Advances in CAR-T Cell Therapy for Treating Acute Myeloid Leukemia: Challenges and Future Perspectives

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Sejarah artikel:
Submission: 10 September 2024
Revision: 30 September 2024
Accepted: 10 October 2024

Kata kunci:
CAR-T Cell therapy, Acute Myeloid Leukemia, Chimeric Antigen Receptor

ABSTRACT

This review focuses on the revolutionary capacity of CAR-T therapy in the context of acute myeloid leukemia (AML). It is an area beset with challenges despite its progress in the treatment of hematologic malignancies. Specifically, it narrows down to identifying ideal target antigens such as CD33, CD123, and FLT3 which are pertinent to the challenge of antigen heterogeneity in AML as well as minimizing on-target off-tumor toxicity. These problems are attempted to be eliminated through innovative concepts like the use of dual-targeting CARs, armoured CAR-T cells, and CRISPR-mediated genetic editing techniques, each having a superior safety and efficacy profile.Dual-target CARs have the advantages of improved selectivity; armoured CAR-T cells can survive in the AML immunosuppressive microenvironment, and CRISPR-mediated CAR-T cells ensure precision with minimal side effects. The clinical outcomes in the last few years and a string of recent preclinical studies show promising, albeit challenging, therapeutic results. In any case, such observations mark significant progress toward ensuring long-term remission while tolerable toxicity levels. This chapter draws upon recent clinical trials and research findings in order to put into sharp relief CAR-T therapy's emergent role as a new, life-saving approach for the treatment of AML. Its understanding of technological advancement in CAR-T therapy places this chapter as an invaluable reference source for researchers and clinicians interested in optimizing CAR-T therapy in the treatment of AML.

INTRODUCTION

Acute myeloid leukemia (AML) is, even today, an especially challenging hematological malignancy to treat after the different advances realized in supportive care and risk stratification in the last five decades. The current standard of care was developed in the 1970s, involving multimodal chemotherapeutic regimens and, for many, allogeneic hematopoietic stem cell transplantation. These, however, pose critical challenges, particularly the resistance of leukemic stem cells to chemotherapy and the associated high toxicity with allo-HSCT (Vanhooren et al., 2023)[1]. The latest available epidemiological data for South Korea, which are from 2011-2020, clearly shows that the pattern of acute myeloid leukemia is changing; it has decreased in terms of age-standardized incidence rates from 1.94 to 1.77 per 100,000 population, but increased in prevalence rates from 8.93 to 9.67 per 100,000 populations (Han, H. J., et al 2024)[2].

Relapsed/refractory AML is very hard to be treated since it contains vast resistance to conventional drugs, and survival outcomes after a long time are poor. Latest studies show that CAR-T therapy may be alternative since traditional therapies have brought out very low efficacy in R/R AML (Ma, J., & Ge, Z. 2021)[3]. Immunotherapy represents a promising avenue in AML therapy. Considering that there is evidence of success with this approach in B-cell malignancies, myeloid disorders may as well be sensitive to T cells on account of the graft-versus-leukemia effect that is observed with allo-HSCT. Of all immunotherapeutic approaches, CAR T-cell therapy has been given much publicity. This unprecedented efficacy of CD19-directed CAR-T cells in B-cell acute lymphoblastic leukemia has led to a growing interest in the adaptability of this method in AML treatment (Vanhooren et al., 2023; Mardiana & Gill, 2020)[1,4]

METHODOLOGY

This review article was specifically performed to investigate the state of affairs in terms of the latest developments, the existing gaps and the clinical results of CAR-T therapy in acute myeloid leukaemia (AML) therapy. The method was based on the methodological approach focused on narrative reviews, which allows a critical compilation of the most pertinent and high-level evidence.

Literature Search Strategy

A stepwise literature search was carried out in PubMed, Scopus, Web of Science and Google Scholar databases for peer-reviewed published articles in the period of time od 2020 and 2024. The search terms used included: “CAR-T therapy”, “acute myeloid leukaemia”, “immunotherapy”, “target antigens in AML”, “dual-target CARs”, “armored CAR-T cells” and “CRISPR in CAR-T therapy”. Boolean operators (AND, OR) were used to connect search arguments. Additionally, the reference lists of relevant articles were searched to find more studies related to the topic.

Inclusion and Exclusion Criteria

A review was done only on articles that fulfilled the following conditions:

Inclusion Criteria:

1. English Language Studies.
2. Research Article, clinical study, or a systematic questionnaire-based study.
3. Literature dealing with CAR-T milestones, targets and strategies for enhanced treatment in AML.

Exclusion Criteria:

1. Gray Literature, Editorial Articles, and Convention Papers.
2. Research not concentrated on CAR T cell therapy targeting AML.
3. Studies for which the full text was not accessible.

DATA EXTRACTION AND ANALYSIS

Details such as study design, target antigens, therapeutic strategies, and clinical outcomes were also recorded and categorized under respective themes. New developments such as dual-targeting CARs, armored CAR-T cells and CRISPR editing were given extra focus. Each study included in the review was evaluated for its quality and relevance and its contribution

CAR-T CELL THERAPY BASICS

A. CAR Structure and Function

CARs are synthetic receptors that integrate the affinities of an antibody and the killing potency of T cells. Traditionally, a CAR typically contains four key components: (Dimitri, A., et al. 2022)[5]

1. **Extracellular Domain**
   - Single-chain variable fragment (scFv) derived from monoclonal antibodies
   - Determines target specificity and binding affinity
   - Can be engineered for optimal antigen recognition
2. **Hinge/Spacer Region**
   - Provides flexibility and optimal distance between T cell and target
   - Influences CAR functionality through spatial orientation
   - Usually derived from CD8α, CD28, or IgG4
3. **Transmembrane Domain**
   - Anchors the CAR to the T cell membrane
   - Influences receptor stability and signaling
   - Typically derived from CD28 or CD8α
4. **Intracellular Signaling Domains**
   - CD3ζ chain for primary activation signal
   - Co-stimulatory domains (CD28, 4-1BB, or OX40) for enhanced persistence
   - Determines T cell activation, proliferation, and survival

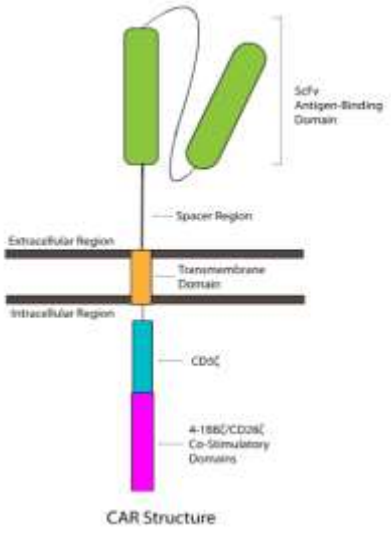

Fig 1: Schematic representation of a CAR T-cell (Dimitri, A., et al. 2022)[6].

B. Target selection in AML

The procedure of generating CAR-T cells is multi-phased (Ayala Ceja, M., et al., 2024)[7].

1. Leukapheresis and T cell isolation
   - Collection of Peripheral blood mononuclear cells
   - Enrichment for T cell via magnetic separation
   - Quality assessment of starting material
2. Activation of T cells
   - Activation by anti-CD3/CD28 beads
   - Addition of cytokines (IL-2, IL-7, IL-15)
   - Follow-up on activation markers
3. Genetic modification to introduce the CAR
   - Delivery viral: lentivirus or retrovirus, or non-viral: transposon
   - CAR transgene incorporation
   - Insertional mutagenesis testing for safety
4. CAR-T cells expansion ex vivo
   - 10-14 days of expansion
   - Following growth, viability, and phenotype
   - Sterility and potency tests
5. Quality control testing
6. Reinfusion to the patient

   This process takes 2-3 weeks. A lymphodepleting chemotherapy might be administered in the course to improve the expansion and persistence of CAR-T cells.

CHALLENGES

Antigen Selection

The main disadvantage with B-cell malignancies is that they express lineage-specific antigens such as CD19; however, most of the targetable antigens found in AML are shared with healthy hematopoietic stem and progenitor cells. This overlap may cause myeloablation and associated complications of infections and bleeding events, as stated by Mardiana & Gill, 2020; Damiani & Tiribelli, 2024.[4,7]

Among these antigens, several have been explored as potential targets for CAR-T therapy in AML, they are CD33, CD123, CLL-1, FLT3 and CD70

Table 1. Detailed Characterization of CAR-T Cell Targets in AML
Data summarised from Zarychta, J., e al 2023.[8]

Antigen	Expression in AML	Normal Tissue Expression	Clinical Trial Status	Key Considerations
CD33	90-99% of cases	Myeloid progenitors, monocytes	Phase I/II completed	-High efficacy potential -Risk of myelosuppression
CD123	50-78% of cases	HSCs, endothelial cells	Multiple Phase I/II	- Lower toxicity profile - Variable expression levels
CLL-1	78-92% of cases	Mature myeloid cells	Phase I ongoing	- LSC-specific targeting - Limited off-target effects
FLT3	54-92% of cases	Early progenitors	Preclinical	- Mutation status dependent - Resistance development
CD70	86% of cases	Activated lymphocytes	Phase I	- Immune checkpoint role - T cell fratricide risk

Notes
HSPCs: Hematopoietic stem and progenitor cells, AML: Acute Myeloid Leukemia, LSC: Leukemic stem cells

Strategies to Mitigate On-Target, Off-Tumor Toxicity

As far as the issue of the overlapping antigen presentation among AML blasts and healthy hematopoietic cells is concerned, several strategies have been made (Greiner, J., et al., 2022)[9]:

1. **Bidirectional CAR-T cells**: These CAR-T cells bind to two different antigens, thus increasing specificity and reducing the off-tumor effect (Khaniya, A., et al 2024)[10]
2. **Transient CAR expression**: mRNA-based CARs express the CAR transiently, therefore controlling the treatment duration and reducing the long-term toxic side effects (Xiao, K., 2024)[11].
3. **Suicide genes or safety switches that kill the cells:** Suicidal characteristics of the cell can be engineered in CAR-T cells so that they are effectively removed from the bloodstream when major toxicities become apparent (Ercilla-Rodríguez, P., et al., 2024)[12].
4. **Precision genome editing:** Antigen from the healthy hematopoietic stem cells can be knocked out with the help of CRISPR/Cas9, making them invulnerable to CAR-T-cell killing (Wei, W., et al., 2023)[13].

Recent Advancements and Future Directions

1. Completed and Ongoing Clinical Trials

As of 2024, numerous clinical studies conducted on CAR-T cell therapy in AML are ongoing. From a search at ClinicalTrials.gov, over 50 active studies targeting various antigens exist
Some of the notable ones include:

1. Targeted CD123 CAR-T cells; phase I/II (NCT02159495)
2. 2.CLL-1 targeted CAR-T cells: a phase I (NCT03631576)
3. 3.CAR T cells targeting CD33 with genome-edited haematopoietic stem cells: a phase I study (NCT04849910)

Table 2. Summary of Key Clinical Trials

Trial ID	Target	Phase	Patients	ORR	CR Rate	Median Survival	Key Toxicities
NCT04230265	CD33	I	12	75%	50%	8.5 months	CRS (Grade 1-2)
NCT03631576	CLL-1	I	15	80%	60%	Not reached	ICANS (Grade 1)
NCT04849910	CD33/CD123	I/II	18	85%	65%	12 months	Manageable CRS

2. Preliminary Outcomes and Toxicities Identified

Thus far, initial clinical outcomes have seemed promising but have presented with concerns, such as:

- CD33-specific CAR-T cells have demonstrated both significant myelotoxicity and anti-leukemic effects. (Pérez-Amill, L., et al 2023)[14].
- CD123-specific CAR-T cells have been found to be effective in a few patients, but of course, long-term hematopoietic toxicity has been of concern here (Loff, S.,2020)[15].
- CAR-T cells, targeting both CD33/CLL-1, and dual-targeting, for example, have shown promising preliminary results with manageable toxicity profiles (Koedam, J., et al., 2022)[16].

3. Common toxicities associated with CAR-T cell therapy in AML include:

1. Cytokine release syndrome (CRS)
2. Immune effector cell-associated neurotoxicity syndrome (ICANS)
3. On-target, off-tumor effects leading to myelosuppression
4. B-cell aplasia for some targetsFuture Directions and Emerging Approaches

4. Allogeneic "Off-the-Shelf" CAR-T Cells

Allogeneic CAR-T cells from healthy donors will have the benefit of off-the-shelf availability, with lower cost in manufacturing. The gene editing technologies advanced rapidly and were accompanied by the development of universal CAR-T cells and conditions that predisposed to reduced risk for graft-versus-host disease (Mohty, R., & Lazaryan, A. 2024)[17].

5. Combination Therapies

Combination of CAR-T cell therapy with others may also enhance the effectiveness of CAR-T cell therapy and results in overcoming resistance mechanisms. Of these, the combinations are as follows

- CAR-T cell in combination with checkpoint inhibitors
- CAR-T cells plus hypomethylating agents
- CAR-T cells as a bridge to, or in addition to, allo-HSCT

6. New Generation of CAR Designs

Currently, researchers are designing new, improved designs for CARs with the aim of efficiency and safety. Logic-gated CARs These CARs act only if there is the presence of two antigens. They increase specificity. Switchable CARs: These resources allow for the tightly regulated control of CAR-T cell activity through small molecule drugs, CAR-NK cells: It can be postulated that CARs engineered into NK cells may offer a safer option compared to T cells as they carry out scarce persistence with lower chances of risk with regard to CRS (Tomasik, J., et al 2022)[18].

CONCLUSION

CAR-T cell therapy will be one of the promising therapies that may be used in AML and with its potential for targeted, potent anti-leukemic activity. There is a way still to go in much daunting challenges, particularly how to identify optimal targets to provide appropriate toxicity reductions. It is probably by further research and with further development in more advanced CAR designs and combination strategies as well as allogenic approaches, which could address the current limitations facing CAR-T cell therapy and would make it an appropriate treatment option for most patients diagnosed with AML. This may complete the knowledge gaps about optimal sequencing or potential replacement of CAR-T therapy with conventional treatments such as allo-HSCT and could open doors to more efficient treatment approaches and/or tailoring to each individual case in AML patients. With this evolution, CAR-T cell therapy would probably play a greater role in the treatment of this difficult disease called AML, and the outcomes could change for patients.

CAR-T cell therapy for AML, which is an area under rapid acceleration with promising development in the selection of a target, safety management, and clinical outcomes. Keeping patient safety and efficacy under strict focus, novel integrations of technologies and combinations may solve some of today's problems. Universal CAR-T platforms and smart designs are among the emerging ones that promise to change the face of AML in the future.

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