Employing Synthetic T-cell Biology to Target AML without On-Target/Off-Cancer Toxicity

M. Paulina Velasquez and Stephen Gottschalk

Summary: Ideal targets for chimeric antigen receptor T-cell therapy for acute myeloid leukemia (AML) remain elusive. In this issue of Blood Cancer Discovery, Richards and colleagues explore CD93 as a potential AML target antigen, and devise an approach to mitigate “on-target/off-cancer toxicity.”

See related article by Richards et al. (5).

With four FDA-approved chimeric antigen receptor (CAR) T-cell products targeting CD19, CD19 CAR T cells have become an integral part of our treatment armamentarium for B-cell malignancies (1). In addition, B-cell maturation antigen (BCMA) CAR T cells for multiple myeloma have been recently approved by the FDA, and early-phase clinical studies targeting CD30 for lymphoma, and CD7 for T-cell malignancies, have shown promising antitumor activity. In contrast, CAR T-cell therapy for acute myeloid leukemia (AML) remains in its infancy, and significant roadblocks have emerged, including suitable antigens for targeting AML blasts as well as the immunosuppressive AML microenvironment (2–4).

In their elegant work in this issue of Blood Cancer Discovery, Richards and colleagues focus on the AML target antigen dilemma (5). Currently, several AML targets are actively being pursued, including Lewis-Y (LeY), CD33, CD38, CD123, and CLL1 (4). These are less than ideal because they are either expressed on hematopoietic progenitor cells (HPC; e.g., CD33 or CD123), mature neutrophils (e.g., CL11), or activated immune cells (e.g., CD70). Nevertheless, several CAR T-cell products targeting CD33, CD123, or LeY have been or are actively being explored in early-phase clinical studies. However, despite potent anti-AML activity in preclinical AML xenograft models, emerging data from clinical studies have shown limited efficacy (6, 7), and are performed due to the inherent, potential “on-target/off-cancer toxicity” in the bridge-to-hematopoietic cell transplant (HCT) setting.

To discover suitable targets for AML-redacted CAR T-cell therapy, several groups have pursued multi-omics-based approaches and have concluded that finding a single AML-specific target might be challenging, and that strategies should rather focus on targeting at least two antigens to achieve the desired specificity (2). Richards and colleagues explore in their article CD93 as a CAR target for AML (5). CD93 is a C-type lectin transmembrane receptor, which is expressed not only in bulk AML blasts but also in leukemia stem cells (LSC; ref. 8). It presents a promising target because it is involved in leukemogenesis and maintenance of the malignant phenotype, which should reduce the risk of immune escape, and is not expressed on normal HPCs. They generate a humanized, CD93-specific single-chain variable fragment (scFv) and design four different CD93 CAR vectors, based on different orientations of the light and heavy chains of the scFv (VH-VL or V L-VH) and the costimulatory domain (CD28 or 41BB). In coculture assays of CD93 CAR T cells with antigen-positive targets, they determined that cytokine secretion correlated with antigen expression density and that there was no difference in cytokine secretion or antitumor activity among the evaluated CAR T-cell populations. However, they did note an increase in PD-1 and TIM3 expression in T cells expressing CD93 CAR with CD28 signaling domains. To confirm these findings in vivo, xenograft mice were sublethally irradiated and injected with a CD93-positive AML cell line. Infusion of CD93 CAR T cells resulted in rapid tumor clearance regardless of costimulatory domain. However, T cells expressing CARs with CD28 signaling domains maintained antitumor activity longer, correlating with increased T-cell presence at day 14. In addition, they established a CD93-positive patient-derived xenograft model and showed that CD93 CAR T cells had robust antitumor activity, with only 3 of 35 mice in the treatment groups succumbing to documented CD93-positive disease.

No toxicity was observed in any of the animal experiments. However, given the lack of cross-reactivity of the used CD93-specific scFv with murine CD93, CD93 CAR T-cell toxicity on HPCs and other tissues was further evaluated in vitro. Colony-forming unit (CFU) assays and coculture assays with cord blood–derived CD34-positive cells either untreated or exposed to effector cells (mock transduced or CD93 CAR T cells) showed no difference among groups, consistent with the lack of CD93 expression on HPCs. IHC evaluation of tissue microarrays revealed CD93 expression in endothelial cells, which was confirmed by gene expression analysis of healthy lung and pancreatic tissue confirmed these findings.
Of interest, the authors found that other AML antigens, which are currently being explored as CAR targets, including CD123 and CD38, are also expressed in endothelial cells. On the basis of these findings, Richards and colleagues set out to evaluate endothelial toxicity of CD93 CAR T cells in vitro. They show that CD93 CAR T cells recognize endothelial cells as judged by cytokine production, and that CD123 and CD38 are upregulated on endothelial cells in the presence of increasing concentrations of IFNγ in contrast to CD93. Their finding that other AML targets are also expressed on the cell surface of endothelial cells is intriguing. It most likely reflects that endothelial cells and HPCs are developmentally linked, sharing a common precursor called the hemangioblast. Thus, endothelial toxicity, rather than toxicity to parenchymal cells, needs to be considered for all antigens that are being explored for AML-redirected CAR T-cell therapy.

Thus, targeting CD93 with a standard CAR T-cell therapy approach does not solve the “AML target antigen dilemma,” because adoptively transferred CD93 CAR T cells target endothelial cells, which could potentially lead to side effects such as capillary leak syndrome. Several approaches have been developed to prevent “on-target/off-cancer” toxicity, including conditional expression systems and Boolean logic–gated CARs. Three logic gates are actively being explored including “AND gate,” “Sequential AND gate,” and “NOT gate” CARs (9). Examples of gated CARs include costimulatory CARs (AND gate), SynNotch receptors (Sequential AND gate), and inhibitory CARs (iCAR; NOT gate; ref. 9). To potentially overcome endothelial toxicity of CD93 CAR T cells, the authors focus here on iCARs, which have the same structure as standard, activating CARs, consisting of antigen-binding, hinge/transmembrane, and signaling domains. However, in contrast to standard, activating CARs, the signaling domain of iCARs contains immunoreceptor tyrosine-based inhibitory motif (ITIM)–based endodomain derived from T-cell inhibitory molecules such as PD1 or TIGIT, rather than tyrosine-based activating motifs (ITAM). Thus, T cells expressing an AML-specific CAR and an endothelial cell antigen-specific iCAR, are inhibited in the presence of endothelial cells even if these cells also express the targeted AML antigen.

Figure 1. NOT-gated CARs to prevent endothelial toxicity of CD93 CAR T cells. A, Concept of NOT-gated, inhibitory CAR (iCAR) T-cell therapy. iCARs have the same structure as standard, activating CARs, consisting of antigen-binding, hinge/transmembrane, and signaling domains. However, the signaling domain is inhibitory, containing immunoreceptor tyrosine-based inhibitory and not activating motifs (ITIM versus ITAM). Thus, T cells expressing an AML-specific CAR and an endothelial cell antigen-specific iCAR, are inhibited in the presence of endothelial cells even if these cells also express the targeted AML antigen. B, Discovery of suitable antigens for iCAR T-cell therapy approach for AML.

In summary, the work by Richards and colleagues presents a significant step forward in our quest to develop a safe and effective CAR T-cell therapy approach for AML. However, based on our experience so far, we still have a long, windy road ahead of us before CAR T-cell therapy will become a mainstay in our treatment armamentarium for AML.

Authors’ Disclosures

M.P. Velasquez reports a patent for PCT/US20/27719 pending. S. Gottschalk reports personal fees from Immatics, Tessa Therapeutics, Catamaran Bio, Nektar Therapeutics, and Novartis outside the submitted work.

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