

Lessons learned from a highly-active CD22-specific chimeric antigen receptor

Adrienne H. Long, Waleed M. Haso and Rimas J. Orentas*

Pediatric Oncology Branch; National Cancer Institute; Center for Cancer Research; National Institutes of Health; Bethesda, MD USA

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CD22 is an attractive target for the development of immunotherapeutic approaches for the therapy of B-cell malignancies. In particular, an m971 antibody-derived, second generation chimeric antigen receptor (CAR) that targets CD22 holds significant therapeutic promise. The key aspect for the development of such a highly-active CAR was its ability to target a membrane-proximal epitope of CD22.

Genetically modifying T cells with chimeric antigen receptors (CARs) is a promising new approach for the immunotherapy of cancer. CARs are hybrid receptors, constructed by linking an extracellular antigen-binding domain (often a single chain variable fragment, scFv) to the intracellular signaling domains of a T-cell molecule (Fig. 1). Thanks to chimeric nature, CARs have the ability to redirect T-cell specificity to target antigens in an MHC-independent fashion. The antitumor efficacy of adoptively transferred CAR-expressing T cells is currently being evaluated in clinical trials. Early studies focusing on anti-CD19 strategies for the treatment of leukemia patients have reported impressive antitumor effects.¹ To this end, we have recently described the development of a CD22-targeting CAR and its pre-clinical characterization in a model of B-cell acute lymphoblastic leukemia (B-ALL).²

Like CD19, CD22 is expressed in a B-cell lineage-restricted fashion. The possibility to employ of CD22 as a target for the therapy of B-cell malignancies has been previously confirmed in clinical trials based on CD22-targeting immunotoxins (BL22 and HA22).^{3,4} Previous studies on the development of a CD22-specific CAR have unveiled potential antitumor effects.⁵ However, maximal efficacy was only obtained when CD22 was modified so that the target epitope was positioned in close

proximity to the cell membrane. Following this initial report, anti-CD22 antibodies that target proximal epitopes of CD22 (e.g., m971) or display a high binding affinity (e.g., HA22) have been described.^{4,6} Given the availability of these reagents and the recent clinical successes achieved by CD19-targeting CAR-based therapeutic approaches, we sought to explore and optimize the design of CD22-specific CARs.

We tested ten different constructs encoding CD22-specific CARs to assess how the following structural modifications and alterations in signaling domains affect CAR efficacy: targeting membrane proximal epitopes (m971-derived CARs), improving scFv binding affinity (BL22- vs. HA22-derived CARs), including an IgG1 CH2CH3 spacer domain, and including different co-stimulatory motifs (second generation vs. third generation CARs). A profound difference was observed when proximal (m971-derived CAR) vs. distal (HA22-derived CAR) epitopes were targeted. The m971-derived CAR consistently provided T cells with higher lytic activity than its HA22-derived counterpart in vitro, matching previous observations on CD19-specific CARs in spite of the fact that CD22 was expressed in lower levels than CD19 on all B-ALL cell lines tested (REH, SEM, NALM6, KOPN8). In B-ALL xenograft models, both m971- and HA22-derived CAR-expressing T cells

improved survival, though the former did so more consistently than the latter. In light of these findings, it is interesting to compare the size of CD22 and CD19. CD22 contains indeed of seven immunoglobulin (Ig) extracellular domains, while CD19 only contains two. Like the m971-derived CD22-targeting CAR, which binds to an epitope found within the three membrane-proximal Ig domains, the CD19-specific CAR targets a proximal epitope.

Modifying other structural properties of the CD22-targeting CAR did not significantly influence efficacy. Binding affinity determines the efficacy of CD22-targeting immunotoxins. Indeed, high affinity HA22-based molecules exert more potent antitumor effects than their BL22-derived counterparts.⁷ However, a similar effect was not observed with CD22-specific CARs. In 2004, Chmielewski et al. reported the influence of affinity in an ERBB2-targeting CAR.⁸ Their work demonstrated that improving scFv binding affinity has no effect on CAR-expressing T-cell function above a certain threshold. Because BL22 was initially selected as a high affinity antibody, it is possible that this threshold has already been surpassed, as the improved affinity of HA22 had little, if any, impact on CAR activity.

The addition of a spacer domain derived from the CH2CH3 region of human IgG1 also had little effect on the efficacy

*Correspondence to: Rimas J. Orentas; Email: Rimas.Orentas@nih.gov

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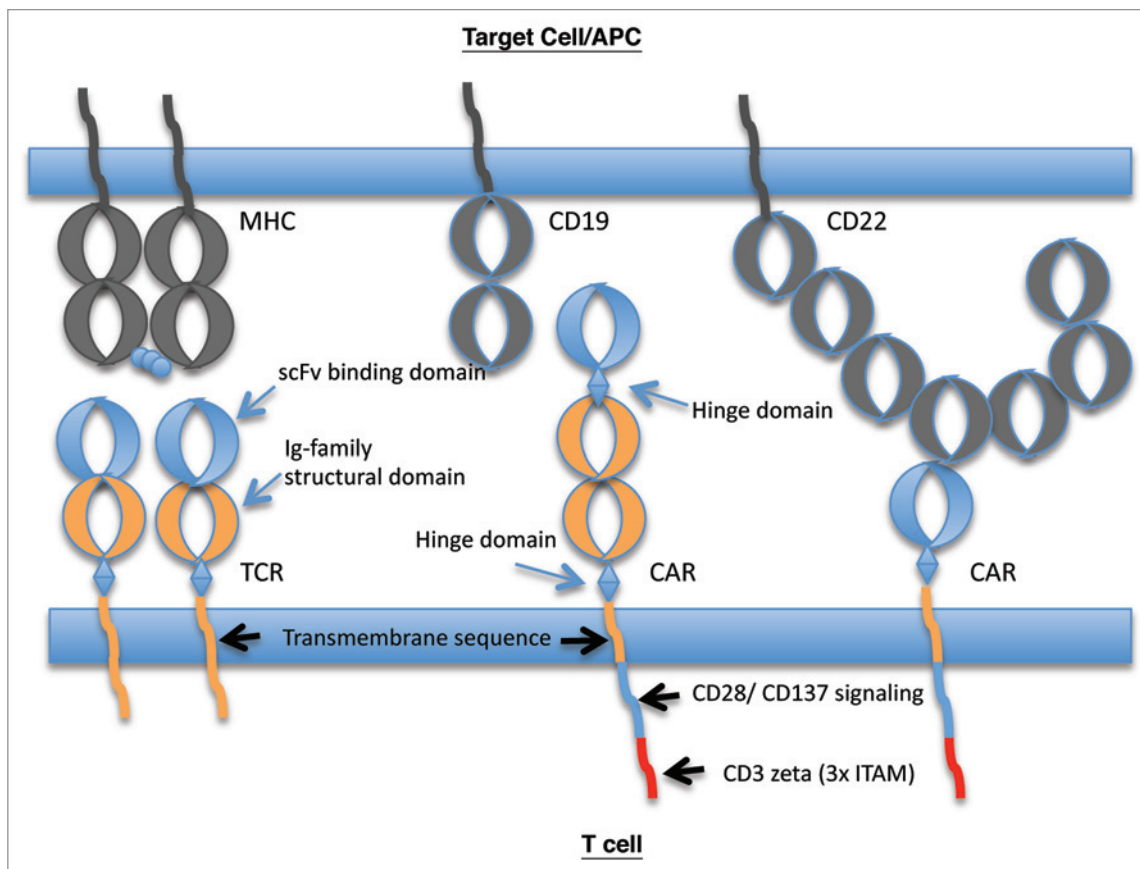


Figure 1. Structural variations in chimeric antigen receptors. Chimeric antigen receptors (CARs) mimic T-cell receptor (TCR)-MHC interactions in that the clustering of the CARs at sites of contact with the antigen induces the activation of T cells. TCRs are composed of various structural elements, immunoglobulin (Ig) superfamily domains and binding domains that interact with peptides presented on MHC molecules. CARs are also composed of single chain variable fragment (scFv)-derived binding elements originated from VDJ recombination events (in blue) as well as structural (in orange), transmembrane, and signaling motifs. All of these elements are linked by random or Ig-derived sequences, adding another important variable to the structure and flexibility of CARs. For illustrative purposes, two different CAR targets are shown: CD19 and CD22, which greatly differ in size and hence may vary in their ability to activate T cells that express CARs of various structural formats. CARs of two different size formats are also shown. In addition to these elements, we now know that the CAR-binding site on CD22, be it proximal or distal relative to the plasma membrane, has profound effects on CAR-mediated T-cell function.

of CD22-targeting CARs. The impact of spacer domains on CAR function has been previously investigated by a number of groups, with conflicting results. In particular, it has been suggested that spacers may play a role in establishing an “ideal” distance between the T-cell and target-cell membranes.⁹ In such a scenario, long spacers may be required to access proximal epitopes. In our hands, the presence of a spacer failed to affect the ability of CD22-specific CAR-expressing T cells to kill their targets, as the addition of a spacer had no significant impact on the targeting of distal epitopes (by HA22-derived CARs) and was not required for effectively targeting proximal epitopes (by m971-derived molecules).

Surprisingly, second generation CARs (containing either CD28 or CD137/4-1BB

signaling domains) outperformed third generation CARs (containing both CD28 and CD137 signaling domains) in almost all studies in vitro and did not enhance antitumor effect in vivo. This was unexpected, as it has been suggested that the inclusion of CD137 or OX40 signaling domains in addition to the CD28 signaling domain increases the lytic activity of T cells and prevents their apoptotic demise. However, it is interesting to note that the mutation of two of the ζ chain ITAM motifs improves CAR function.¹⁰ This suggests that additional activation signals obtained with third generation CARs may result in T-cell “tuning” by compensatory negative signals that prevent optimal CAR function. Alternatively, xenogeneic mouse models may favor rapid strong responses as opposed to the persistence of

antitumor effector cells. In the end, studies on patients affected by different malignancies are required to define the best CAR for the clinical management of indolent vs. acute tumors.

In summary, our study demonstrates that T cells expressing a CD22-targeting CAR exhibit a therapeutic potential for the treatment of B-ALL. Targeting membrane-proximal epitopes is critical for developing highly active CD22-targeting CARs. We conclude that m971-derived, second generation CD22-specific CARs hold significant promise and we plan to evaluate this approach to the treatment of B-ALL patients in a Phase I clinical trial.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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