Best Practice & Research Clinical Haematology 32 (2019) 101104

Contents lists available at ScienceDirect

Best Practice & Research Clinical Haematology

journal homepage: www.elsevier.com/locate/issn/15216926

How close are we to CAR T-cell therapy for AML?

Saar I. Gill

University of Pennsylvania, 3400 Civic Center Blvd, Philadelphia, PA, 19104, USA

ARTICLE INFO

Keywords:
Acute myeloid leukemia
AML
CAR
CART
Chimeric antigen receptor
Chimeric antigen receptor T cell
CD33
CD123

ABSTRACT

Chimeric antigen receptor (CAR) T-cell therapy for acute myeloid leukemia (AML) has thus far been elusive, in part owing to the absence of truly AML-specific surface antigens, making AML difficult to target. However, progress has been made toward the use of CAR T-cell therapy in this disease, prompting the topic of this paper. Discussion and clinical examples of potential solutions to creating a safe and effective CAR T cell for AML include: (1) Decreasing the potency or activity of CAR T cells to enhance the therapeutic window; (2) Using transient or depletable CAR T cells as part of pre-transplant conditioning; and (3) Using a gene-edited allogeneic donor hematopoietic stem cell transplant in order to allow safe and protracted anti-AML CAR T-cell function.

Introduction

The experience with chimeric antigen receptor (CAR) T-cell therapy (CART) in B-cell malignancies has provided some guidance for its use in acute myeloid leukemia (AML). Accumulating data suggest that, at least in the setting of B-cell acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL), functional CART cell persistence correlates with aplasia of normal and malignant B cells (the targets of anti-CD19 CAR T cells). In turn, relapse risk appears to be higher in those patients whose CART cells fail to persist [1,2].

CAR T-cell therapy of AML

AML cells express a variety of stem cell and myeloid differentiation antigens on the cell membrane, such as CD33, CD34, CD123, CD135 (FLT3) (Fig. 1). The same antigens are also expressed on normal myeloid cells— for example CD33 is also expressed on mature neutrophils, monocytes, and tissue-resident macrophages and CD123 is expressed on lymphoid progenitors as well as some differentiated lymphoid and myeloid cells. Based on clinical observations from clinical CART-19 data, it stands to reason that myeloid ablation should occur with the use of potent CAR T cells directed against CD33 and pan-myeloablation could occur if anti-CD123 CAR T cells were used.

This prediction is borne out by some experimental data [3,4]. We previously published our experience using a model of Non-Obese Diabetic Severe Combined Immuno Deficiency $\gamma_{chain}{}^{-/-}$ (NSG) mice that were engrafted with primary human AML cells or normal human CD34^{+} cells, and then treated with control T cells or with anti-CD33 or anti-CD123 CAR T cells. While AML was eradicated in mice treated with CAR T cells, we also noted eradication of normal hematopoiesis. Our overall conclusion from these studies was that the central problem of potent, antigen-specific immunotherapy for AML is the absence of truly AML-specific surface antigens. There are several theoretical solutions to this dilemma:

E-mail address: saargill@pennmedicine.upenn.edu.

https://doi.org/10.1016/j.beha.2019.101104

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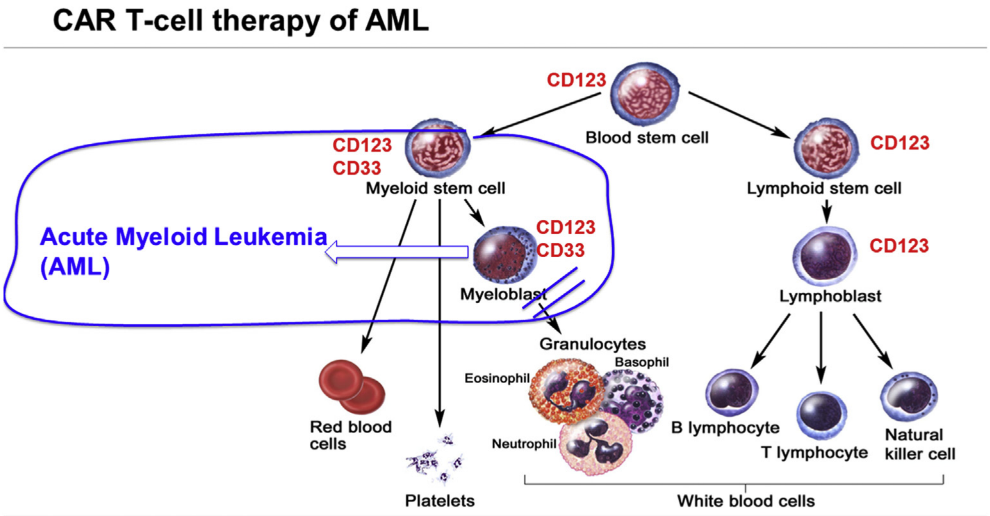

Fig. 1. Schematic of hematopoietic differentiation tree.

Fig. 1 Legend: In addition to other antigens, AML cells express CD33 and CD123 on their surface. These same antigens are also expressed on normal myeloid cells. Hypothetically, myeloid ablation would occur with the use of CAR T cells against CD33, and quite possibly pan myeloablation if anti-CD123 CAR T cells were used.

Solution 1: AML CART cells with attenuated activity

The first possible solution could be useful if there were truly differential levels of expression of the target antigen between normal and malignant cells [5]. Anti-AML CAR T cells could then be made with attenuated activity in the hope that a therapeutic window could be found that would allow targeting high CD123-expressing malignant cells without targeting medium-expressing CD123 normal cells. This sort of differential expression, however, does not exist for most myeloid antigens. Furthermore, this would likely be accompanied by antigen-dim relapse, a phenomenon that has been well-documented in a phase 1 trial using anti-CD22 CAR T cells in patients with pre-B cell acute lymphoblastic leukemia (ALL) [6] where most patients.

Investigators at the City of Hope are conducting a phase 1 trial of anti-CD123 CAR T therapy in AML [7]. Most patients had relapsed after prior allogeneic transplant and received escalating doses of donor-derived anti-CD123 CAR T cells at a dose that is likely in the therapeutic range (if one were to extrapolate from the CART-19 literature). Although the results have not yet been published, a review of the results from conference proceedings suggests that the minority of patients achieved a durable and deep complete remission.

There are several examples of pilot trials or case reports using CAR T cells for the treatment of AML. These targeted Lewis Y [8], CD33 [9], or CD123 [10,11] Yi Luo et al., Blood 2015; 126:3778 (American Society of Hematology annual meeting 2015) [AU: Please provide complete ref for Chang 2015]. We recently completed a study using "biodegradable" T cells that were electroporated with anti-CD123 CAR mRNA in order to diminish CAR persistence and thereby obviate the risk of myeloablation or endothelial toxicity [12]. A general conclusion from the few AML patients treated with CART cells worldwide is that, to date, anti-AML CAR T cells have shown limited activity. Therefore, strategies to reduce the activity of CAR T cells in order to potentially enhance the safety are not likely to work.

Solution 2: AML CART as conditioning regimen

A possible second solution is to use AML CAR T cells as a novel conditioning regimen prior to allogeneic stem cell transplantation. Since CAR T cells in xenograft models can induce myeloablation, an attractive strategy may be to construct potent but depletable anti-myeloid CAR T cells that could be used for elimination of AML and the residual normal hematopoiesis. Thus, a potential liability of myeloid-specific CART cells could be turned to our advantage. The patient would then undergo allogeneic stem cell transplant to reconstitute hematopoiesis.

A preliminary report from a Chinese group of a bispecific CAR T cell provided encouraging support for this strategy [13]. The bispecific CART targeted CD33 as well as CLL1 (also known as CLEC12A, a C-type lectin domain family 12 member A that is present on some AML cells). Two patients with relapsed/refractory AML received fludarabine and cyclophosphamide and then underwent two infusions of 1×10^6/kg of the bispeb bispecific CART cells. Both patients attained a minimal residual disease (MRD)-negative remission within 3 weeks of infusion, accompanied by the expected myeloablation. Both patients then successfully underwent anti-thymocyte globulin (ATG)-based allogeneic stem cell transplantation and were said to engraft normally. We postulate that incorporation of a T-cell depleting antibody in the conditioning regimen prevented the CART cells from rejecting the stem cell graft.

Four trials are being conducted at present in the United States with potentially depletable CAR T cells. Details of the four trials can be seen in Table 1.

Table 1
Clinical trials with transient or depletable CAR T cells.

Disease and Key Inclusion/Exclusion Criteria	Location	Trial Number	Intervention	AE Mitigation Strategy, Including Myeloablation
R/R AML - > 18 yo - AlloHSCT eligible with stem cell source identified - If relapsed after prior alloHSCT, must be off immune suppression and have no active GVHD (> Gr II)	University of Pennsylvania, Pennsylvania, USA	NCT03766126	Autologous lentivirally transduced anti CD123 CAR T cells (CD123CAR-41BB-CD3ζ)	- Fractionated dosing of CART-123 - Patient must have suitably matched donor or stem cell source available, alloHSCT expected to be required in responding patients
R/R AML or relapsed BPDCN - > 12 yo - AlloHSCT eligible with stem cell source identified - If relapsed after prior alloHSCT, must be off immune suppression	City of Hope Medical Center, California, USA	NCT02159495	Autologous lentivirally transduced anti CD123 CAR T cells (CD123CAR-CD28^{-}CD3ζ-EGFRt)	- EGFRt in CAR construct allows for in vivo eradication of CAR T-cell population if needed with anti-EGFR mAb - Patient must have a suitably matched donor or stem cell source available
R/R AML or ELN adverse AML in up-front treatment - 18–65 yo - If relapsed after prior alloHSCT, must be off immune suppression for 6 weeks, and have no evidence of GVHD - CD123(+) blasts by standard flow cytometry	MD Anderson Cancer Center, Texas, USA	NCT03190278	Universal (TCR KO) allogeneic anti CD123 CAR T-cells (UCART123)	- TCR KO to reduce risk of GVHD from allogeneic CAR T cells - Patient must have a suitably matched donor or stem cell source available
R/R AML - Pediatric 1–18 yo - Adult > 18–80 yo - If relapsed after prior alloHSCT, must be at least 3 mo post alloHSCT, be off immune suppression, and have no evidence of GVHD	MD Anderson Cancer Center, Texas, USA	NCT03126864	Autologous lentivirally transduced anti CD33 CAR T-cells	- Incremental dosing of CART-33 (starting dose in both cohorts is $> 1.5 \times 10^5$/kg and $< 4.5 \times 10^5$/kg)

Abbreviations: alloHSCT, allogeneic hematopoietic stem cell transplant; AML, acute myeloid leukemia; mo, month; BPDCN, blastic plasmacytoid dendritic cell neoplasm; EGFR, epidermal growth factor receptor; EGFRt, truncated epidermal growth factor receptor; Gr, grade; GVHD, graft-vs-host disease; KO, knockout; mAb, monoclonal antibody; R/R, relapsed/refractory; TCR, T-cell receptor; yo, years old.

The first trial is being conducted at the University of Pennsylvania (Penn) (NCT03766126) using an autologous, lentivirally transduced anti-CD123 CAR T cell. The strategy to mitigate potential adverse effects is to use fractionated dosing of the CAR T cells over 3 days. Patients must have a suitable transplant donor should they experience myeloablation from the CART therapy.

The second trial is being conducted at the City of Hope (NCT02159495) with a lentivirally transduced anti-CD123 CAR, incorporating an extracellular EGFR domain that could, if needed, be used to eliminate the T cells using an anti-EGFR antibody (cetuximab). Other active trials in the United States use autologous lentivirally transduced CD33 CAR T cells (NCT03126864), or target CD123 using healthy donor T cells that are engineered to lack the T-cell receptor (and should therefore be unable to cause graft-versus-host disease) (NCT03190278).

Transient or depletable CAR T cells, however, will not provide CAR T-cell-mediated immunosurveillance, as they do in B-cell malignancies. As noted above, in B-cell leukemias CART-19 cells have been shown to be capable of years-long functional persistence accompanied by aplasia of both normal and malignant B cells. Thus, limiting anti-myeloid CAR T cell persistence by depletion or subsequent allogeneic stem cell transplantation would likely deprive the patient of durable immunosurveillance.

Solution 3: anti-myeloid CAR T cells

A potential third solution is to make anti-myeloid CAR T cells and then co-infuse them with a hematopoietic system that is engineered to be resistant to CAR T cells. This strategy would effectively synthesize de novo a leukemia-specific antigen. Currently, CAR T cells are unable to discriminate malignant from normal stem progenitor cells (HSPC), since both populations bear the same antigen (for example, CD33). This leads to disease elimination that is accompanied by bone marrow toxicity. However, if allogeneic donor hematopoietic stem cells could be edited to lack a shared antigen without significant impairment of their function, the disease could be eliminated and “normal” hematopoiesis regenerated. This would then permit persistent circulating anti-myeloid CART cells that protect the patient from relapse without causing bone marrow failure.

This approach raises a number of questions: Can CD33 be removed from normal HSPC's without impairing their ability to self-renew and differentiate? Will CD33 knockout myeloid cells retain normal function? And will CD33 knockout HSPCs be resistant to CD33-targeted therapy? We recently published work that addresses all these questions [14]]. We showed that after CRISPR/Cas9-based editing, human CD34 $^{+}$ cells lacking CD33 differentiate and function normally in vitro and in xenograft models. We also found that the myeloid progeny of these HSPCs function normally and that the CD33 knockout myeloid cells are resistant to CD33 CAR T cells. In rhesus macaques, we have found normal engraftment without toxicity [14]. With a follow-up of 2 years, no leukemogenesis has occurred in the primates. A clinical trial using allogeneic donor-derived CD34-selected, CD33-knockout HSCT followed by CART-33 in patients with relapsed refractory AML is now being devised.

Next steps must include a careful assessment of potential off-target editing in HSPCs. Using an unbiased experimental off-target prediction model we have found a high degree of specificity for editing CD33 and we are working to validate these observations. The feasibility of this strategy is supported by several current clinical trials that edit hematopoietic stem cells for the treatment of thalassemia or sickle cell disease (eg, NCT03745287, NCT03655678). We predict that within a few years there will accrue a significant body of clinical experience using a variety of genetic tools to manipulate human CD34 $^{+}$ cells.

Conclusion

CAR T-cell therapy for AML is already here, but there remains a great deal of work to be done. Based on the last 8 years of research and investigation, we conclude that (i) no leukemia-specific surface marker exists in all patients, (ii) decreasing the potency or activity of CAR T cells is unlikely to generate a useful therapeutic window, and (iii) myeloablation is a likely consequence of potent anti-myeloid CAR T-cell therapy. Thus, two approaches seem feasible: the first is to use transient or depletable CAR T cells as part of pre-transplant conditioning. The second is to use a gene-edited allogeneic donor hematopoietic stem cell transplant in order to allow safe and protracted anti-AML CAR T-cell function, thereby effectively synthesizing a leukemia-specific antigen.

Disclosures

Research Funding: Novartis, Tmunity Therapeutics, Carisma Therapeutics.
Stock and Ownership: Carisma Therapeutics.
Consulting: Aileron, Fate, Sensei.

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