Chimeric antigen receptor T-cell therapy for acute myeloid leukemia: how close to reality?

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Introduction

The approval of the anti-CD19 chimeric antigen receptor (CAR) T-cell product tisagenlecleucel (Kymriah®) by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for relapsed pediatric B-lineage acute lymphoblastic leukemia (B-ALL) was a landmark event in acute leukemia therapy. The approval was based on data from a phase II global trial in which 75 pediatric and young adult B-ALL patients received tisagenlecleucel, demonstrating safety, feasibility and biological response, with complete remissions (CR) at three months in 81% of patients, and event-free survival rates of 73% and 50% at six and 12 months, respectively.1 A detailed summary of the design and basic biology of CAR T cells was recently published, providing an excellent summary of the history and the current state of the field of CAR T-cell therapy for the treatment of malignant diseases.2 Unfortunately, the successes of CAR T cells in treating B-ALL have not yet been translated to the treatment of acute myeloid leukemia (AML), where progress has been delayed by the lack of a suitable targetable surface antigen. In B-ALL and other B-cell malignancies, elimination of malignant B cells occurs alongside that of normal B cells/B-cell progenitors. B-cell depletion has been clinically tolerated for years, since the ensuing hypogammaglobulinemia is easily corrected. In stark contrast, elimination of normal myeloid cells/progenitors is unlikely to be tolerated for long, as the targeted AML antigen is frequently co-expressed on healthy hematopoietic stem/progenitor cells (HSPC), leading to ablation of all myeloid progeny. Creative solutions are being sought to overcome these obstacles in order to make CART therapy a viable option for patients with AML.

The current state of play: anti-acute myeloid leukemia chimeric antigen receptor T cells in the clinic

Thirteen CART trials for patients with AML are currently enrolling patients (Table 1), though none have yet yielded mature published data. The first trial demonstrating biological activity for CART in AML was published in 2013, evaluating a second-generation (CD28 co-stimulatory domain) retrovirally transduced anti-Lewis Y CAR T-cell.3 Four of five enrolled patients with relapsed/refractory (RR) AML received a mean 4.46x10⁹/kg CAR-positive T cells after lymphodepleting chemotherapy. The best responses achieved for each patient were: stable disease in two patients (for 49 days and 23 months respectively), reduction in blasts in one patient, and a cytogenetic remission in a patient with abnormal cytogenetics as the sole abnormality (i.e. blast count not elevated). Although all patients eventually progressed, this study was important as it demonstrated biological activity of CAR T cells against AML without significant hematopoietic toxicity, and the possibility of targeting a non-protein antigen.4

Two attractive targets for CART therapy in AML are CD33 and CD123. Both antigens are almost ubiquitous on AML blasts, though both are also present on normal HSPC.5 Published data for CART33 are limited; a case report from 2015 describes a 41-year old patient who had a transient response to CART33,7 and two patients who demonstrated a clinical response to a combined CD33-CLL1 CART (CLL1=C-type lectin molecule-1) were reported at the American Society of Hematology (ASH) annual meeting in 2018,8 though no data have been presented to date for the rest of this cohort (clinicaltrials.gov identifier: 03795779).

CD123 is a particularly attractive antigen due to both its expression on other hematologic malignancies,9 and its credentials as a potential marker of leukemia-initiating cells (LIC).10 Given the shared expression of CD123 on both healthy and malignant blasts, it is anticipated that patients responding to CART123 are likely to require a rescue allogeneic stem cell transplant (alloHSCT), a hypothesis supported by our pre-clinical data indicating myeloablation and AML eradication by
# Table 1. Chimeric antigen receptor (CAR) T-cell therapy trials for patients with acute myeloid leukemia open for enrollment.

| Disease key inclusion/ exclusion criteria | Location | Trial number | Intervention | Strategy to mitigate potential adverse events including myeloablation |
|------------------------------------------|----------|--------------|--------------|---------------------------------------------------------------------|
| RR AML                                   | The University of Pennsylvania, PA, USA | NCT03766126 | Autologous lentivirally transduced anti CD123 CARTs (CD123CAR-41BB-CD3) | - Fractionated dosing of CART-123  
- Patient must have a suitably matched donor or stem cell source available, alloHSCT expected to be required in responding patients |
| RR AML or relapsed BPDCN                 | City of Hope Medical Center, CA, USA | NCT02154945 | Autologous lentivirally transduced anti CD123 CARTs (CD123CAR-CD28-CD3-EGFRt) | - EGFRt in CAR construct allows for in vivo eradication of CART population if needed with anti-EGFR mAb  
- Patient must have a suitably matched donor or stem cell source available |
| RR AML or ELN adverse AML in up-front treatment | MD Anderson Cancer Center, TX, USA | NCT03190278 | Universal (TCR KO) allogeneic anti CD123 CARTs (UCART123) | - TCR KO to reduce risk of GvHD from allogeneic CARTs  
- Patient must have a suitably matched donor or stem cell source available |
| Relapsed AML after alloHSCT              | MD Anderson Cancer Center, TX, USA | NCT03126864 | Autologous lentivirally transduced anti CD33 CARTs | - Incremental dosing of CART-33 (starting dose in both cohorts is >1.5 x10^5/kg and <4.5 x10^9/kg) |
| Relapsed AML after alloHSCT              | City of Hope Medical Command, Chengdu, China | NCT03114670 | Allogeneic (donor derived) lentivirally transduced anti CD123 CARTs (CD123CAR-41BB-CD3-EGFRt) | - EGFRt in CAR construct allows for in vivo eradication of CART population if needed with anti-EGFR mAb |
| RR AML, MDS, MPN, CML or other hematologic malignancy | The General Hospital of Western Theater Command, Fujian, China | NCT03785779 | Lentivirally transduced anti CD33 and CLL1 compound CARTs* | - Not stated |
| RR AML                                  | Fujian Medical University Union Hospital, Fujian, China | NCT03531576 | Anti CD123 and CLL1 compound CARTs** | - Not stated |
| RR AML                                  | 307 Hospital of PLA, Beijing, China | NCT03556982 | Allogeneic or autologous anti CD123 CARTs* | - Not stated |
| RR AML                                  | Hebei Yanda Ludaopei Hospital, Hebei, China | NCT03786390 | Autologous lentivirally transduced anti CD123 CARTs | - Not stated |
| RR AML                                  | Southern Medical University Zhuijiang Hospital, Guangdong, China | NCT03473457 | CARTs targeting CD33, CD38, CD56, CD117, CD123, CD34 or Mucl | - Not stated |

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CART123 go hand-in-hand.\(^{-11}\) In addition to likely hematopoietic toxicity, we and others have detected CD123 expression on the endothelium of small-calibre blood vessels.\(^{-12}\) This raises the concern for additional toxicity in the form of vascular leak, and indeed, the first patient who was treated with an anti-CD123 “universal” CART (UCART123) died from cytokine release syndrome (CRS) and capillary leak syndrome (CLS) on day 9 post infusion.\(^{-13}\) It is unclear if his death was due to CD123 vascular expression, or was multi-factorial due to CRS exacerbated by the patient’s age (78 years) and the extent of disease burden. It is important to note that severe CRS has clinical overlap with CLS. The FDA allowed the trial to re-open with a log-fold reduction in UCART123 dose, reduced lymphodepleting chemotherapy dose, and an upper limit for age of enrollment of 65 years old. In order to mitigate the risk of vascular toxicity, the first CART123 trial at the University of Pennsylvania was conducted using serial infusions of “bio-degradable” CART123 cells (clinicaltrials.gov identifier: 02623582). Rather than being transduced with lentivirus, which would endow the CART population the (usually desirable) capacity of exponential expansion \(in \text{vivo}\), these CART123 cells were manufactured by electroporation of mRNA encoding the CAR. Thus, a CART123 cell stimulated by encountering its antigen would have a finite capacity to expand, since CAR mRNA is diluted between daughter T cells. Though its antigen would have a finite capacity to expand, since CART123 go hand-in-hand.\(^{-11}\) In addition to likely hematopoietic toxicity, we and others have detected CD123 expression on the endothelium of small-calibre blood vessels.\(^{-12}\) This raises the concern for additional toxicity in the form of vascular leak, and indeed, the first patient who was treated with an anti-CD123 “universal” CART (UCART123) died from cytokine release syndrome (CRS) and capillary leak syndrome (CLS) on day 9 post infusion.\(^{-13}\) It is unclear if his death was due to CD123 vascular expression, or was multi-factorial due to CRS exacerbated by the patient’s age (78 years) and the extent of disease burden. It is important to note that severe CRS has clinical overlap with CLS. The FDA allowed the trial to re-open with a log-fold reduction in UCART123 dose, reduced lymphodepleting chemotherapy dose, and an upper limit for age of enrollment of 65 years old. In order to mitigate the risk of vascular toxicity, the first CART123 trial at the University of Pennsylvania was conducted using serial infusions of “bio-degradable” CART123 cells (clinicaltrials.gov identifier: 02623582). Rather than being transduced with lentivirus, which would endow the CART population the (usually desirable) capacity of exponential expansion \(in \text{vivo}\), these CART123 cells were manufactured by electroporation of mRNA encoding the CAR. Thus, a CART123 cell stimulated by encountering its antigen would have a finite capacity to expand, since CAR mRNA is diluted between daughter T cells. Though there was no measurable anti-leukemic activity responses in this trial, evidence of CART bioactivity was manifest by fever, CRS and transient CART detection \(in \text{vivo}\), without evidence of vascular toxicity or CLS.\(^{-14}\) This favorable safety data paved the way for a phase I trial of lentivirally-transduced second-generation CART123 (CD123CAR-4BB-CD3ζ) which has begun enrollment at the University of Pennsylvania (clinicaltrials.gov identifier: 03766126). CART123 are infused with the intention of a strategy and inter-patient dose escalation from 50x10⁶ CAR⁺ cells (dose level 1, DL1) to 200x10⁶ CAR⁺ cells (dose level 2, DL2). Interim data were reported at the end of 2018; seven patients with AML have now been treated.\(^{-15}\) Of the two patients treated at DL1, one achieved a morphological leukemia-free state (MLFS) lasting 70 days, and at recurrence of disease received a second CART123 infusion which reduced the blast count (from 77.9% to 0.9% by flow cytometry). Of the five patients treated at DL2, one patient achieved a complete remission with incomplete count recovery (CRi) at day 28, and one had a CR at day 84. Three patients had stable disease. No dose-limiting toxicities were reported, and all treatment-related cytopenias resolved by 12 weeks post treatment. No CD123-negative relapses have been observed to date, and longer-term data are awaited from this pioneering study.

### New paradigms of chimeric antigen receptor T cells in acute myeloid leukemia

**NKG2D ligand chimeric antigen receptor T cells**

Given the paucity of suitable ‘traditional’ cell surface antigens in AML, alternative strategies to harness the potential of CART therapy for AML are needed. Natural killer group 2D (NKG2D) ligands are expressed on malignant cells and have a role in stimulating anti-tumor immunity, but have limited expression on healthy tissues, providing a potential target for CART therapy.\(^{-16,17}\) However, many different types of cellular stress (including inflammation) can up-regulate NKG2D ligands on normal tissues,\(^{-18,19}\) potentially reducing specificity of NKG2D-CARs for malignant tissues due to CART-induced CRS. Investigators at the Dana-Farber Cancer Institute of Eps8 or WT1 peptide specific events including myeloablation.

**RR AML or ALL**

- Child, adult, older adult (ages not stated)
- if relapsed post prior alloHSCT must be off immune suppression at least 2 wks

**RR AML or ALL**

- 18-80 yo
- High expression of Eps8 or WT1
- Ineligible for or declining salvage alloHSCT

**RR AML / ALL or MDS**

- 18-80 yo
- High expression of Eps8 or WT1
- Ineligible for or declining salvage alloHSCT

**RR AML or ALL**

- 3 - 80 yo
- CD123 (+) in >50% of blasts

| Disease and key inclusion/ exclusion criteria | Location | Trial number | Intervention | Strategy to mitigate potential adverse events including myeloablation |
|---------------------------------------------|----------|--------------|--------------|---------------------------------------------------------------------|
| RR AML / ALL or MDS                         | Southern Medical University | NCT03291444 | CARTs** (antigen target not stated) followed by intradermal injection of Eps8 or WTI peptide specific dendritic cells | Not stated |
| RR AML or ALL                               | Second Affiliated Hospital of Xi’an, Shaanxi, China | NCT03672851 | Anti CD123 CARTs** | Not stated |
| RR AML                                      | Xian Lu, Beijing, China | NCT03585517 | Anti CD123 CARTs** (IM23) | Not stated |

Information available from [www.clinicaltrials.gov](http://www.clinicaltrials.gov) using search term “CART” and “AML” in January 2019. *Source of cells (autologous vs. allogeneic): not stated. #Method of chimeric antigen receptor (CAR) transduction not stated. AML: acute lymphoblastic leukemia; alloHSCT: allogeneic hematopoietic stem cell transplantation; AML: acute myeloid leukemia; BPCD3: blastic plasmacytoid dendritic cell neoplasm; CML: chronic myeloid leukemia; EGFRt: epidermal growth factor receptor; ELN: European LeukemiaNet; Gr: grade; GvHD: graft-versus-host disease; IHC: immunohistochemistry; KO: knock-out; mAb: monoclonal antibody; MDS: myelodysplastic syndrome; mo: months; MPN: myeloproliferative neoplasms; PBMC: peripheral blood mononuclear cells; RR: relapsed refractory; TCR: T-cell receptor; wks: weeks; yo: years old.
Institute reported a phase I clinical trial of autologous NKG2D-CAR T-cells in seven patients with AML.\(^1\) Their first-generation construct (CAR-NKG2D-CD3\(\zeta\)) uses the naturally-occurring NKG2D receptor as the antigen-binding domain, with endogenous DAP10 expression providing co-stimulation.\(^2\) CART-NKG2D cells were successfully manufactured in all seven patients, and median infused cell counts showed AML cell killing in vitro when two targets were present on the same cells, and are thus potentially amenable to a combinatorial CAR approach.\(^3\) The antigen CLL-1, which was targeted in the above-mentioned combinatorial approach in combination with CD33 by Liu \textit{et al.}\(^4\) also has potential utility as a stand-alone antigen, with pre-clinical data suggesting that differential expression between malignant and healthy blasts may be sufficient to preserve hematopoiesis.\(^5,6\) No clinical CAR T-cell trials targeting CLL-1 alone are currently open, though a phase I trial evaluating a CLL1-CD3\(\zeta\) conjugate have been the expected hematopoietic toxicity, and also hepatotoxicity and veno-occlusive disease, with both being likely attributable to the calicheamicin component of the therapy, rather than due to targeting of CD33 hepatic cells.\(^7\) There is evidence to suggest that CD33 has a role in the modulation of inflammatory and immune response,\(^8\) and the potential for this to impact the incidence of severity of CRS response when CART33 are administered after engraftment with CD33KO-HSCP will be actively considered during the conduct of the trial.

**Identifying new target antigens**

The lack of ideal antigens for CART therapy in AML spurred a search for new ones. Investigators from the Memorial Sloan-Kettering Cancer (MSKCC) sought to identify sets of antigens suitable for targeting with a combinatorial CART strategy,\(^9\) by which CAR T cells express a dual-specific CAR (or 2 different CAR transduced into the same cell) directed against two different target antigens with non-overlapping expression in normal tissues. When both antigens are encountered by the dual-specific CART, the potency of cell killing is increased relative to that seen if only one antigen is present; the design of the combinatorial CART must therefore comprise two target antigens that are specifically co-expressed in malignant cells. The MSKCC group identified four promising targets, ADGRE2, CCR1, CD70 and LILRB2, which satisfied their criteria for suitable off-tumor expression that also demonstrated AML cell killing \textit{in vitro} when two targets were present on the same cells, and are thus potentially amenable to a combinatorial CAR approach.\(^10\)

**Bone marrow transplant with a gene-edited allograft followed by chimeric antigen receptor T cells**

The provision of a rescue alloHSCT after \textit{in vivo} depletion of the CART population is a potential solution to the problem of hematopoietic toxicity, though it may create a new problem, which is placing a limit on the duration of \textit{in vivo} persistence of the CART population. The accrued experience with CART-19 in B-ALL suggests that the optimal period of time for CART persistence for disease response is at least 2-3 months,\(^11\) or potentially as long as 8-10 months.\(^12\) A new approach to allow long-term CART against myeloid antigens, such as CD33, is to edit out the antigen from allogeneic donor HSCP, which are then transplanted into the patient with AML. Following engraftment, the patient may then be treated with CART33 manufactured from the same allogeneic donor. Our research group has demonstrated that CD33 can be removed from HSPC using CRISPR/Cas9 without impairment of hematopoietic or immunological function, and that the CD33 knock-out HSCP (and their progeny) are impervious to attack by CART33 both \textit{in vitro} and \textit{in vivo} in murine and non-human primate models.\(^13\) This treatment approach, currently under development at the University of Pennsylvania (see Figure 1 for a summary) would allow for long-term CART persistence while also protecting normal hematopoiesis. It remains to be seen if this approach is feasible from both a manufacturing and a clinical perspective, and indeed what the clinical consequences of CD33 depletion would be in the bone marrow compartment, and by the depletion of CD33+ tissue resident macrophages. Several publications have demonstrated that donor-derived cells eventually repopulate the visceral resident macrophage niche; specifically, Kupffer cells,\(^14\) pulmonary alveolar cells,\(^15\) and microglia.\(^16\) Data are limited as to the time taken for repopulation of these cells, with published data in the lung suggesting this may take 2-3 months after alloHSCT alone.\(^17\) It is noted that the main toxicities associated with gemtuzumab ozogamicin (Mylotarg\(^\text{®}\), an anti-CD33 antibody-drug conjugate) have been the expected hematopoietic toxicity, and also hepatotoxicity and veno-occlusive disease, with both being likely attributable to the calicheamicin component of the therapy, rather than due to targeting of CD33 hepatic cells.\(^18\) There is evidence to suggest that CD33 has a role in the modulation of inflammatory and immune response,\(^19\) and the potential for this to impact the incidence of severity of CRS response when CART33 are administered after engraftment with CD33KO-HSCP will be actively considered during the conduct of the trial.

**Other immunotherapy options for acute myeloid leukemia**

Given the challenges of treating AML with CART T cells, as outlined above, many investigator groups are also exploring other types of adoptive cellular therapy that have different therapeutic mechanisms, and may be less hampered than this issue of antigen specificity that are currently limiting CAR T-cell therapy for AML. By transducing T cells with the \(\alpha\) and \(\beta\) chains of a known specificity T-cell receptor (TCR), engineered TCR cells (TCR-T) can be endowed with specificity to known tumor-associated antigens (TAA) or conceivably, neoantigens. The TCR chains may be cloned from patients or normal donors that exhibit an immune response to the TAA, and may be further affinity-enhanced in order to confer enhanced reactivity to the target.\(^20,21\)
A unique advantage of TCR-T over CAR-T is their ability to recognize intracellular antigens that are presented on the MHC of cancer cells, thus theoretically increasing their anti-tumor specificity. While AML has a relatively low mutational burden of AML, and thus relatively few neoantigens that could be targeted, a possibly viable target is the TAA tumor-associated antigen Wilms’ tumor 1 (WT1). Several groups have demonstrated safety and a measure of efficacy in early phase trials of patients with AML/MDS, though disease responses were transient.

A new phase I/II trial evaluating WT1 TCR-T in combination with IL-2 in patients with AML/MDS was recently completed (clinicaltrials.gov identifier: 02550535) and the results are eagerly awaited.

Given the enormous efforts to drug the PD/PDL1 axis in solid cancers, interest has also turned to this approach in AML. PD-L1 was shown to be up-regulated on blasts in an analysis of 55 samples selected for their high white cell count, with the hypothesis that up-regulated PD-L1 was coupled with leukocytosis due to failure of the immune response. However, PD1-inhibition in AML has so far failed to yield convincing responses.

Another potential avenue for controlling AML with the immune system is by vaccination, though strong clinical data here are also currently lacking. Vaccination against WT1 with a leukemia-specific peptide combined with an adjuvant failed to show any demonstrable effect of clinical response in a small trial of AML patients and another similarly designed trial was stopped after the first four patients failed to show any clinical response (clinicaltrials.gov identifier: 00433745). An alternative form of vaccination is a novel cell therapy involving the generation of autologous dendritic cell / AML fusion cells ex vivo which are then re-infused with the intention of expanding AML-reactive T cells in vivo. Safety and tolerability were demonstrated in a small trial of AML patients who achieved remission after standard induction chemotherapy (patients who did not achieve a CR were excluded from the trial), and in a recent update, 12 of 17 vaccinated patients (71%) remained in remission with a median follow up of 57 months. These findings suggest that vaccination could be a useful consolidative therapy for patients achieving a CR, perhaps in those at high risk for relapse. This approach is currently being evaluated in a post-alloHSCT setting, both as single agent therapy and in combination with decitabine (clinicaltrials.gov identifier: 03679650). Overall, however, data that vaccination can directly mediate an anti-leukemia response in patients with active disease are lacking.

How will acute myeloid leukemia respond to chimeric antigen receptor T-cell therapy?

The potential for T-cell mediated killing of AML is manifest in the induction of long-term remissions in some patients after alloHSCT and donor lymphocyte infusions, suggesting AML can indeed be susceptible to T-cell mediated effects, and by extension, to CART therapy. However, alloHSCT fails to induce a sufficient graft-versus-leukemia effect in some patients with AML having demonstrated numerous mechanisms of resistance to therapy over the years, including loss of HLA molecules in relapse post alloHSCT, upregulation of anti-apoptotic proteins, downregulation of antigen expression, and changes in T-cell populations, including T-cell exhaustion and the expansion of regulatory T cells. It remains to be seen what immune evasion mechanisms AML will generate in response to CART therapy, though it is likely that many of the mechanisms of resistance to or relapse following CART therapy in other diseases will also be seen in AML, such as relapse with antigen loss, generation of
an immuno-suppressive microenvironment, failure of persistence of the CART population, or unacceptable on-target off-tumor toxicity. As early results from CART trials for AML become available in the coming years, we will gain a better understanding of their relative role vis-à-vis that of other recently approved therapies for this disease targeted against specific genetic lesions (FLT3, IDH1/2) or survival pathways (BCL2). The T-cell manufacturing process is complicated and time-consuming, with the median time from enrollment to infusion in the ELIANA trial of CART-19 being 45 days (range 30-105 days), and so the availability of well-tolerated, targeted, non-immunosuppressive therapies may improve the feasibility of bridging patients to potentially curative immunotherapy by providing disease control and clinical stability.

How close are we ... really?

Although the first CART trials for AML are now appearing in the clinical sphere, it is likely that other barriers will need to be overcome before this therapy becomes widely available. It remains to be seen if the ‘bridge to transplant’ approach is feasible, if it provides a sufficient duration of CART persistence, and how the on-target off-tumor toxicity of the various constructs, combinations and target antigens will be tolerated. We suspect that CART therapy will not be suitable or efficacious for all patients with AML, for example, frail elderly patients who are at higher risk of toxicity, or for those lacking access to expensive personalized therapies, by virtue of geographic and economic factors. However, select patients with AML are now being treated on CART trials and in the next 1-2 years data are likely to tell us more about the patient, disease and treatment characteristics that can predict success in this arena. We think of the future of CART cell therapy for AML as the next step in alloHSCT: a complex, resource-intensive but feasible approach intended to provide curative therapy to selected patients. In addition, the lessons learned in treating AML with CAR T cells may reveal other targetable pathways to be exploited in combination with immune-based or pharmacological therapies. We await the impact of CART therapy on AML with cautious optimism, noting the recent shower of drug approvals that followed a long dry spell in AML therapeutics. We are hopeful that the combination of alloHSCT and CAR T-cell therapy (the old master and new arrival in adoptive cellular therapy) may prove to be the key to unlocking relapsed-refractory AML.

We, and many others, continue to create and develop new solutions to make CAR T cells for AML a safe, deliverable and effective reality.

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