

Chimeric Antigen Receptor-Engineered T Cell Therapy in Acute Myeloid Leukaemia

EDITOR'S
PICK

This interesting, very well written article by Paubelle et al. on the use of chimeric antigen receptor (CAR) T cell therapy in acute myeloid leukaemia (AML) offers a clear, understandable, direct, and comprehensive overview of the CAR T therapy story, while highlighting the difficulties that have been encountered by researchers along the way. Once again, it is demonstrated that medical research is paved with difficulties and barriers that must be negotiated before achieving success.

CAR T therapy has so far registered impressive, although not yet definitive, advances for the treatment of lymphoid diseases; however, little has been reported on CAR T cell use in AML, a field in which clinical advances are urgently required. By reading this article, you will gain a clear idea of CAR T therapy developments, together with the future potential and problems associated with the use of the treatment for AML patients.

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Abstract

Acute myeloid leukaemia (AML) is a disease with a very poor outcome and remains an area of significant unmet need, necessitating novel therapeutic strategies. The progress made in the field of immunotherapy, in particular chimeric antigen receptor (CAR)-engineered T cells, has given rise to many hopes for pathologies such as B cell acute lymphoblastic leukaemia and B cell lymphoma, and many studies have attempted to translate these successes to AML. This review summarises the recent advances in, and defines an ideal target for, CAR T cell therapy in AML.

INTRODUCTION

Acute myeloid leukaemia (AML) is an aggressive malignant disease affecting 2.5–3.5 per 100,000 adults each year in Western countries.^{1,2} Over the last decade, considerable advances have been made in the identification of molecular markers, leading to the improvement of risk stratification, especially in AML patients with a normal karyotype.³ However, in the past 5 years, the cure rate has been 35–40% for AML patients <60 years old and 5–15% for patients >60 years old.⁴ The elderly, who are unfit to have intensive chemotherapy, have a median overall survival of 4–10 months.² Despite improved understanding of leukaemogenesis, AML still has poor outcomes due to high disease and treatment-related mortality. To date, allogeneic haematopoietic stem cell transplantation (alloHSCT) remains the most effective treatment for relapse prevention in non-favourable-risk patients with AML^{5,6} and has remained the most successful immunotherapeutic treatment for AML patients, especially with the advances made in using alternative donors;⁷ however, this therapeutic strategy has a high toxicity without guaranteeing the absence of relapse.⁸ Thus, it is a public health issue that new, more effective, and less toxic treatments should emerge for the treatment of AML. In this regard, chimeric antigen receptor (CAR)-engineered T cells have been developed and used in paediatric acute lymphoblastic leukaemia (ALL), resulting in complete remission without graft versus host disease (GvHD).⁹

CAR T cells are artificial molecules comprising an extracellular antigen-binding domain, commonly derived from a monoclonal antibody in the form of a single chain fragment variable (scFv), and an intracellular signalling domain associated with ≥ 1 costimulatory signalling modules.^{10,11} The first generation of CAR T cells were developed in ovarian metastatic carcinomas¹² and built on the model scFv-spacer-CD3 ζ . Unlike the T cell receptors, they recognise the antigen in the absence of major histocompatibility complex proteins; therefore, CAR T cells can specifically target various surface antigens on malignant cells.¹³

Nonetheless, first-generation CAR T cells exhibit a lack of cytokine secretion, insufficient proliferation, and low persistence *in vivo*.

To improve these shortcomings, the next generation of CAR T cells involved the addition of costimulatory signalling domains, such as CD28 and 41BB or CD3 ζ ,¹⁴ to the cytoplasmic tail of the CAR to provide additional signals to T cells. The addition of these costimulatory proteins limited apoptosis of CAR T cells because of the synthesis of IL-2.¹⁵ Preclinical studies have indicated that this second generation improves the antitumour activity of T cells.¹⁵ Recently, third-generation CAR T cells combine multiple signalling domains, such as CD3 ζ -CD28-41BB or CD3 ζ -CD28-OX40, to improve activity;¹⁶ however, this improvement has not demonstrated a significant clinical impact when compared with the prior generation of CAR T cells. For example, the CAR T cell strategy is highly specific in redirecting T cells towards predefined target cells but reaches its limits when targeting solid tumours with phenotypic heterogeneity.¹⁷ After initial tumour reduction by CAR T cells, antigen-negative cancer cells not recognised by CAR may give rise to tumour relapse. This situation might be overcome by CAR-mediated activation of T cells in the tumour, releasing inducible IL-12 that increases tumour-infiltrating T cell activation and attracts and activates innate immune cells to eliminate the antigen-negative cancer cells in the targeted lesion; these fourth-generation CAR T cells are called TRUCK T cells.¹⁸ The evolution of CAR T cells is shown in [Figure 1](#).

Recently, the U.S. Food and Drug Administration (FDA) gave approval for two third-generation CAR T cells targeting CD19;¹⁹ the first was tisagenlecleucel in B cell-lineage ALL, followed by axicabtagene ciloleucel in diffuse large B cell lymphoma and B cell-lineage ALL. This therapeutic strategy may be applicable to a large number of tumoural pathologies, including AML, for which there is a need for treatment in view of the poor results observed in adults and children. This review therefore focusses on CAR T cells for the treatment of AML.

TARGETING OF CHIMERIC ANTIGEN RECEPTOR T CELLS IN ACUTE MYELOID LEUKAEMIA

CAR T cell therapy targeting CD19 has yielded remarkable outcomes in paediatric and young adult patients with relapsed or refractory

diffuse large B cell lymphoma and ALL.²⁰⁻²⁷ Many groups have attempted to reproduce these encouraging results in other pathologies, such as AML; however, because of the lack of expression of CD19 on the surface of AML cells, CAR T cells directed against CD19 have no efficacy in this pathology.²⁸ An ideal target for CAR T cells in AML should be highly expressed in most of the malignant cells, including leukaemic stem cells (LSC), and should be shared by most AML patients. Unlike naïve T lymphocytes, which can signal through the T cell receptor in response to very low antigen density, CAR T cells need a higher density of antigen to be effective.²⁹⁻³² Expression of the target by normal cells may or may not be associated with high toxicity but, to prevent toxicity, the target should not be shared by vital tissues such as the heart, liver, and normal haematopoietic

stem cells (HSC). An approach to remedy this pitfall is the use of an algorithm integrating a large dataset of transcriptomics and proteomics from malignant and normal tissues to identify potential targets expressed in LSC but not in normal CD34+CD38- haematopoietic cells, T cells, or vital tissues.³³ However, using CAR T cells in AML remains difficult due to the non-restricted expression of AML-associated antigens. Moreover, CAR T cells may persist in the organism for several years,³⁴ thus an unwise target choice may lead to prolonged aplasia. **Table 1** summarises the different clinical trials currently underway employing CAR T cells in AML.³⁵⁻⁴²

CD33

CD33 (also known as Siglec-3) is a transmembrane receptor expressed on cells of myeloid lineage and on approximately 90% of AML cells.⁴³⁻⁴⁵

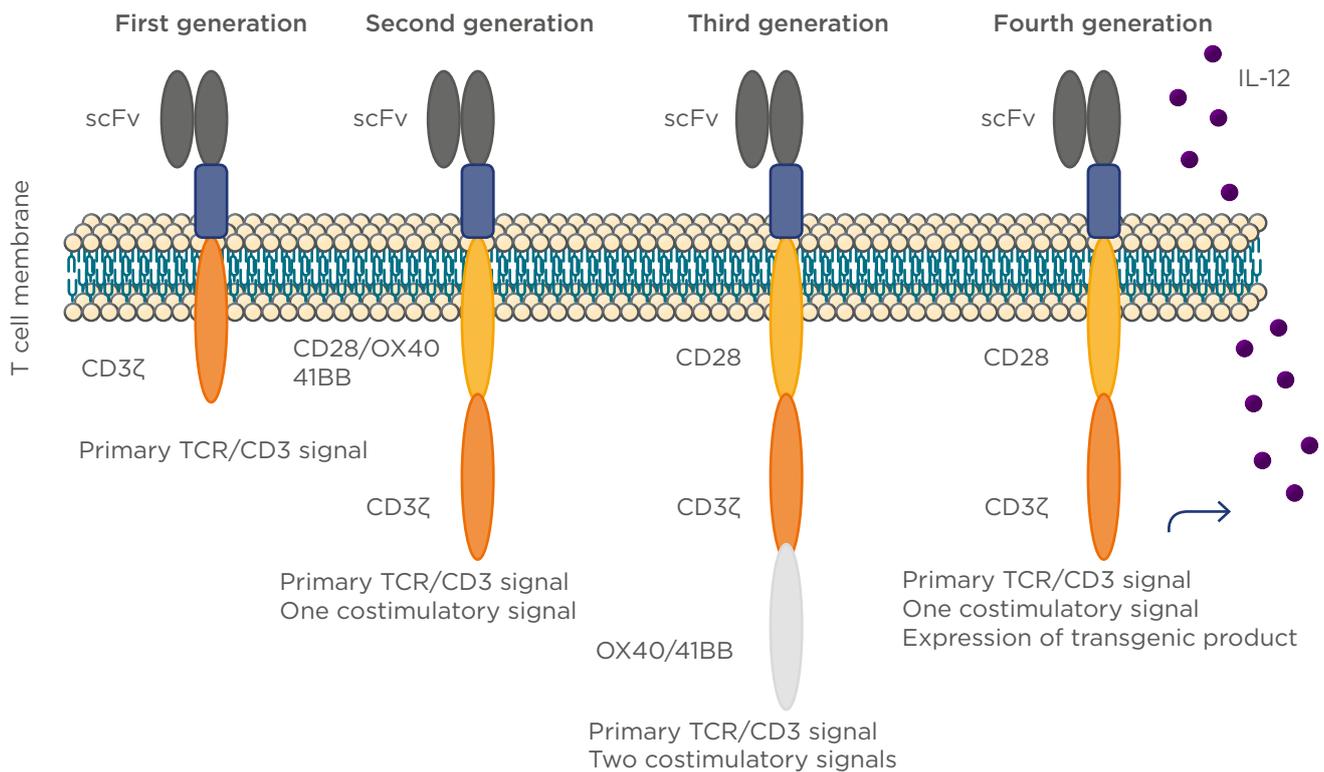


Figure 1: The evolution of chimeric antigen receptor T cells.

First-generation CAR T cells consist of a single-chain fragment of variable region antibody for target binding linked by a spacer domain to the transmembrane and intracellular signalling domain of CD3ζ derived from the T cell receptor. Addition of a costimulatory domain, mostly of the CD28 family, makes up second-generation CAR T cells. Third-generation CAR T cells contain two costimulatory domains. Fourth-generation CAR T cells (also known as TRUCK T cells) are additionally modified with a constitutive or inducible expression cassette for a transgenic protein, for instance a cytokine, which is released by the CAR T cell to modulate the tumour T cell response.

CAR: chimeric antigen receptor; scFv: single-chain fragment variable region; TCR: T cell receptor.

Table 1: Current clinical trials using chimeric antigen receptor T cells for acute myeloid leukaemia.

Study number	AML type	Target	Costimulation domain	Dosage	Associated conditioning	Clinical phase
NCT03473457 ³⁵	R/R	CD38/CD33/ CD56/CD123/ CD117/CD133/ CD34/Muc1-	N/A	0 ⁶ -10 ⁷ /kg	N/A	II
NCT03222674 ³⁶	R/R	Muc1/CD33/ CD38/CD56/ CD117/CD123	N/A	N/A	N/A	I/II
NCT03114670 ³⁷	Relapsed after alloHSCT	CD123	41BB-CD3ζ	N/A	N/A	I/II
NCT03126864 ³⁸	R/R	CD33	N/A	5x10 ⁸ - 5x10 ¹⁰ /kg	Fludarabine- cyclophosphamide	I
NCT03190278 ³⁹	R/R	CD123	N/A	6.25x10 ⁵ - 6.25x10 ⁶ /kg	N/A	I
NCT02159495 ⁴⁰	All AML	CD123	CD28-CD3ζ	N/A	Fludarabine- cyclophosphamide	II
NCT01864902 ⁴¹	R/R	CD33	CD137-CD3ζ	N/A	N/A	I
NCT01716364 ⁴²	MM, AML, MDS	LeY	CD28	N/A	N/A	I

ALL: acute lymphoblastic leukaemia; alloHSCT: allogeneic haematopoietic stem cell transplantation; AML: acute myeloid leukaemia; LeY: Lewis antigen Y; MDS: myelodysplastic syndrome; MM: multiple myeloma; N/A: not applicable; R/R: relapsed/refractory.

It is usually considered myeloid-specific but can also be found on lymphoid cells and non-haematological tissues.⁴⁵ Gemtuzumab ozogamicin (Mylotarg®, Pfizer, New York City, New York, USA) is an antibody-targeted chemotherapy agent consisting of a humanised murine anti-CD33 monoclonal antibody (clone P67.6) linked to a calicheamicin Y1 derivative via a hydrolysable bifunctional linker.⁴⁶ Gemtuzumab ozogamicin induces cell death in CD33-positive cells by internalisation of the calicheamicin drug and then cleavage of double-stranded DNA,⁴⁷ and has shown promising results in AML.^{48,49} Using an antiCD33 scFv, CAR T cells targeting CD33 have been developed for the treatment of AML, and promising results of murine AML models^{50,51} and humans⁵² have been reported. Several clinical trials are currently ongoing in AML with CAR T cells directed against CD33.

CD123

CD123 is the alpha chain of the IL-3 receptor and is a cluster-like protein of differentiation encoded by *IL3RA* on the human X chromosome. It is

expressed on the surface of several leukaemic cells, including cells of AML, plasmacytoid,⁵³ hairy cell leukaemia,⁵⁴ and Hodgkin's lymphoma,⁵⁵ thus constituting a potential target for the treatment of these diseases. Although CD123 presents low expression levels on normal haematopoietic cells,⁵⁶ it may be a possible target for AML.⁵⁷ CAR T cells directed against CD123 in AML mouse models resulted in a reduction of leukaemic burden but showed contrasting results on normal haematopoiesis.⁵⁸⁻⁶² Modifying the scFv directed against CD123 may be able to reduce myelotoxicity in an AML murine model,⁶² and clinical studies are underway in AML patients with CAR T cells targeting CD123.

Lewis Y Antigen

The Lewis antigen system is a human blood group system based on genes of chromosome 19. The Lewis antigen Y (LeY) is an oligosaccharide overexpressed on most epithelial cancer cells and haematological malignant cells, including AML cells,^{63,64} but is weakly expressed by healthy tissues.^{65,66} A Phase I study with second-generation CAR

T cells directed against LeY in four AML patients reported two cytogenetic remissions without Grade 3 or 4 side effects and with long-term persistence of the CAR T cells.⁶⁷ Moreover, *in vivo* models have shown promising results of CAR T cells targeting LeY in epithelial cancers without significant toxicity.⁶⁸

FMS-Like Tyrosine Kinase 3

FMS-like tyrosine kinase 3 (FLT-3/CD135) is a receptor-type tyrosine-protein kinase, or fetal liver kinase-2, that belongs to the receptor tyrosine kinase Class III family of cytokine receptors and binds the FLT-3 ligand. It is expressed on the surface of many haematopoietic progenitor cells and its signalling is important for the normal development of HSC and progenitor cells. The *FLT3* gene is one of the most frequently mutated genes in AML,⁶⁹ high levels of wild-type FLT-3 have been reported for blast cells of some AML patients without *FLT3* mutations. These high levels of wild-type FLT-3 may be associated with worse prognosis.⁷⁰ It was recently reported that CAR T cells directed against FLT-3 in *in vivo* and *in vitro* models had a potent reactivity against AML cell lines and primary AML blasts expressing either wild-type FLT-3 or FLT-3 with internal tandem duplication. However, as anticipated, FLT-3-CAR T cells recognised normal HSC *in vitro* and *in vivo* and disrupted normal haematopoiesis in colony-formation assays, suggesting that adoptive therapy with FLT-3-CAR T cells will require subsequent CAR T cell depletion and alloHSCT to reconstitute the haematopoietic system.⁷¹

Folate Receptor β

Folate receptor β (FOLR2) is a protein encoded by the *FOLR2* gene,⁷² and is expressed on myeloid lineage cells and frequently upregulated in AML cells.⁷³ Preclinical models using CAR T cells directed against FOLR2 have shown promising results on AML cells while preserving normal HSC; the concomitant use of all-trans retinoic acid, which allows an upregulation of FOLR2, improved efficiency against AML.⁷⁴

Hyaluronate Receptor

The hyaluronate receptor (CD44) is a cell-surface glycoprotein involved in cell-cell interactions,

cell adhesion, and migration, and is expressed on many mammalian cell types. CD44 variant domain 6 (CD44v6) is a variant isoform expressed in multiple myeloma⁷⁵ and AML.⁷⁶ CAR T cells directed against CD44v6 have shown antileukaemic effects in mouse models, although at the cost of leukopenia.⁷⁷ Further steps may involve adding a suicide gene to this construct.⁷⁸

CD38

CD38, also known as cyclic ADP ribose hydrolase, is a glycoprotein⁷⁹ found on the surface of many immune cells (specifically white blood cells), including T, B, and natural killer lymphocytes. CD38 also functions in cell adhesion, signal transduction, and calcium signalling.⁸⁰ CD38 is expressed on AML cells but not on normal HSC.^{81,82} As for FOLR2, all-trans retinoic acid enhances CD38 expression and its action is synergistic with CAR T cells targeting CD38.⁸³

Natural Killer Group 2D

Natural killer group 2D (NKG2D) is a transmembrane protein belonging to the CD94/NKG2 family of C-type lectin-like receptors.⁸⁴ NKG2D is highly expressed in AML tissue but weakly on healthy tissue.⁸⁵ Several CAR T cells directed against NKG2D have been clinically developed, leading to complete remissions without neurotoxicity, cytokine release syndrome (CRS), or treatment-related mortality.⁸⁶

CD7

CD7 is a transmembrane protein and a member of the immunoglobulin superfamily. This protein is found on thymocytes and mature T cells and plays an essential role in T cell interactions and in T cell-B cell interaction during early lymphoid development.⁸⁷ In AML, its expression correlates with chemoresistance.⁸⁸ CAR T cells directed against CD7 have shown potent efficiency *in vitro* and in murine AML models with low toxicity.⁸⁹

C-type Lectin Domain Family 12 Member A

C-type lectin domain family 12 member A (*CLEC12A*) is a member of the C-type lectin/C-type lectin-like domain superfamily. Members

of this family share a common protein fold and have diverse functions, such as cell adhesion, cell-cell signalling, glycoprotein turnover, and roles in inflammation and immune response. The protein encoded by this gene is a negative regulator of granulocyte and monocyte function⁹⁰ and is overexpressed on the surface of AML cells; CLEC12A could therefore serve as a target for CAR T cells to negate minimal residual disease⁹¹ or target LSC.⁹²

TOLERANCE OF CHIMERIC ANTIGEN RECEPTOR-ENGINEERED T CELLS IN ACUTE MYELOID LEUKAEMIA

Off-Target Side Effects and Haematological Tolerance

Given the lack of specificity of the target antigens for the tumour cells, CAR T cells may have an action on normal tissues expressing these antigens. In addition, a number of these target antigens could be expressed by normal HSC and therefore myelosuppression would be expected as a consequence. However, the target selection process and the representability of the murine models made it possible to avoid major haematological toxicity in clinical trials with CAR T cells targeting CD33⁵² and LeY.⁶⁷

Cytokine Release Syndrome

By linking target antigen-expressing malignant cells to cytotoxic T cells, T cell-engaging therapies harness the cell-mediated immune response and direct it against malignant cells, bypassing the major histocompatibility complex. Clinical trials using CAR T cells have demonstrated high complete response rates;^{10,26,89} however, complete remissions have been associated with CRS, the intensity of which ranges from mild to life-threatening.^{9,34,93} Mild CRS symptoms are characteristic of flu-like syndrome, with fevers and myalgias, while some patients experience a severe inflammatory syndrome, including vascular leak, hypotension, pulmonary oedema, and coagulopathy, resulting in multiorgan system failure. The pathophysiology of CRS is related to a significant increase in the release of cytokines, such as IL-10 and IL-6, due to the activation and proliferation of T lymphocytes.⁹⁴ Among the cytokine storm, IL-6 appears to be the central mediator of

CRS⁹⁴ and clinical studies have shown that the use of corticosteroids or an IL-6 receptor antagonist antibody (tocilizumab) allows CRS control induced by CAR T cells.⁹ Other approaches, such as adding a suicide gene and kill-switch strategies,¹⁵ are possible. Due to the increased experience of corticosteroid and tocilizumab use, a consensus is emerging for the management of CRS.⁹⁴ CRS does not spare AML patients treated with CAR T cells and has been reported after the use of CAR T cells against CD33⁵² and CD123.⁹⁵

Other Toxicities

The development of neurologic toxicities, including confusion, delirium, myoclonus, aphasia, and seizure, has been reported in patients receiving CD19-specific CAR T cells.^{23,24} However, these side effects are also found with blinatumomab⁹⁶ and seem specific to CD19, so should therefore not be found in AML. Anaphylaxis can also occur because CAR T cells contain antigen-recognition domains derived from murine antibodies⁹⁷ and efforts are ongoing to humanise the components of expressed proteins. The risk of insertional oncogenesis in human cells has also been established in the context of gene therapy of HSC.⁹⁸ Insertion of a transgene into T cells may induce malignant transformation; however, no cases of transformation have been reported following the infusion CAR T cells.

CONCLUSION AND REMAINING CHALLENGES

The progress of immunotherapy brings new hope for cancer treatment. Like in ALL, CAR T cells provide much hope for the treatment of AML. However, unlike in ALL, there is no target antigen such as CD19 or CD22 in AML, and the target choice can lead to off-target effects. CD123 and CD33 are the major targets, either separately or on constructions targeting both at the same time,⁹⁹ and results of the numerous ongoing clinical trials need to be awaited to weigh the potential benefits versus side effects of these new strategies. Moreover, CAR T cells are currently reserved for relapsed or refractory AML patients; therefore this new strategy will have to find its place in a therapeutic arsenal that already contains

alloHSCT as well as new targeted therapies. In order to reduce off-target toxicities, several strategies may be used. The identification of novel leukaemia-associated antigens or neoantigens could provide more specific targets and transcriptomic and proteomic analyses are ongoing to fully characterise the AML 'surfaceome'. Furthermore, dual-targeting or sequential approaches could improve treatment specificity while relying on combinations of AML antigens. In addition, the understanding and prevention of the escape mechanisms of CAR T cells in AML remains a challenge. The strategy to address the relapses

due to antigen escape involves designing CAR T cells able to target multiple antigens simultaneously or sequential strategies. Moreover, relapses in AML are caused by the persistence of LSC, which do not necessarily express the same antigens as the bulk cells, and therefore LSC could persist despite the use of CAR T cells. The exorbitant cost of these new strategies will also be a societal challenge, but the democratisation of CAR T cells may lead to lower costs. Therefore, CAR T cells could competitively challenge the prominent place of alloHSCT for the treatment of AML.

References

- Lowenberg B et al. Acute myeloid leukemia. *N Engl J Med.* 1999;341(14):1051-62.
- Döhner H et al. Acute myeloid leukemia. *N Engl J Med.* 2015;373(12):1136-52.
- Grimwade D et al. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. *Blood.* 2016;127(1):29-41.
- Burnett A et al. Therapeutic advances in acute myeloid leukemia. *J Clin Oncol.* 2011;29(5):487-94.
- Cornelissen JJ, Blaise D. Hematopoietic stem cell transplantation for patients with AML in first complete remission. *Blood.* 2016;127(1):62-70.
- Stelljes M et al. Allogeneic transplantation versus chemotherapy as postremission therapy for acute myeloid leukemia: A prospective matched pairs analysis. *J Clin Oncol.* 2014;32(4):288-96.
- Gupta V et al. Allogeneic hematopoietic cell transplantation for adults with acute myeloid leukemia: Myths, controversies, and unknowns. *Blood.* 2011;117(8):2307-18.
- Duval M et al. Hematopoietic stem-cell transplantation for acute leukemia in relapse or primary induction failure. *J Clin Oncol.* 2010;28(23):3730-8.
- Grupp SA et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med.* 2013;368(16):1509-18.
- Eshhar Z et al. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc Natl Acad Sci USA.* 1993;90(2):720-4.
- Gross G et al. Chimaeric T-cell receptors specific to a B-lymphoma idiotype: A model for tumour immunotherapy. *Biochem Soc Trans.* 1995;23(4):1079-82.
- Kershaw MH et al. A Phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin Cancer Res.* 2006;12(20 Pt 1):6106-15.
- Eshhar Z. The T-body approach: Redirecting T cells with antibody specificity. *Handb Exp Pharmacol.* 2008;(181):329-42.
- Bridgeman JS et al. CD3zeta-based chimeric antigen receptors mediate T cell activation via cis- and trans-signalling mechanisms: Implications for optimization of receptor structure for adoptive cell therapy. *Clin Exp Immunol.* 2014;175(2):258-67.
- Zhang C et al. Engineering CAR-T cells. *Biomarker Res.* 2017;5:22.
- Sadelain M et al. The basic principles of chimeric antigen receptor design. *Cancer Discov.* 2013;3(4):388-98.
- Lamers CH et al. Treatment of metastatic renal cell carcinoma (mRCC) with CAIX CAR-engineered T-cells-A completed study overview. *Biochem Soc Trans.* 2016;44(3):951-9.
- Chmielewski M, Abken H. TRUCKs: The fourth generation of CARs. *Expert Opin Biol Ther.* 2015;15(8):1145-54.
- Liu Y et al. Tisagenlecleucel, an approved anti-CD19 chimeric antigen receptor T-cell therapy for the treatment of leukemia. *Drugs Today.* 2017;53(11):597-608.
- Brentjens RJ et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Sci Transl Med.* 2013;5(177):177ra38.
- Kochenderfer JN et al. Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. *Blood.* 2013;122(25):4129-39.
- Kochenderfer JN et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J Clin Oncol.* 2015;33(6):540-9.
- Lee DW et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: A Phase 1 dose-escalation trial. *Lancet.* 2015;385(9967):517-28.
- Maude SL et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med.* 2014;371(16):1507-17.
- Qasim W et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. *Sci Transl Med.* 2017;9(374).
- Turtle CJ et al. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. *J Clin Invest.* 2016;126(6):2123-38.
- Turtle CJ et al. CD19-Targeted chimeric antigen receptor-modified T-cell immunotherapy for B-cell malignancies. *Clin Pharmacol Ther.* 2016;100(3):252-8.
- Francis J et al. CD19 expression in acute leukemia is not restricted to the cytogenetically aberrant populations. *Leuk Lymphoma.* 2013; 54(7): 1517-20.
- Drent E et al. A rational strategy for reducing on-target off-tumor effects of cd38-chimeric antigen receptors by affinity optimization. *Mol Ther.* 2017;25(8):1946-58.

30. Turatti F et al. Redirected activity of human antitumor chimeric immune receptors is governed by antigen and receptor expression levels and affinity of interaction. *J Immunother.* 2007;30(7):684-93.
31. Weijtens ME et al. Functional balance between T cell chimeric receptor density and tumor associated antigen density: CTL mediated cytotoxicity and lymphokine production. *Gene Ther.* 2000;7(1):35-42.
32. Walker AJ et al. Tumor antigen and receptor densities regulate efficacy of a chimeric antigen receptor targeting anaplastic lymphoma kinase. *Mol Ther.* 2017;25(9):2189-201.
33. Perna F et al. Integrating proteomics and transcriptomics for systematic combinatorial chimeric antigen receptor therapy of AML. *Cancer Cell.* 2017;32(4):506-19 e5.
34. Porter DL et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med.* 2015;7(303):303ra139.
35. Zhujiang Hospital. CAR-T cells therapy in relapsed/refractory acute myeloid leukemia (AML). NCT03473457. <https://clinicaltrials.gov/ct2/show/NCT03473457>.
36. Shenzhen Geno-Immune Medical Institute. Multi-CAR T cell therapy for acute myeloid leukemia. NCT03222674. <https://clinicaltrials.gov/ct2/show/NCT03222674>.
37. Affiliated Hospital to Academy of Military Medical Sciences. Donor-derived Anti-CD123-CART Cells for Recurred AML After Allo-HSCT. NCT03114670. <https://clinicaltrials.gov/ct2/show/NCT03114670>.
38. M.D. Anderson Cancer Center. Study of adoptive cellular therapy using autologous T cells transduced with lentivirus to express a CD33 specific chimeric antigen receptor in patients with relapsed or refractory CD33-positive acute myeloid leukemia. NCT03126864. <https://clinicaltrials.gov/ct2/show/NCT03126864>.
39. Cellectis S.A. Study evaluating safety and efficacy of UCART123 in patients with acute myeloid leukemia (AML123). NCT03190278. <https://clinicaltrials.gov/ct2/show/NCT03190278>.
40. City of Hope Medical Center. Genetically modified T-cell immunotherapy in treating patients with relapsed/refractory acute myeloid leukemia and persistent/recurrent blastic plasmacytoid dendritic cell neoplasm. NCT02159495. <https://clinicaltrials.gov/ct2/show/NCT02159495>.
41. Chinese PLA General Hospital. Treatment of relapsed and/or chemotherapy refractory CD33 positive acute myeloid leukemia by CART-33 (CART33). NCT01864902. <https://clinicaltrials.gov/ct2/show/NCT01864902>.
42. Peter MacCallum Cancer Centre, Australia. Safety study of anti lewis x chimeric antigen receptor in myeloma, acute myeloid leukemia or myelodysplastic syndrome. NCT01716364. <https://clinicaltrials.gov/ct2/show/NCT01716364>.
43. Griffin JD et al. A monoclonal antibody reactive with normal and leukemic human myeloid progenitor cells. *Leuk Res.* 1984;8(4):521-34.
44. Walter RB et al. CD33 expression and P-glycoprotein-mediated drug efflux inversely correlate and predict clinical outcome in patients with acute myeloid leukemia treated with gemtuzumab ozogamicin monotherapy. *Blood.* 2007;109(10):4168-70.
45. Hernandez-Caselles T et al. A study of CD33 (SIGLEC-3) antigen expression and function on activated human T and NK cells: Two isoforms of CD33 are generated by alternative splicing. *J Leukoc Biol.* 2006;79(1):46-58.
46. Pagano L et al. The role of gemtuzumab ozogamicin in the treatment of acute myeloid leukemia patients. *Oncogene.* 2007;26(25):3679-90.
47. van Der Velden VH et al. Targeting of the CD33-calicheamicin immunoconjugate Mylotarg (CMA-676) in acute myeloid leukemia: In vivo and in vitro saturation and internalization by leukemic and normal myeloid cells. *Blood.* 2001;97(10):3197-204.
48. Castaigne S et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): A randomised, open-label, Phase 3 study. *Lancet.* 2012;379(9825):1508-16.
49. Hills RK et al. Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: A meta-analysis of individual patient data from randomised controlled trials. *Lancet Oncol.* 2014;15(9):986-96.
50. Kenderian SS et al. CD33-specific chimeric antigen receptor T cells exhibit potent preclinical activity against human acute myeloid leukemia. *Leukemia.* 2015;29(8):1637-47.
51. O'Hear C et al. Anti-CD33 chimeric antigen receptor targeting of acute myeloid leukemia. *Haematologica.* 2015;100(3):336-44.
52. Wang QS et al. Treatment of CD33-directed chimeric antigen receptor-modified T cells in one patient with relapsed and refractory acute myeloid leukemia. *Mol Ther.* 2015;23(1):184-91.
53. Hwang K et al. Immunohistochemical analysis of CD123, CD56 and CD4 for the diagnosis of minimal bone marrow involvement by blastic plasmacytoid dendritic cell neoplasm. *Histopathology.* 2013;62(5):764-70.
54. Del Giudice I et al. The diagnostic value of CD123 in B-cell disorders with hairy or villous lymphocytes. *Haematologica.* 2004;89(3):303-8.
55. Fromm JR. Flow cytometric analysis of CD123 is useful for immunophenotyping classical Hodgkin lymphoma. *Cytometry Part B, Clinical Cytometry.* 2011;80(2):91-9.
56. Munoz L et al. Interleukin-3 receptor alpha chain (CD123) is widely expressed in hematologic malignancies. *Haematologica.* 2001;86(12):1261-9.
57. Li F et al. Characterization of SGN-CD123A, A potent CD123-directed antibody-drug conjugate for acute myeloid leukemia. *Mol Cancer Ther.* 2018;17(2):554-64.
58. Mardiros A et al. T cells expressing CD123-specific chimeric antigen receptors exhibit specific cytolytic effector functions and antitumor effects against human acute myeloid leukemia. *Blood.* 2013;122(18):3138-48.
59. Giordano Attianese GM et al. In vitro and in vivo model of a novel immunotherapy approach for chronic lymphocytic leukemia by anti-CD23 chimeric antigen receptor. *Blood.* 2011;117(18):4736-45.
60. Pizzitola I et al. Chimeric antigen receptors against CD33/CD123 antigens efficiently target primary acute myeloid leukemia cells in vivo. *Leukemia.* 2014;28(8):1596-605.
61. Gill S et al. Preclinical targeting of human acute myeloid leukemia and myeloablation using chimeric antigen receptor-modified T cells. *Blood.* 2014;123(15):2343-54.
62. Thokala R et al. Redirecting specificity of T cells using the sleeping beauty system to express chimeric antigen receptors by mix-and-match of VL and VH domains targeting CD123+ tumors. *PLoS One.* 2016;11(8):e0159477.
63. Zhang S et al. Selection of tumor antigens as targets for immune attack using immunohistochemistry: II. Blood group-related antigens. *Int J Cancer.* 1997;73(1):50-6.
64. Sakamoto J et al. Expression of Lewis x, Lewis b, X, and Y blood group antigens in human colonic tumors and normal tissue and in human tumor-derived cell lines. *Cancer Res.* 1986;46(3):1553-61.
65. Kobayashi K et al. Lewis blood group-related antigen expression in normal gastric epithelium, intestinal metaplasia, gastric adenoma, and gastric carcinoma. *Am J Gastroenterol.* 1993;88(6):919-24.
66. Yuriev E et al. Three-dimensional structures of carbohydrate

- determinants of Lewis system antigens: implications for effective antibody targeting of cancer. *Immunol Cell Biol.* 2005;83(6):709-17.
67. Ritchie DS et al. Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. *Mol Ther.* 2013;21(11):2122-9.
 68. Peinert S et al. Gene-modified T cells as immunotherapy for multiple myeloma and acute myeloid leukemia expressing the Lewis Y antigen. *Gene Therapy.* 2010;17(5):678-86.
 69. Yamamoto Y et al. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood.* 2001;97(8):2434-9.
 70. Bullinger L et al. An FLT3 gene-expression signature predicts clinical outcome in normal karyotype AML. *Blood.* 2008;111(9):4490-5.
 71. Jetani H et al. CAR T-cells targeting FLT3 have potent activity against FLT3(-)ITD(+) AML and act synergistically with the FLT3-inhibitor crenolanib. *Leukemia.* 2018;32(5):1168-79.
 72. van Heyningen V, Little PF. Report of the fourth international workshop on human chromosome 11 mapping 1994. *Cytogenet Cell Genet.* 1995; 69(3-4):127-58.
 73. Ross JF et al. Folate receptor type beta is a neutrophilic lineage marker and is differentially expressed in myeloid leukemia. *Cancer.* 1999;85(2):348-57.
 74. Lynn RC et al. Targeting of folate receptor beta on acute myeloid leukemia blasts with chimeric antigen receptor-expressing T cells. *Blood.* 2015;125(22):3466-76.
 75. Liebisch P et al. CD44v6, a target for novel antibody treatment approaches, is frequently expressed in multiple myeloma and associated with deletion of chromosome arm 13q. *Haematologica.* 2005;90(4): 489-93.
 76. Legras S et al. A strong expression of CD44-6v correlates with shorter survival of patients with acute myeloid leukemia. *Blood.* 1998;91(9):3401-13.
 77. Casucci M et al. CD44v6-targeted T cells mediate potent antitumor effects against acute myeloid leukemia and multiple myeloma. *Blood.* 2013;122(20):3461-72.
 78. Di Stasi A et al. Inducible apoptosis as a safety switch for adoptive cell therapy. *N Engl J Med.* 2011;365(18):1673-83.
 79. Orciani M et al. CD38 is constitutively expressed in the nucleus of human hematopoietic cells. *J Cell Biochem.* 2008;105(3):905-12.
 80. Nata K et al. Human gene encoding CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase): Organization, nucleotide sequence and alternative splicing. *Gene.* 1997;186(2):285-92.
 81. Terstappen LW et al. Flow cytometric characterization of acute myeloid leukemia: IV. Comparison to the differentiation pathway of normal hematopoietic progenitor cells. *Leukemia.* 1992;6(10):993-1000.
 82. Konopleva M et al. CD38 in hematopoietic malignancies. *Chem Immunol.* 2000;75:189-206.
 83. Yoshida T et al. All-trans retinoic acid enhances cytotoxic effect of T cells with an anti-CD38 chimeric antigen receptor in acute myeloid leukemia. *Clin Transl Immunology.* 2016;5(12):e116.
 84. Houchins JP et al. DNA sequence analysis of NKG2, a family of related cDNA clones encoding type II integral membrane proteins on human natural killer cells. *J Exp Med.* 1991;173(4):1017-20.
 85. Huang B et al. Modulation of NKG2D-ligand cell surface expression enhances immune cell therapy of cancer. *J Immunother.* 2011;34(3): 289-96.
 86. Sentman CL, Meehan KR. NKG2D CARs as cell therapy for cancer. *Cancer J.* 2014;20(2):156-9.
 87. Liu TQ et al. [Features of Immunophenotypes and Characteristics of Molecular Biology and Cellular Genetics of AML Patients with CD4 and CD7 Expression]. *Zhongguo shi yan xue ye xue za zhi* 2016;24(6):1627-32. (In Chinese).
 88. Chang H et al. Prognostic relevance of immunophenotyping in 379 patients with acute myeloid leukemia. *Leuk Res.* 2004;28(1):43-8.
 89. Png YT et al. Blockade of CD7 expression in T cells for effective chimeric antigen receptor targeting of T-cell malignancies. *Blood Adv.* 2017;1(25):2348-60.
 90. Drickamer K. C-type lectin-like domains. *Curr Opin Struct Biol.* 1999;9(5):585-90.
 91. Laborda E et al. Development of a chimeric antigen receptor targeting C-type lectin-like molecule-1 for human acute myeloid leukemia. *Int J Mol Sci.* 2017;18(11).
 92. Darwish NH et al. Acute myeloid leukemia stem cell markers in prognosis and targeted therapy: Potential impact of BMI-1, TIM-3 and CLL-1. *Oncotarget.* 2016;7(36): 57811-20.
 93. Porter DL et al. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med.* 2011;365(8):725-33.
 94. Lee DW et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood.* 2014;124(2):188-95.
 95. Luo Y et al. First-in-Man CD123-Specific chimeric antigen receptor-modified T Cells for the treatment of refractory acute myeloid leukemia. *Blood.* 2015;126:3778.
 96. Topp MS et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: A multicentre, single-arm, Phase 2 study. *Lancet Oncol.* 2015;16(1):57-66.
 97. Curran KJ et al. Chimeric antigen receptors for T cell immunotherapy: Current understanding and future directions. *J Gene Med.* 2012;14(6):405-15.
 98. Hacein-Bey-Abina S et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science.* 2003;302(5644):415-9.
 99. Petrov JC et al. Compound CAR T-cells as a double-pronged approach for treating acute myeloid leukemia. *Leukemia.* 2018;32(6):1317-26.