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Spotlight

CD70 CAR T cells in AML: Form follows function

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Using a multimodal approach toward developing a new CD70-targeted Chimeric antigen receptor (CAR) T cell in acute myeloid leukemia, Leick et al.[1] report on their synergetic strategy, which incorporates both CAR T cell construct modifications with enhancement of leukemia antigen expression to improve CAR T cell functionality.

The development of effective therapies for those with relapsed/refractory (r/r) acute myeloid leukemia (AML) remains challenging, warranting a need for novel approaches. Chimeric antigen receptor (CAR) T cells represent one such strategy. Based on the impressive outcomes with CAR T cells in B cell malignancies and targetable antigens in AML, extending the therapeutic index of CAR T cells to AML holds promise.

A major limitation in targeting AML, however, is that on-target, off-tumor toxicities may not be tolerable, particularly because antigens expressed on AML (e.g., CD123 and CD33) are often expressed on normal hematopoietic progenitors and CAR T cell targeting may impair subsequent hematopoiesis. Additionally, the early experience with CAR T cells in AML has been largely disappointing,[2] collectively necessitating further study in identifying more optimal targets and ways to improve efficacy.

To address the challenge of CAR T cells in AML, Leick and colleagues[1] targeted the CD70-CD27 axis, specifically because CD70 is both absent on normal hematopoietic cells and highly expressed on AML blasts,[3] and the experience with antibody-based approaches targeting CD70 has been promising.[4] They subsequently used an orthogonal approach to both rationally design CAR T cells with improved efficacy and incorporate pharmacodynamic regulation of leukemia antigen expression to synergize with CAR T cell function (Figure 1).

Focusing first on CAR T cell design, they homed in on the hinge region, which was once regarded simply as a linker between the CAR binding and transmembrane domains. However, the CAR hinge has recently been subject to intense investigation as it can modulate CAR responsiveness against antigen^{low} tumors.[5] Specifically, a CD19 CAR T cell containing a CD28-derived hinge exhibited greater cytotoxic potential against CD19^{low} tumor compared to CARs with a CD8α hinge.[6] Moreover, increasing CD8α hinge length in a CD19 CAR T cell decreased cytokine production while maintaining *in vivo* cytotoxicity in a murine model.[7]

Thus, utilizing truncated CD27 as the CAR antigen-binding domain, which may have improved functionality over alternate scFvs,[3] Leick and colleagues[1] incorporated a CD8α hinge to CD70-targeting CAR T cells. Additionally, because CD27 can be proteolytically cleaved from the cell membrane by matrix metalloproteases,[3] potentially limiting its efficacy, an *in silico* tool to approximate the location of the cleavage site to the CD27 hinge[8] was used to generate a panel of CARs with cleavage-resistant hinges. Using these strategies, there was augmented avidity, resulting in improved CAR T cell expansion and enhanced efficacy, thereby sustaining durable *in vivo* responses in mice.[1] Furthermore, despite equal *in vitro* cytotoxic potential, *in vivo* treatment of AML-bearing mice with CD8α hinge-containing CAR T cells resulted in improved anti-leukemia efficacy and a significantly increased probability of survival.

This important distinction—differentiating *in vitro* assays from *in vivo* responses—is critical to the field, but how these results translate to or predict human response remains uncertain. In the present study, cleavage-resistant constructs harboring a flexible linker as compared to the CD8α hinge could not be differentiated by conventional cytotoxicity assays. However, acoustic force microscopy, ranking the avidity of CAR T cells by their ability to resist increasing acoustic forces and remain bound to plate-bound leukemic cells,[9] provided a good projection of *in vivo* responses. Furthermore, using a microfluidics platform, they were able to directly evaluate CAR-ligand interactions, highlighting the importance of biophysical approaches in evaluating CAR T cell function.[9] Thus, the authors highlight potentially important strategies for future preclinical testing of novel CAR T cell constructs.

Lastly, with recognition that sole dependence on CAR T cells may not be sufficient for efficacy in AML and that antigendim escape can result in treatment failure, pharmacologic modulation aimed at enhancing CAR T cell functionality by augmenting tumor antigen expression was pursued. By increasing CD70 expression on AML through the hypomethylation of the CD70 promoter,[4] pre-treatment with azacytidine (AZA) enhanced CD70 expression and anti-CD70 CAR T cell activity in preclinical *in vivo* models, supporting the utilization of AZA with a CD70-targeted CAR T cell therapy. Supported by the positive results from the combination of AZA with cusatuzumab, an anti-CD70 antibody,[4] and the widespread use of AZA in r/r patients with AML to control disease, AZA may also serve as a useful therapeutic in bridging patients with r/r AML to CD70-targeted CAR T cells.

Although the adage of "form follows function" typically applies to the field of design, this premise has broad

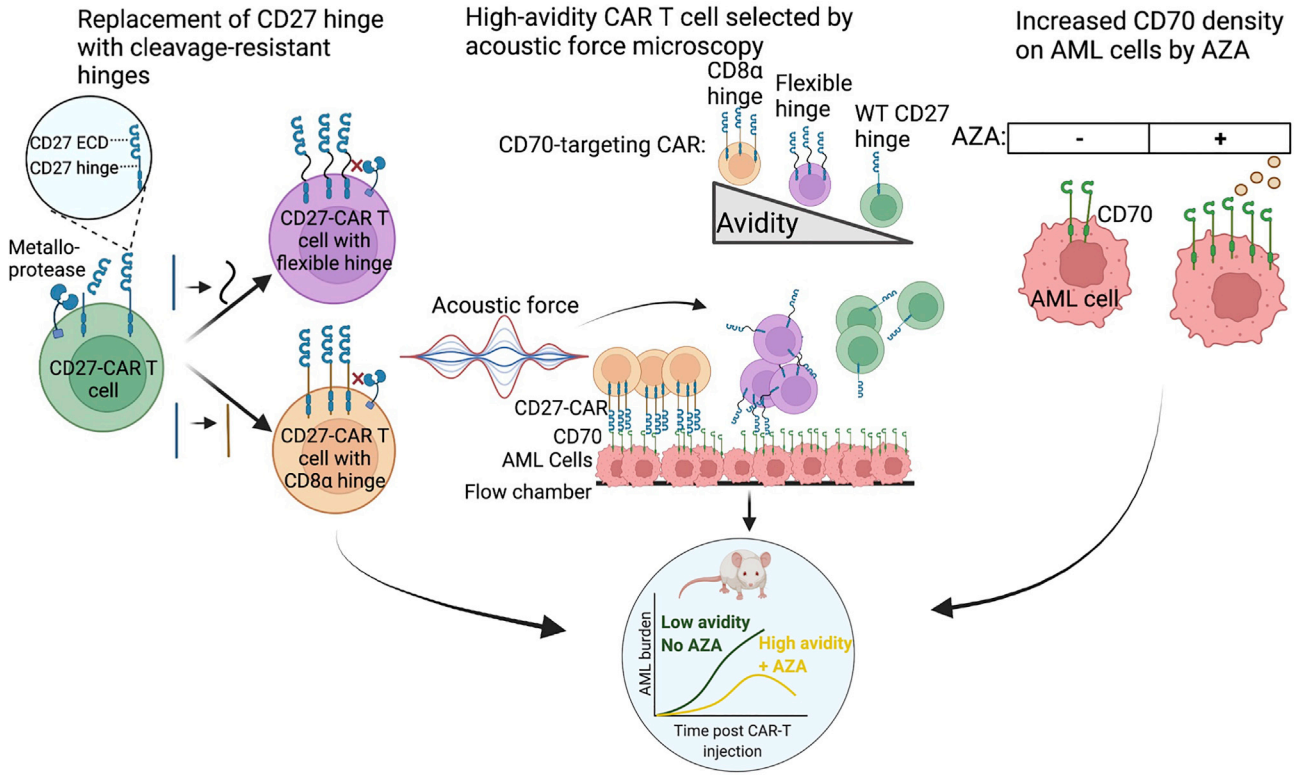

Figure 1. Engineering CD70-targeting CAR T cells to enhance *in vivo* activity against AML

CD70 is targeted through a CAR bearing its ligand, CD27. To prevent metalloprotease-mediated cleavage of CD27, its hinge was replaced with a panel of hinges. Leick et al. found that a CD27 CAR with both a flexible hinge and a CD8α hinge had cytotoxic potential, but notably, the avidities of the two CARs differed significantly, as measured by acoustic force microscopy. This higher avidity CD27-CD8α-CAR exhibited stronger *in vivo* activity when CD70 antigen density on the AML cells was increased by AZA. These modifications contributed to durable and predictable *in vivo* results.

applicability in CAR T cell development. While significant work delineating the parameters of the hinge that influence CAR avidity and immune synapse formation remains, CAR T cell construction has to be configured in the context of eradicating the disease that it is targeting. How other factors influencing avidity (e.g., cytoskeleton, co-receptor binding, and cell-cell adhesion) can be leveraged to further enhance CAR activity warrants further study. Importantly, the work by Leick et al.[1] represents an important step forward in addressing the roadblocks faced by CAR T cell therapeutics for AML. The strategic design, together with the use of AZA to increase CD70 expression on leukemia, represents a synergistic approach toward testing a new therapeutic option in patients with r/r AML.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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