressed in about 30% of adult AML patients, is typically seen in activated T cells but is absent on normal myeloid and erythroid cells. CD7-deficient mice retain normal T cell function, making its function possibly redundant. When present in AML, it is associated with chemotherapy resistance and a more aggressive disease course. Preclinical data have shown that CD7 CAR-T cells provide protection against severe leukemia in xenograft models, and clinical trials using CD7 CAR-T products are in progress [17,18].

CD33 is sialic acid-binding immunoglobulin primarily expressed in cells of the myeloid lineage, including myeloid progenitor cells, and in around 85–90% of AML cases, making it a potential target for CAR-T cell therapy. A phase I trial to assess the safety and efficacy of CAR-T-33 cells showed marked disease regression in a 41-year-old with relapsed/refractory (r/r) AML but with early disease relapse [19]. Another single-center phase I clinical trial assessing the safety and feasibility of CD33 CAR-T cells in r/r AML enrolled ten patients, three of whom eventually received the CAR-T cells. Disease response was not achieved with CD33 CAR-T in any of the three patients. Cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), tumor lysis syndrome, respiratory distress syndrome, and septic shock were some of the adverse effects noted with CD33 CAR-T in the trial [20]. Fig. 2 describes some ongoing clinical trials using CD33 CAR-T cells.

CD123, also called IL-3 receptor subunit alpha, is seen in 70–80% of patients with AML and is primarily expressed on myeloid lineage cells. Anti-CD123 CAR-T cells can successfully ablate leukemic blasts in preclinical studies. The first-in-human clinical trial of CD123 CAR-T cell therapy enrolled six patients with refractory AML following allo-HSCT therapy. Three out of six patients had complete remission, and no myeloablative effects were

Table 1. Investigational AML-specific target antigens for CAR-T/NK cell therapy.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Cellular location of expression</th>
<th>Expression in AML</th>
<th>Expression in HSCs</th>
<th>Expression in other non-leukemic healthy cells</th>
<th>Use in clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD7 [17,18]</td>
<td>Transmembrane</td>
<td>Yes, 30% of cases</td>
<td>Yes (T cell precursors); Absent in erythroid or myeloid cells</td>
<td>Mature T cells/NK cells</td>
<td>CAR-T (NCT04762485)</td>
</tr>
<tr>
<td>CD44v6 [24]</td>
<td>Surface</td>
<td>Yes, 60% of cases</td>
<td>No</td>
<td>Keratinocytes, monocytes, activated T cells</td>
<td>CAR-T (NCT04097301)</td>
</tr>
<tr>
<td>FLT3 [26,27]</td>
<td>Transmembrane</td>
<td>Yes</td>
<td>Yes</td>
<td>Dendritic cells, NK cells</td>
<td>CAR-T (NCT05023707)</td>
</tr>
<tr>
<td>NKG2D ligand</td>
<td>Surface</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td>CAR-T [30]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CAR-NK (NCT05247957)</td>
</tr>
</tbody>
</table>
observed [21]. Currently, several clinical trials with CAR-T targeting CD123 are ongoing (Fig. 2).

CLL-1, also known as C-type-lectin-like molecule 1, is expressed on >80% of AML blasts. It has become a popular target for CAR cells as it is notably absent on granulocyte progenitors and non-hematologic tissues. Several preclinical studies have established the anti-leukemic activity of CLL-1-directed CARs without destroying normal HSCs. A case report by Zhang et al. showed morphological, immunophenotypic, and molecular complete remission for over ten months in a patient with AML treated with anti-CLL1 CAR-T therapy [22]. Moreover, in a phase I/II anti-CLL1 CAR-T cell therapy trial that enrolled four pediatric patients with r/r AML, three patients achieved complete remission with only low-grade adverse events [23]. Several trials assessing the utility of anti-CLL-1 CAR clinical trials are ongoing (Fig. 2).

Another potential target is CD44v6, which is present in up to 60% of AML cases. CD44 is an adhesive receptor expressed on multiple tissue types, but its splice variant CD44v6 is relatively tumor restricted. Casucci et al. showed that T cells targeting CD44v6 with a newly designed CAR-T cell therapy mediated a potent anti-leukemia effect without harming HSCs in xenograft mouse models [24]. A clinical trial using CAR-T cells targeting CD44v6 in relapsed refractory AML and MM was initiated in Italy but terminated early, without enrollment of any AML patients (NCT04097301).

Fms-related tyrosine kinase 3 (FLT3) is a gene that encodes a transmembrane tyrosine kinase receptor, which helps in hematopoiesis and regulates the survival of normal HSCs [25]. FLT3-ITD and FLT3-TKD mutations are associated with poor prognosis in AML. Agents like giltertinib, quizartinib, and midostaurin are FDA approved for FLT3 mutated AML and MM was initiated in Italy but terminated early, without enrollment of any AML patients (NCT04097301).

The Natural Killer Group 2 member D (NKG2D) ligand on tumor cells and provides a costimulatory signal to T cells. However, it relies on antigen recognition via the T cell receptor (TCR) to deliver the primary activating signal. In contrast, NKG2D ligand recognition through the NKG2D-CAR-T cells mediates primary T cell activation. A phase 1 clinical trial by Baumeister et al. in patients with AML/Myelodysplastic Syndrome (MDS)/Multiple Myeloma (MM) using NKG2D CAR-T cells without lymphodepleting chemotherapy found limited expansion and persistence of CAR-T cells but did detect functional activity. No objective anti-tumor responses were seen with a single dose; however, no dose-limiting toxicity occurred [30]. A preclinical study by Driouk et al. demonstrated increased NKG2D-ligand expression in AML cells after exposure to histone deacetylase (HDAC) inhibitors. This effect was not noted on healthy cell lines. Furthermore, this HDAC-mediated upregulation in NKG2D-ligand expression translated to improved anti-leukemic efficacy of NKG2D CAR-T cells in vitro, thus providing a rationale for combining HDAC inhibitors with NKG2D-CAR-T cell therapy in the treatment of AML [31]. Efforts to utilize NKG2D ligand as a target by CAR-T cells are ongoing in multiple clinical trials (Fig. 2).

2.1.2. Intracellular targets

A comprehensive analysis of the Cancer Genome Atlas Research Network identified several immunogenic AML-associated neoantigens, such as mutations of enzymes IDH1, IDH2, and NPM1 [32]. Proteins associated with these mutations are expressed intracellularly and inaccessible to conventional CAR-T/NK cell therapies typically directed towards surface antigens. To overcome this problem, an innovative approach of gene-transduced tumor-specific T cell receptor (TCR) T cells is being studied in multiple clinical trials. In contrast to CAR-T cells that target naturally occurring antigens, TCR T cells are engineered to express receptors specific to certain tumor-associated antigens formed from a complex of a tumor peptide and an MHC molecule. For instance, a study by Chapuis et al. investigated the prophylactic use of TCR T cells directed against Wilms tumor 1 (WT1) antigen as a novel mechanism to reduce relapse risk after hematopoietic cell transplantation in AML patients. Twelve patients received WT-1-directed T cells and reported 100% relapse-free survival at the median of 44 months, which was significantly higher compared with the 54% rate in the comparative group [33]. Several preclinical studies have suggested the possible role of shared neoantigens (mutated antigens common amongst different
cancer patients but not expressed in the normal genome, such as Nucleophosmin1 (NPM1) mutations, core-binding factor-β, and myosin heavy chain 11 (CBFB-MYH11), in TCR-based immunotherapy of AML [34,35].

Another target for TCR-based immunotherapy in AML patients who experience disease relapse post allogeneic hematopoietic cell transplantation is the minor histocompatibility (H) antigen HA1, which is hematopoietic restricted and expressed in leukemia cells [36]. The rationale behind this approach is that TCR-transduced lymphocytes will target HA1 present on leukemic cells but not donor HSCs or non-hematopoietic host cells, thus having a selective anti-leukemia effect without causing graft-vs-host disease. A phase 1 trial using this approach in multiple hematologic malignancies, including AML, is currently active (NCT03326921).

2.2. Limiting CAR-T cell persistence to prevent prolonged myeloablation

Another potential approach to reduce the risk of protracted bone marrow ablation is limiting the long-term in vivo persistence of non-leukemic specific antigen-directed CARs. We will discuss various approaches that are under investigation to address this below.

One possible method is to alter the costimulatory domain in the CAR-T cell constructs, such as using CD28 instead of the 4-1BB signaling domain, as this may result in shorter CAR-T cell persistence [37,38]. Another approach to limit CAR-T cell persistence is utilizing mRNA electroporation instead of viral transduction to incorporate CAR into T cells, as degradation of mRNA results in CAR-T cell death. A study by Cummins et al. using RNA CD123 CAR-T cells in relapsed/refractory AML showed a good safety profile; however, no objective anti-tumor response was observed. Several drawbacks, such as poor persistence, longer manufacturing time, and lower CAR-T cell viability, have limited the clinical utility of this approach [39].

Incorporating a “safety switch” in CAR-T cells is a strategy assessed in both preclinical and clinical studies that can eliminate T cells if needed. The HSV-thymidine kinase suicide gene has been investigated as a safety switch that resulted in the depletion of cells by converting a prodrug into a toxic compound that halts DNA replication and results in cell death [40]. Unfortunately, this approach is not without limitations. The long latency before activation limits its utility in acute toxicity requiring immediate termination. This approach also makes ganciclovir unusable in a patient population at high risk of life-threatening viral infections [41].

More recently, an inducible caspase 9 (iCasp9₇₉) has been developed to co-express in CAR-T cells as a safety switch, consisting of a modified human caspase 9 fused to a human FK506 binding protein (FKBP) [42]. Exposure to a small-molecule pharmacological results in conditional dimerization and activation of iCasp9₇₉ that induces apoptosis of transduced T cells both in vitro and in vivo. Compared with other suicide gene approaches, this system has the advantages of low potential for immunogenicity, selective elimination of transduced T cells, and viable function in T cells over-expressing antiapoptotic molecules. In a population of five pediatric patients who developed GVHD after receiving CD19 CAR-T cells for rr ALL, a single dose of AP1903 eliminated >90% of T cells within 30 minutes [43]. The iCasp9₇₉ suicide system has been incorporated in the CAR construct of several trials (NCT02992210 and 01822652) [44]. Engineering T cells to co-express a well-characterized surface antigen that are targets for clinically approved monoclonal antibodies is another option for a safety switch. Examples include truncated EGFR/cetuximab and CD20/rituximab or depleting CAR-T cells using targetable endogenous receptors, such as CD52 with alemtuzumab [45,46].

While suicide gene systems may be useful in limiting toxicities from the persistence of CAR-T cells, they may also result in an increased risk of disease recurrence. To overcome this potential limitation, controllable CAR-T cells whose function can be reversed is another possible strategy to limit toxicity [47]. Tetracycline (Tet)-ON/Tet-OFF inducible CAR19-T cells responsive to tetracycline have been developed for B cell lymphomas in vitro. These systems can be reactivated in the setting of declining response or relapse if within the life span of CAR-T cells [48].

2.3. Creation of AML-specific antigens through genetic manipulation of HSCs before allogeneic hematopoietic cell transplantation

Kim et al. suggested a novel approach to create AML-specific antigens by editing out the CD33 antigen from normal HSCs prior to allogeneic HCT. Post-engraftment treatment with CD33 CAR-T cells engineered from donor cells targeted CD33 positive AML blasts and stem cells and spared HSCs lacking this antigen. This would ensure normal hematopoiesis despite the persistence of CAR-T cells. This model showed promising results in preclinical studies [14]. Currently, a phase I/II study is in
<table>
<thead>
<tr>
<th>Clinical trial No.</th>
<th>Status</th>
<th>Location</th>
<th>AML type</th>
<th>Study type and Age group</th>
<th>Intervention</th>
<th>Outcomes</th>
</tr>
</thead>
</table>
| NCT04884984       | Recruiting        | The First Affiliated Hospital of Soochow University, China                | CLL1 + r/r AML | Single arm, single-center                 | Anti-CLL1 CAR-T                  | Primary: Treatment-related adverse events  
Secondary: 2-year ORR, OS, EFS, CIR  
Primary: Treatment-related adverse effects up to 12 months after infusion  
Secondary: Up to 1 year ORR, PFS, OS, change of CAR copies, and CAR-T cell counts |
| NCT04923919       | Recruiting        | 920th Hospital of Joint Logistics Support Force of People's Liberation Army of China | CLL1 + r/r AML | Single arm Early phase 1                  | Anti-CLL1 CAR-T                  | Primary: Treatment-related adverse events up to 12 months after infusion  
Secondary: Up to 1 year ORR, PFS, OS, change of CAR copies, and CAR-T cell counts |
| NCT03631576       | Unknown           | Fujian Medical University, China                                          | r/r AML        | Single arm, phase 2,3 Age: <70 years      | Anti-CD123/CLL1 CAR-T            | Primary: 1-year LFS  
Secondary: TRAE at 1 year |
| NCT03114670       | Unknown           | Affiliated Hospital to Academy of Military Medical Sciences, China       | CD123+ AML relapsed post allogenic stem cell transplant | Single arm, Phase 1              | Anti-CD123 CAR-T                  | Primary: Incidence of TRAEs  
Secondary: CAR-T cell persistence in vivo, CAR-T specific antibody level, OS and disease response  
Primary: TRAEs, CAR-T-manufacturing feasibility  
Secondary: Day 28 ORR, reduction in peripheral blood and marrow blast count, OS, PFS, DOR, need for rescue alloSCT  
Primary: Severity and frequency of adverse effects, Percentage of manufacturing products that do not meet release criteria  
Secondary: OS, PFS, DOR, need for rescue alloSCT  
Primary: Tumor load (upto 12 months) |
| NCT04678336       | Recruiting        | University of Pennsylvania, USA                                          | r/r AML        | Single group phase 1 Age: 1–29 years      | Anti CD123 CAR-T                 | Primary: Treatment-related adverse events  
Secondary: 2-year ORR, OS, EFS, CIR  
Primary: Treatment-related adverse effects up to 12 months after infusion  
Secondary: Up to 1 year ORR, PFS, OS, change of CAR copies, and CAR-T cell counts |
| NCT03766126       | Active, not recruiting | University of Pennsylvania, USA                                          | r/r AML        | Single group phase 1 Age: ≥ 18 years      | Anti CD123 CAR-T                 | Primary: Treatment-related adverse events  
Secondary: 2-year ORR, OS, EFS, CIR  
Primary: Treatment-related adverse effects up to 12 months after infusion  
Secondary: Up to 1 year ORR, PFS, OS, change of CAR copies, and CAR-T cell counts |
| NCT03796390       | Unknown           | Hebei Yanda Ludaopei Hospital, China                                     | r/r AML        | Single group phase 1 Age: 2–65 years      | Anti CD123 CAR-T                 | Primary: Treatment-related adverse events  
Secondary: 2-year ORR, OS, EFS, CIR  
Primary: Treatment-related adverse effects up to 12 months after infusion  
Secondary: Up to 1 year ORR, PFS, OS, change of CAR copies, and CAR-T cell counts |

(continued on next page)
<table>
<thead>
<tr>
<th>Clinical trial No.</th>
<th>Status</th>
<th>Location</th>
<th>AML type</th>
<th>Study type and Age group</th>
<th>Intervention</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT03971799</td>
<td>Recruiting</td>
<td>Center for International Blood and Marrow</td>
<td>CD33+ r/r AML</td>
<td>Single arm, multi-center phase 1/2</td>
<td>Anti-CD33 CAR-T</td>
<td>Primary: Maximum tolerated dose, rates of morphologic remission at day 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transplant Research, USA</td>
<td></td>
<td>Age: 1–35 years</td>
<td></td>
<td>Secondary: 1 year OS, PFS, Treatment-related mortality</td>
</tr>
<tr>
<td>NCT05008575</td>
<td>Recruiting</td>
<td>Xinqiao Hospital, China</td>
<td>r/r AML</td>
<td>Single group phase 1</td>
<td>Anti-CD33 CAR-NK cells</td>
<td>Primary: Incidence of ORR, DLT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Age: 18–70 years</td>
<td></td>
<td>Secondary: PFS, ORR, OS</td>
</tr>
<tr>
<td>NCT02944162</td>
<td>Unknown</td>
<td>Hefei Binhu Hospital, China</td>
<td>r/r AML</td>
<td>Single group phase 1/2</td>
<td>Anti-CD33 CAR-NK cells</td>
<td>Primary: TRAE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Age: 3–80 years</td>
<td>Anti CD38 CAR-NK cells</td>
<td>Secondary: ORR</td>
</tr>
<tr>
<td>NCT01864902</td>
<td>Unknown</td>
<td>Chinese PLA General Hospital</td>
<td>CD33 + r/r AML</td>
<td>Single arm, phase 2/3</td>
<td>Anti CD33 CAR-T</td>
<td>Primary: TRAEs until week 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Age: 5–90 years</td>
<td>Anti CD7 CAR-T</td>
<td>Secondary: Anti-leukemic Responses to CAR-T upto 24 weeks, in vivo existence of CAR-T at 1 year</td>
</tr>
<tr>
<td>NCT05215015</td>
<td>Recruiting</td>
<td>Wuxi People’s Hospital, China</td>
<td>r/r AML</td>
<td>Single group early phase 1</td>
<td>Anti-CD33/ CLL1CAR-NK cells</td>
<td>Primary: Incidence of DLT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Age: 18–75 years</td>
<td>Anti CD19 CAR-T</td>
<td>Secondary: OS, ORR, DOR, MRD</td>
</tr>
<tr>
<td>NCT03896854</td>
<td>Unknown</td>
<td>The First Affiliated Hospital of Soochow</td>
<td>CD19 + r/r AML</td>
<td>Single group phase 1/2</td>
<td>Anti CD38 CAR-T</td>
<td>Primary: Number of adverse events in 12 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>University, China</td>
<td></td>
<td>Age: 6–65 years</td>
<td></td>
<td>Secondary: 2-year ORR, EFS and CIR</td>
</tr>
<tr>
<td>NCT04351022</td>
<td>Recruiting</td>
<td>The First Affiliated Hospital of Soochow</td>
<td>CD38+ r/r AML</td>
<td>Single group phase 1/2</td>
<td>Anti CD7 CAR-T</td>
<td>Primary: Number of adverse events</td>
</tr>
<tr>
<td></td>
<td></td>
<td>University, China</td>
<td></td>
<td>Age: 6–65 years</td>
<td></td>
<td>Secondary: ORR, CIR, persistence of CAR-T cells in vivo</td>
</tr>
<tr>
<td>NCT04762485</td>
<td>Recruiting</td>
<td>The First Affiliated Hospital of Soochow</td>
<td>r/r CD7+ Acute</td>
<td>Single group phase 1/2</td>
<td>Anti CD276 CAR-T</td>
<td>Primary: 3-month ORR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>University, China</td>
<td>leukemia</td>
<td>Age: 12–65 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT04692948</td>
<td>Recruiting</td>
<td>Anhui provincial hospital, China</td>
<td>r/r AML</td>
<td>Single group</td>
<td>Anti CD276 CAR-T</td>
<td>Primary: Incidence of DLT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hebei Yanda Lu Daopei Hospital, China</td>
<td></td>
<td>Age: 18–70</td>
<td>NKG2DL-specific CAR-NK cells</td>
<td>Maximal tolerable dose</td>
</tr>
<tr>
<td>NCT05247957</td>
<td>Recruiting</td>
<td>Hebei Yanda Lu Daopei Hospital, China</td>
<td>r/r AML</td>
<td>Single group phase 1</td>
<td>NKG2DL-specific CAR-NK cells</td>
<td>Secondary: LFS</td>
</tr>
</tbody>
</table>
progress evaluating the safety of VOR33, an allelo-
geneic CRISPR/Cas9 genome-edited HSC lacking
CD33 followed by gemtuzumab ozogamicin, in
AML patients at high risk for relapse after HCT
(NCT04849910). Editing other antigens, such as
CD123 and CLL-1, from HSCs prior to transplant is
also being studied. A preclinical evaluation of
engineered HSPCs with ablated CD123 or CLL-1
proteins obtained from healthy donors’ peripheral
blood showed successful functional hematopoiesis
resistant to CD123 or CLL-1 targeted therapies,
providing a next-generation HCT option that would
facilitate safe and effective use of antigen-directed
immunotherapy treatments for AML [49].

2.4. Mitigating downregulation of target antigens
and antigen escape

Similar to the irreversible loss of CD19 as a
mechanism for relapse after CAR-T cell therapy in
relapsed/refractory B-ALL [50], there is a concern
that downregulation of target antigen or antigen
escape could reduce the efficacy of CAR-T/NK cell
therapies for AML. Several potential approaches to
overcome this issue are under investigation,
including creating adoptive cell therapy against
multiple antigens and methods to upregulate the
target antigens.

Targeting a single antigen may be insufficient for
CAR-T cell treatment, and creating tandem CARs
that recognize two epitopes can circumvent this
concern. A preclinical study assessing the efficacy of
CD123b-CD33b CCAR composed of anti-CD123 and
anti-CD33 CAR units showed ablation of leukemic
cells expressing CD123, CD33b, or both ex vivo in
AML cells and in vivo in xenograft models [15].
Similarly, CLL1-CD33 compound CAR-T cells have
shown promise in eliminating AML cells in pre-
clinical studies and a phase I dose escalation trial
[51]. Clinical trials assessing the utility of compound
CAR-T cells are currently ongoing.

Another potential method to overcome AML
target antigen downregulation is using therapies
that upregulate these antigens. A study by Li et al.
showed that decitabine increases CD19 expression
in lymphoma cells, and cells pretreated with deci-
tabine responded better to CAR-T cells targeting
CD19. This method succeeded in two patients with
relapsed/refractory B cell lymphoma who achieved
complete remission [52]. This method is yet to be
applied to AML CARs. Similarly, a study by Kha-
wanky et al. showed that CD123 expression in-
creases in leukemia cells on exposure to
hypomethylating agents, such as azacitidine, in
AML xenograft models [53]. Another agent known

---

**Table: Clinical Trials**

<table>
<thead>
<tr>
<th>Trial ID</th>
<th>Status</th>
<th>Phase</th>
<th>Age</th>
<th>Prognosis</th>
<th>Primary:</th>
<th>Secondary:</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT05092451</td>
<td>Not yet recruiting</td>
<td>Single group phase 1/2</td>
<td>18–80 years</td>
<td>r/r AML</td>
<td>CD70 + r/r AML</td>
<td>TRAE, Treatment-related adverse effects</td>
</tr>
<tr>
<td>NCT05023707</td>
<td>Recruiting</td>
<td>Single arm phase 1, phase 2</td>
<td>Single arm phase 1</td>
<td>r/r AML</td>
<td>FLT3 + r/r AML</td>
<td>OS: Overall survival; EFS: Event-free survival; TRAE: Treatment-related adverse events; CR: Cumulative incidence of relapse; LFS: Leukemia-free survival; DOR: Duration of response; alloSCT: Allogeneic stem cell transplant; DLT: Dose-limiting toxicity.</td>
</tr>
</tbody>
</table>

**Abbreviations:** ORR: Objective response rate; OS: Overall survival; EFS: Event-free survival; TRAE: Treatment-related adverse effects; CR: Cumulative incidence of relapse; LFS: Leukemia-free survival; DOR: Duration of response; alloSCT: Allogeneic stem cell transplant; DLT: Dose-limiting toxicity.
to increase the expression of leukemia antigens is all-trans-retinoic acid (ATRA). It increases the expression of CD38 in AML cells, and a study by Yoshida et al. assessing the enhancement of CD38 expression of AML cells using ATRA showed increased efficacy of anti-CD38-CAR in vitro, suggesting the potential utility of this modality [54].

3. Clinical trials using adoptive cellular therapy

Table 2 summarizes the currently active clinical trials using experimental CAR-T and CAR-NK therapies in treating AML. The target antigens include CD33, CD38, CLL1, FLT3, CD123, CD19, CD70, and CD276. Most of these are in phase 1–2, with the primary aim of identifying treatment-related toxicities.

4. Conclusion

Adoptive cellular therapy in AML continues to face several challenges, especially treatment-related toxicity. However, with several strategies in clinical trials, there is certainly a promise of its potential for AML treatment being realized soon.

Author contributions

SA, PA and NM wrote the initial draft of the manuscript. AR, CB and NM reviewed and suggested changes to the initial draft. All authors read and approved the final version of the manuscript.

Financial support

No financial support was utilized in the preparation of this manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

References


[33] Li H, Zhao Y. Increasing the safety and efficacy of chimeric antigen receptor T cell therapy. Protein Cell 2017;8(8):573–89.


