# Chimeric antigen receptor T-cells for B-cell malignancies

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**The adoptive transfer of T-lymphocytes modified to express chimeric antigen receptors (CAR-Ts) has produced impressive clinical responses among patients with B-cell malignancies. This has led to a rapid expansion in the number of clinical trials over the past several years. Although CD19-specific CAR-Ts are the most extensively evaluated, CAR-Ts specific for other B-cell–associated targets have also shown promise. However, despite this success, toxicities associated with CAR-T administration remain a significant concern. There continues to be substantial heterogeneity among CAR-T products, and differences in both CAR designs and CAR-T production strategies can substantially affect clinical outcomes. Ongoing clinical studies will further elucidate these differences and many other innovative approaches are being evaluated at the preclinical level. In this review, we will discuss the background and rationale for the use of CAR-Ts, provide an overview of advances in the field, and examine the application of CAR-Ts to the treatment of B-cell malignancies, including a summary of clinical trials published to date. (Translational Research 2017;187:59–82)**

**Abbreviations:** ALL = acute lymphoblastic leukemia; B-ALL = B-cell acute lymphoblastic leukemia; CAR-T = chimeric antigen receptor T-cell; CAR = chimeric antigen receptor; CD = cluster of differentiation; CHOP = Children's Hospital of Philadelphia; CLL = chronic lymphocytic leukemia; CR = complete remission or complete response; CRISPR-Cas9 = clustered regularly interspaced short palindromic repeats CRISPR-associated protein 9; CRS = cytokine release syndrome; CTLA-4 = cytotoxic T-lymphocyte–associated protein 4; DLBCL = diffuse large B-cell lymphoma; DNA = deoxyribonucleic acid; FHCRC = Fred Hutchinson Cancer Research Center; FL = follicular lymphoma; HIV = human immunodeficiency virus; iC9 = inducible caspase-9; IFN$\gamma$ = interferon gamma; IL = interleukin; ITAM = immunoreceptor tyrosine-based activation motif; JAK/STAT = Janus kinase/signal transducers and activators of transcription; MCL = mantle cell lymphoma; MHC = major histocompatibility complex; MRD = minimal residual disease; mRNA = messenger ribonucleic acid; MSKCC = Memorial Sloan Kettering Cancer Center; NCI = National Cancer Institute; NHL = non-Hodgkin lymphoma; ORR = overall response rate; PBMCs = peripheral blood mononuclear cells; PD-1 = programmed cell death protein 1; PR = partial remission or partial response; scFv = single-chain variable fragment; TALEN = transcription activator-like effector; TBI = total body irradiation; TCR = T-cell receptor; TM = transmembrane; ZFN = zinc-finger nuclease

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## INTRODUCTION

The adoptive transfer of chimeric antigen receptor T-cells (CAR-Ts) has emerged as an extremely promising immunotherapeutic approach for the treatment of B-cell malignancies, and the remarkable versatility of this technique makes it potentially applicable to a wide range human diseases. Traditional CAR molecules couple the single-chain variable fragment (scFv) of a monoclonal antibody to one or more intracellular signaling domains derived from the T-cell receptor (TCR) complex and costimulatory molecules. When stably expressed on the surface of T-cells, CARs combine the antigen-binding properties of a monoclonal antibody with the effector and self-renewal properties of T-lymphocytes. The native TCR recognizes intracellular peptide antigens that are presented on the cell surface by the major histocompatibility complex (MHC). Because of the highly polymorphic nature of the MHC gene, a TCR specific for a given peptide-MHC complex would be unlikely to recognize the same antigen when presented by another patient's MHC. In contrast, the scFv of a CAR targets antigens directly expressed on the surface of tumor cells (without processing and MHC presentation), allowing the same receptor to be used for all patients. Furthermore, in addition to proteins, the scFv of a CAR can also recognize carbohydrates and glycans, thereby greatly expanding the array of targetable antigens. Finally, the MHC-independent recognition of targeted antigens by CAR-Ts overcomes one of the major mechanisms of tumor escape, that is, the downregulation of MHC on tumor cells.

Immune surveillance and targeted immune responses are important in both preventing and suppressing many cancers; however, tumors may ultimately escape immune recognition and destruction via multiple mechanisms.[1-3] It has, therefore, been a long-standing goal to devise ways to engineer components of the immune system to overcome tumor-associated evasion and suppression strategies. Adoptively acquired immunity was first described in 1954,[4] and the development of allogeneic hematopoietic stem cell transplantation (HSCT) beginning in the late 1960s represented the first major breakthrough in cellular immunotherapy. The concept of CAR-Ts was described in the mid-1980s.[5,6] Many technical challenges prevented early clinical application however,[7] and it is only in the past 5-years that the efficacy of CAR-Ts has been demonstrated in multiple clinical trials. The past decade has also seen significant advances in other areas of cancer immunotherapy, including the emergence of immune checkpoint inhibitors and bi-specific antibodies. Taken together, these therapies have firmly established the enormous potential benefits of redirecting the immune system to target cancer cells, and the unprecedented success of CD19-directed CAR-Ts has clearly demonstrated the efficacy of adoptive cellular immunotherapies in accomplishing this goal.

## DEVELOPMENT OF CHIMERIC ANTIGEN RECEPTORS

**First-generation CARs.** The native TCR is a membrane-bound heterodimer comprised of an $\alpha$-chain and a $\beta$-chain. Antigen recognition by the endogenous TCR of $CD4^+$ and $CD8^+$ T-cells is restricted to peptides bound to polymorphic MHC molecules on the surface of cells. Recognition of an MHC-bound peptide antigen by the TCR leads to downstream activation of kinase pathways via a complex of noncovalently associated transmembrane (TM) signaling molecules, including a heterodimer of CD3$\varepsilon$ and CD3$\delta$, a heterodimer of CD3$\varepsilon$ and CD3$\gamma$, and a homodimer of $\zeta$-chains. TCR engagement alone is not sufficient to fully activate T-cells, however, and a requisite second activation signal is provided when a costimulatory receptor on the T-cell binds to its cognate ligand on an antigen-presenting cell. CD28 is the most well-characterized costimulatory receptor and binds to either CD80 or CD86 on the surface of antigen-presenting cells.[8] CD80, CD86, and other costimulatory molecules are generally not expressed by tumor cells, however, and chronic TCR activation in the absence of costimulation (or in the presence of signaling via coinhibitory receptors such as cytotoxic T-lymphocyte–associated protein 4 and programmed cell death protein 1) leads to T-cell anergy or death.[9,10]

The first CARs developed in the mid-1980s consisted of the variable region of a monoclonal antibody coupled to the constant region ($\alpha$- and $\beta$-chains) of the TCR.[5,6] The extracellular component (ectodomain) was later modified to include only a recombinant scFv from the antigen-binding variable domains of both the heavy and light chains of a monoclonal antibody. The scFv was coupled to a TM domain, followed by an immunoreceptor tyrosine–based activation motif (ITAM) from the TCR-$\zeta$ endodomain. Eshhar et al. initially demonstrated that this chimeric protein, which they referred to as a "T-body," could induce antigen-dependent, non-MHC-restricted T-cell activation, leading to both interleukin-2 (IL-2) secretion and target cell lysis.[11] This basic structure was used in many subsequent studies and is now referred to as the first-generation CAR.

Clinical trials of first-generation CAR-Ts, initially targeting the human immunodeficiency virus[12] and later targeting antigens such as carbonic anhydrase IX, CD171, folate receptor $\alpha$, and GD2[13-17] expressed in solid tumors, as well CD19 and CD20 expressed by B-cell malignancies,[18,19] did not induce substantial