

# Acute myeloid leukemia therapeutics CARs in the driver's seat

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Acute myeloid leukemia remains a difficult disease to cure and novel therapeutic approaches are needed. To this end, we developed CD123 chimeric antigen receptor (CAR) redirected T cells which exhibited potent antileukemic activity. We discuss what we learned during the development of CD123 CARs and future directions for this immunotherapy.

Chimeric antigen receptors (CARs) are synthetic molecules consisting of an extracellular antigen-binding domain (most often derived from variable heavy and light regions of a monoclonal antibody) fused via a spacer region to intracellular signaling domains. Second generation CARs contain 2 intracellular signaling domains: the endodomain from a T cell costimulatory molecule such as CD28, 4-1BB, or OX40, and the intracellular domain of CD3 $\zeta$ . CAR-expressing T cells target cell surface antigens in a major histocompatibility complex independent manner. Several groups have developed CAR T cells specific for the B cell antigen CD19, and have observed encouraging antitumor responses in phase I clinical trials (Reviewed in ref. 1). Jordan and collaborators were the first to demonstrate that the interleukin-3 receptor  $\alpha$  chain (IL3RA, also known as CD123) is overexpressed on acute myeloid leukemia (AML) cells as compared to normal bone marrow.<sup>2</sup> This seminal finding initiated the development of CD123-targeting reagents including monoclonal antibodies and recombinant immunotoxins which showed promise in preclinical evaluations.<sup>3,4</sup> Phase I clinical trials utilizing these reagents have reported antileukemic responses in some patients highlighting the potential of immunotherapies for AML. Hence, the promise of CAR T cells, the unique

expression patterns of CD123, and the need for additional immunotherapies specific for AML prompted our group to develop CAR T cell products targeting CD123 (Fig. 1).<sup>5</sup>

Several recent studies demonstrate the importance of evaluating multiple CARs consisting of various single chain variable fragments (scFvs). In the case of CD22 and receptor tyrosine kinase-like orphan receptor 1 (ROR1), the use of a particular scFv led to superior antitumor effects observed both in vitro and in vivo.<sup>6,7</sup> Although we observed minor differences in cytokine secretion and in vitro killing of primary AML samples between the 2 CD123 CARs we generated (26292 and 32716), these differences might not be predictive for CAR T cell activity in vivo. Therefore, we tested both CAR constructs in all in vivo experiments as well. In the KG1a xenogeneic mouse model presented in our study and in a MV4-11 xenogeneic model of human AML (unpublished data) we have yet to observe significant differences in the overall survival of mice treated with 26292- and 32716-CAR expressing T cells. Compared to CD22, the CD123 extracellular domain is relatively short. Allocation of the epitope where the CAR binds on CD123 may not result in qualitative changes in distance between the T cell and its target as is the case with CD22. Furthermore, the binding

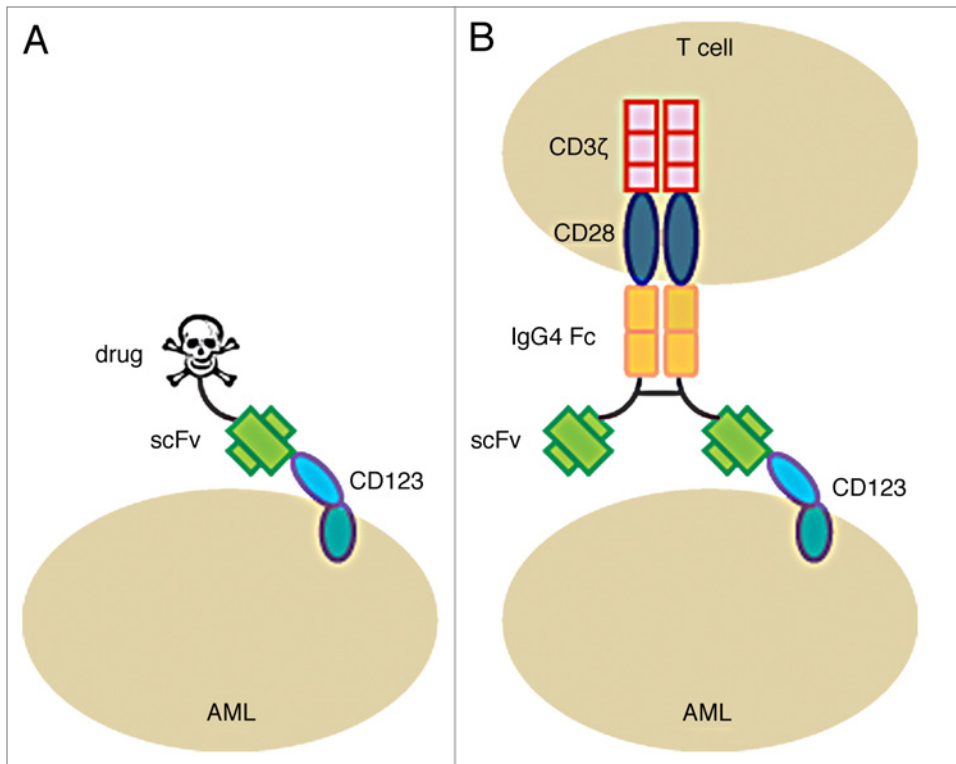
affinities of 26292 and 32716 scFvs for CD123 differ by less than 3-fold, which likely is not large enough to influence effector activity as seen with ROR1 CARs using scFvs with an approximately 50-fold difference in binding affinity.<sup>7</sup> An interesting finding in our study is that the 32716-scFv-based CAR T cell exhibited potent antileukemia activity while a previous study demonstrated that a 32716-scFv-based immunotoxin had little activity against a CD123<sup>+</sup> cell line.<sup>3</sup> This result raises the possibility of resurrecting scFvs that were once thought to lack potency as immunotoxins and give them new life as CARs.

Two common costimulatory signaling domains used in CAR design are derived from either CD28 or 4-1BB. The apparent in vivo differences between these 2 costimulatory domains are highlighted in recent findings from acute leukemia patients treated with second generation CD19 CAR T cells.<sup>8,9</sup> CAR T cells receiving costimulation from 4-1BB exhibited superior persistence compared to CAR T cells with CD28 derived costimulation. Nonetheless, dramatic antitumor responses were observed regardless of the costimulatory endodomain used. With respect to targeting CD123, long-term persistence of CAR T cells may not be desirable since CD123 is expressed on common myeloid progenitor (CMP) cells. Continuous

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**Figure 1.** Single chain variable fragment-based targeting of CD123. **(A)** The CD123-specific single chain variable fragments (scFvs) 26292 and 32716 were originally incorporated as recombinant immunotoxins targeting CD123<sup>+</sup> acute myeloid leukemia (AML). **(B)** Schematic representation of our CD123 chimeric antigen receptor (CAR) T cell binding to CD123 on the surface of an AML cell. The CARs we developed include intracellular CD28 derived costimulatory and CD3 $\zeta$  signaling domains. The extracellular portion of our CD123 CARs consists of a modified hinge-CH2-CH3 spacer derived from the Fc domain of IgG4 and either of 2 scFvs targeting CD123 (26292 or 32716).

killing of CMPs may result in unwanted prolonged cytopenias. Therefore, we plan to move forward with a CD28-derived costimulatory domain in our CD123 CAR design.

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Disease relapse and resistance to currently used chemotherapeutics are major obstacles in the treatment of AML.<sup>10</sup> In our study, 7 of 9 primary AML patient samples used were from

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patients with relapsed or refractory diseases of various risk features. All chemotherapy-resistant samples were susceptible to CAR T cell mediated killing, albeit at varying levels. Chromosomal abnormalities did not predict the susceptibility of leukemic cells to CAR T cell cytolysis. Together, these results suggest that chemoresistance does not confer immunologic resistance, and that CD123 CAR T cells have the potential advantage to treat a broad spectrum of AMLs refractory to conventional therapeutics.

Collectively, we demonstrate that CD123 CAR T cells exhibit robust antitumor activity against a variety of AML samples in a preclinical setting. Careful planning of dosage and timing of CD123 CAR T cells will be critical in utilizing these potent antileukemic T cells in treating AML while minimizing unwanted toxicities to hematopoiesis. Our findings establish a rationale for proceeding with CD123 CAR T cells for the treatment of AML. Planning of a phase I clinical trial to treat patients with relapsed or refractory AMLs using our CD123 CAR T cells is underway.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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