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Pre-clinical development of chimeric antigen receptor T-cell immunotherapy: Implications of design for efficacy and safety

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ABSTRACT

Following the landmark approvals by the United States Food and Drug Administration, the adoptive transfer of CD19-directed chimeric antigen receptor (CAR) T-cells has now entered mainstream clinical practice for patients with chemotherapy-resistant or refractory B-cell malignancies. These approvals have followed on from a prolonged period of pre-clinical evaluation, informing the design of clinical trials that have demonstrated unprecedented efficacy in this difficult to treat patient population. However, the delivery of autologous CAR-engineered T-cell therapy is complex, costly and not without significant risk. Here we summarize the key themes of CAR T-cell preclinical development and highlight a number of innovative strategies designed to further address toxicity and improve efficacy. In concert with the emerging promise of precision genome editing, it is hoped these next generation products will increase the repertoire of clinical applications of CAR T-cell therapy in malignant and perhaps other disease settings.

1. Introduction

Chimeric Antigen Receptor (CAR) T-cell immunotherapy has shown substantial anti-tumor activity against refractory B-cell malignancy [1]. Indeed, therapeutic efficacy is unprecedented for a new cancer medicine with response rates of up to 90% for patients with relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL) and over 60% for patients with non-Hodgkin's lymphoma (NHL) [2]. Immunotherapy using CAR T-cells is generally undertaken following the administration of lymphodepleting chemotherapy. This intervention increases access to supportive cytokines (e.g. IL-7 and IL-15) [3], creates a viable spatial compartment for CAR T-cell growth and persistence [4] and depletes systemic and tumor-resident $CD4^+$ $CD25^+$ regulatory T-cells (Tregs) [5]. Nevertheless, toxicity of CAR T-cell immunotherapy administered in the context of prior lymphodepletion can be life threatening, a factor that requires careful consideration during pre-clinical development.

2. Structure of chimeric antigen receptors

Chimeric antigen receptors are engineered proteins that contain an antigen recognition domain - most commonly a single chain variable fragment (scFv) - linked via a hinge or spacer and transmembrane domain to a bespoke signaling domain [6] (Fig. 1). In initial configurations, a T-cell receptor (TCR)-like signal 1 alone was provided, generally using a module that contains one or more

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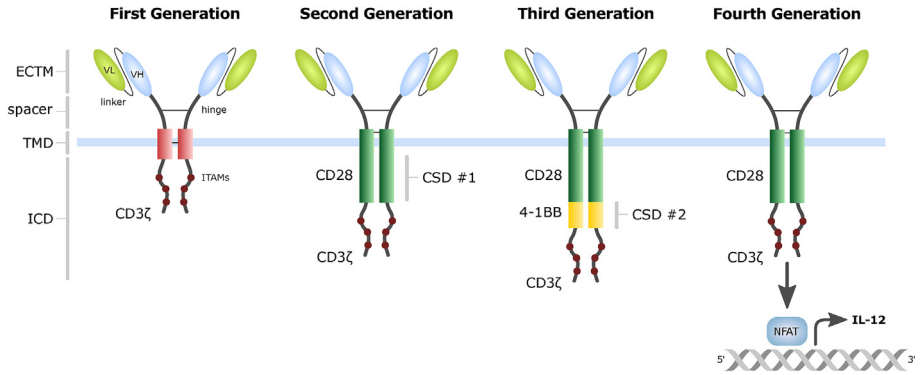

Fig. 1. Evolution of first, second, third and fourth generation CARs. First generation CARs consist of a scFv typically fused to a CD3ζ activation domain. Second generation CARs contain an additional intracellular costimulatory domain, usually CD28 or 4-1BB (CD137). Third generation CARs combine two or more costimulatory domains. Fourth generation CARs are engineered with an activation inducible element (e.g. NFAT-responsive expression cassette) to enable the secretion of a transgenic product such as IL-12. CSD, costimulatory domain; ECTM, extracellular targeting moiety; ICD, intracellular domain; NFAT, nuclear factor of the activated T-cell; scFv, single chain variable fragment; TMD, *trans*-membrane domain.

immunoreceptor tyrosine-based activation motifs (ITAM), such as CD3ζ. However, immunotherapy using T-cells that were engineered to express these "first generation" CARs proved ineffective, largely owing to inadequate T-cell persistence and expansion *in vivo* [7]. Second and third generation CARs respectively incorporate one or two intracellular co-stimulatory domains such as CD28, CD137 (4-1BB), OX40 or ICOS, thereby providing signal 2 for T-cell activation [7]. This development translated into improved CAR efficacy and functionality, although it remains doubtful as to whether third generation CARs are truly superior to their second generation counterparts. More recently, a range of fourth generation CARs have been described in which additional signals are delivered to enhance potency (e.g. inducible release of interleukin (IL)-12). Since CAR T-cells bind in an antibody-like manner, targeting is independent of human leukocyte antigen (HLA) haplotype or tumor-associated HLA downregulation. Furthermore, engagement of non-protein antigens such as carbohydrates and gangliosides can be achieved.

3. Selection of scFv for CAR T-cell targeting

Target selection is crucial in the design of CARs to achieve optimal safety and efficacy [8]. Success of CAR T-cell immunotherapy of B-cell malignancy reflects the widespread expression of CD19 on the transformed cell population, but not on hematopoietic stem cells or terminally differentiated plasma cells. Building on this, CARs targeting other B-cell antigens (e.g. CD20 and CD22) for the treatment of leukemia and lymphomas have been developed. This is especially important as loss of CD19 expression has been reported in relapsed patients and CD19 negative populations have been found in malignancies.

Almost all anti-CD19 CARs currently undergoing clinical evaluation contain murine scFvs. While durable remissions have been achieved, $CD19^+$ disease relapse may also ensue, accompanied by the loss of circulating CAR T-cells [9]. While this may be influenced by choice of co-stimulatory domain [10] and tonic signaling [11], T-cell responses against epitopes derived from murine scFvs may occur. This highlights the desirability of selection of humanized and fully human scFvs, as recently evaluated in patients with advanced NHL [12]. A novel approach to enhance target antigen repertoire involves the re-direction CAR specificity against HLA-restricted peptide antigens that are derived from intracellular tumor antigens, such as Wilms Tumor (WT)1. Use of scFvs that bind specifically to individual HLA/peptide complexes significantly expands the range of potential CAR targets and holds great potential to expand the successful and safe delivery of CAR T-cell immunotherapy [13].

4. Selection of hinge/spacer and transmembrane elements

Selection of hinge or transmembrane region can also influence activity of CAR T-cell immunotherapy. Illustrating this, second generation CD19-specific CAR T-cells that contain a CD28 hinge and transmembrane domain elicited greater target-dependent cytokine release and activation-induced cell death compared to CAR T-cells in which these elements originated from CD8α [14]. While *in vivo* potency was similar, use of a CD8α hinge and transmembrane region may cause reduced cytokine release and limit toxicity in patients [14].

5. Costimulatory domains

Clinically evaluated second generation CARs utilize either a CD28 or 4-1BB costimulatory module and both have shown impressive outcomes [9,15]. Initial pre-clinical comparisons of anti-CD19 CD28ζ and 4-1BBζ CARs demonstrated comparable anti-tumor activity, although cytokine release appears to be slower and of lower magnitude with 4-1BBζ CARs [16,17]. Many pre-clinical studies have employed high T-cell doses, leading to complete tumor eradication, meaning that subtle differences may remain undetected. By lowering T-cell doses, Zhao et al. demonstrated that anti-CD19 4-1BBζ CAR T-cells mediate slower tumor elimination