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Designing chimeric antigen receptors to effectively and safely target tumors

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The adoptive transfer of T cells engineered to express artificial chimeric antigen receptors CARs) that target a tumor cell surface molecule has emerged as an exciting new approach for cancer immunotherapy. Clinical trials in patients with advanced B cell malignancies treated with CD19-specific CAR-modified T cells (CAR-T) have shown impressive antitumor efficacy, leading to optimism that this approach will be useful for treating common solid tumors. Because CAR-T cells recognize tumor cells independent of their expression of human leukocyte antigen (HLA) molecules, tumors that escape conventional T cells by downregulating HLA and/or mutating components of the antigen processing machinery can be eliminated. The ability to introduce or delete additional genes in T cells has the potential to provide therapeutic cell products with novel attributes that overcome impediments to immune mediated tumor elimination in immunosuppressive tumor microenvironments. This review will discuss recent concepts in the development of effective and safe synthetic CARs for adoptive T cell therapy (ACT).

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Introduction

The adoptive transfer of T cells engineered to express artificial chimeric antigen receptors (CARs) that target a tumor cell surface molecule is an exciting new approach for cancer immunotherapy. Clinical trials in patients with advanced B cell malignancies treated with CD19-specific CAR-modified T cells (CAR-T) have shown impressive antitumor efficacy [1••,2,3••,4,5], leading to optimism that this approach can be applied to treat common solid tumors [6]. This review will discuss recent advances in the development of effective and safe synthetic CARs for adoptive T cell therapy (ACT).

Structural elements of chimeric antigen receptors

Ligand binding

CARs consist of fusion molecules and are typically comprised of an extracellular single chain variable fragment (scFv) of a monoclonal antibody (mAb) specific for a surface molecule on the tumor cell, a spacer domain that provides flexibility and optimizes T cell and target cell engagement, a transmembrane domain, and signaling modules that trigger T cell effector functions (Figure 1). The use of scFvs for ligand binding takes advantage of the high specificity and prevalence of mAbs for tumor associated molecules, although other novel ligand binding domains have been utilized or are under development for clinical applications [7].

In contrast to T cell receptors (TCRs) that have been perfected through evolution to safely and efficiently distinguish self from non-self, CARs are constructed synthetically and assembly of an optimal receptor construct is largely empiric (Box 1). Ligand binding of a CAR differs from that of a TCR binding to peptide/MHC (pMHC) in receptor affinity, antigen density, and spatial properties; and experimental approaches to designing an optimal CAR for a specific target molecule have relied on functional assays of transduced T cells *in vitro* or in human tumor xenograft models. Few studies have evaluated the effect of affinity by designing CARs from scFvs of the same specificity but with different affinities. In one study, a CAR constructed from a higher affinity scFv specific for an epitope in the Ig-like/Frizzled region of ROR1 exhibited superior antitumor activity against human tumor xenografts than a lower affinity CAR specific for the same region of ROR1 [8]. However, based on studies of class I restricted TCRs that revealed a threshold of affinity beyond which antigen and CD8 co-receptor engagement result in activation induced T cell death and loss of therapeutic activity [9], it is likely that for each target molecule there will be an affinity threshold for CARs, beyond which T cell effector function and/or survival may be compromised.

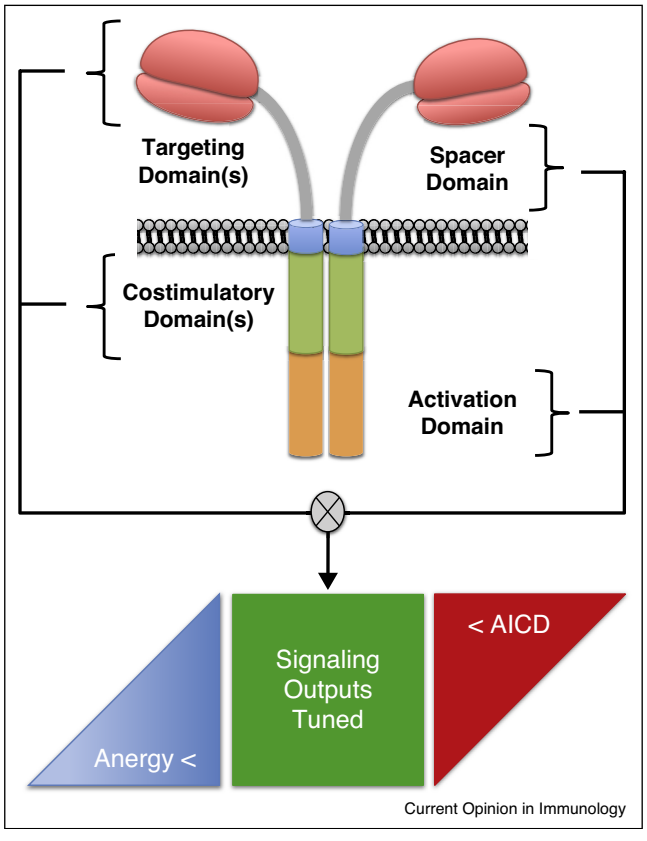
The number of molecules that are expressed on the tumor cell surface and available to bind the CAR can vary substantially for individual targets, and is typically much higher than the number of pMHC molecules available for

Box 1 CAR assembly

- Select humanized scFv binding domains to membrane proximal epitopes in target molecules.
- Examine scFvs of different affinities.
- Screen spacer length variants for optimal function *in vitro* and in NSG mice.
- Screen costimulatory domains for desired function *in vitro* and in NSG mice.
- Alter CAR fusion sites to minimize potential immunogenicity.
- Safety testing in animal models if feasible.

binding of TCRs. Because it is unlikely that CARs will serially engage target molecules and cluster in organized synapses as is observed with TCR/pMHC recognition, it is assumed that a higher ligand density is required for CAR recognition than for TCRs [10]. TCR signaling is further enhanced by the small size of the TCR/pMHC complex, which results in their physical segregation from tyrosine phosphatases that have large ectodomains [11,12]. By contrast, the spatial interaction between a CAR-T cell and its target cell differs depending on the structure and location of the epitope on the target molecule and the design of the extracellular domain of the CAR. Indeed, studies have shown that it is critical to tailor the length and composition of the extracellular spacer domain for individual target molecules to optimize tumor cell recognition, T cell proliferation, and cytokine production [13,14•].

Figure 1



Structural elements of chimeric antigen receptors. Biophysical components of chimeric antigen receptors work in concert to affect qualitative and quantitative signaling outputs to the T cell. Signal output tuning based on scFv binding affinity, extracellular spacer size adapted to the target epitope location, and cytoplasmic costimulatory and ITAM elements are interdependent. The net effect of any combination of components is to tune the CAR to be compatible with effector T cell physiology between thresholds that elicit hypoactivity (anergy) or hyperactivity (activation induced cell death).

Tumor escape from CAR-T cells can occur by the selective outgrowth of antigen loss variants and might be overcome by targeting two different tumor associated molecules. It is feasible to express two ligand binding domains in tandem separated by a flexible linker, or as single CAR constructs in the same or different T cells [15]. The expression of scFvs in tandem is appealing and has been shown to work in principle. However, different spatial requirements for CAR binding for each target may limit the applicability of this approach for some targets or compromise the efficacy of tandem CARs that do not optimally signal through one of the binding domains.

Signaling modules

Distinct constellations of intracellular signaling domains have been incorporated into CARs to activate effector functions in the T cell. CARs were initially designed with CD3ζ or FcR domains as the only intracellular signaling module, but clinical trials of ACT with such 'first' generation CAR-T cells targeting L1CAM and CD20 did not confer significant antitumor activity or result in proliferation and persistence of CAR-T cells *in vivo* [16,17]. Subsequently, CARs were designed to enhance cytokine production by incorporating one or more costimulatory domains fused to CD3ζ, such as CD28, CD137, or OX-40, and these 'second' and 'third' generation receptors were superior for inducing cytokine production and proliferation of CAR-T cells *in vitro*, and for mediating tumor regression in xenograft models in Nod/Scid/$\zeta c^{-/-}$ (NSG) mice compared to CARs with CD3ζ alone [18–23]. Direct comparison of a CD19-specific CAR containing CD28/CD3ζ and one containing only CD3ζ has been performed in one clinical trial and as predicted from preclinical data, T cells expressing the CD28/CD3ζ construct exhibited superior persistence [24]. Surprisingly, antitumor activity was not as dramatic in this study as reported for other trials with CD19-specific CAR-T cells, potentially reflecting other variations in CAR design and/or T cell product composition. Strategies for further augmenting potency and supporting survival of CAR-T cells by co-expressing additional costimulatory receptors, their ligands, and/or cytokines may be necessary for effective CAR-T cell therapy in solid tumors where suppressive tumor microenvironments interfere with T cell function [25,26].

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