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 γ = 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 of 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 α -chain and a β -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 ϵ and CD3 δ , a heterodimer of CD3 ϵ and CD3 γ , and a homodimer of ζ -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 (α - and β -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- ζ 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 virus¹² and later targeting antigens such as carbonic anhydrase IX, CD171, folate receptor α , and GD2¹³⁻¹⁷ expressed in solid tumors, as well as CD19 and CD20 expressed by B-cell malignancies,^{18,19} did not induce substantial

clinical responses, but did serve to highlight several challenges to be considered in subsequent iterations of CAR designs. One of the most significant problems was the limited persistence of CAR-Ts after infusion. Other setbacks included unexpected on-target but off-tumor toxicities.^{15,20} These trials have also provided us with long-term follow-up data related to the safety of certain aspects of CAR-T generation. Most CAR-T trials published to date have utilized γ -retroviral or lentiviral vectors to express CARs in human T-cells. Based on earlier experience in which γ -retroviral vector-based gene therapy in hematopoietic stem cells led to the development of T-cell leukemia,^{21,22} there has been concern that the use of such vectors could cause insertional mutagenesis in CAR-Ts as well. It is thus reassuring that long-term follow-up of patients treated with first-generation human immunodeficiency virus-targeted CAR-Ts (which had much better long-term persistence than most other first-generation CARs) did not reveal any evidence of vector-induced immortalization of cells or enrichment of T-cells with CAR transgene integration near genes implicated in growth control.²³

Second- and third-generation CARs. The next stage of CAR design involved attempts to improve the activation and proliferation of CAR-Ts in vivo by the incorporation of costimulatory domains. The poor in vivo persistence of first-generation CAR-Ts was felt to be partially explained by T-cell anergy and death in the absence of costimulation. To test this hypothesis, Finney et al. designed a CAR that incorporated the CD28 cytoplasmic endodomains in tandem with the TCR- ζ endodomain. In vitro studies revealed that, in response to antigen stimulation, CAR-Ts which included the CD28 endodomain produced 20-times more IL-2 than those with the TCR- ζ endodomain alone.²⁴ Stronger evidence supporting the benefits of incorporating the CD28 costimulatory domains was provided by a study in humans directly comparing an anti-CD19- ζ (first generation) and an anti-CD19-CD28- ζ (second generation) CAR. In that study, patients with non-Hodgkin lymphoma (NHL) were simultaneously infused with both CAR-T products, and it was demonstrated that CD19-CD28- ζ CAR-Ts exhibited significantly greater expansion and persistence.²⁵

Given promising results with the incorporation of the CD28 endodomain, alternative (or additional) signaling domains have also been evaluated. 4-1BB is a member of the tumor necrosis factor receptor family and was initially selected due to data supporting its role in preventing activation-induced death, inducing expansion of CD8⁺ cells, enhancing CD8⁺ T-cell responses during

viral infection and allograft rejection, and contributing to effective antitumor responses in animal models of cancer.²⁶ Imai et al. demonstrated in vitro that anti-CD19-4-1BB- ζ CAR-Ts exhibited significantly greater cytotoxicity toward B-lineage acute lymphoblastic leukemia (B-ALL) cells and significantly greater IL-2 release, as compared with anti-CD19- ζ CAR-Ts.²⁶ Other costimulatory domains (eg, OX-40) have also been evaluated. CAR constructs which add a single costimulatory domain to the first-generation backbone are known as second-generation CARs, whereas those including two costimulatory domains are referred to as third-generation CARs. In addition to combining CD28 and 4-1BB, third-generation CARs have also incorporated other costimulatory domains such as OX-40.^{27,28} Most clinical trials conducted to date have utilized second-generation CARs with either a CD28 or 4-1BB costimulatory signaling domain. Although the optimal choice of costimulatory domains has not been fully elucidated, a recent study at the Baylor College of Medicine in 6 patients with diffuse large B-cell lymphoma (DLBCL) evaluated the simultaneous administration of second-generation CD19-specific CD28-containing CAR-Ts and third-generation CD19-specific CD28-plus 4-1BB-containing CAR-Ts and found 23-fold (range 1.1- to 109-fold) higher expansion and correspondingly longer persistence for the third-generation CAR-Ts.²⁹ As discussed below, there are many additional design factors that affect the function of CAR-Ts, which in addition to variable clinical trial designs, make it difficult to directly compare existing studies.

Additional design factors affecting CAR function. The potential effects of various structural modifications on CAR function have been extensively reviewed elsewhere, and a detailed discussion is beyond the scope of this article.^{20,30} Briefly, considerations in designing the ectodomain of a CAR include the scFv used, its affinity for the target antigen, and the specific binding site on this antigen. Although most CARs have been generated by using a scFv, other designs have utilized single antigen-binding region domains,³¹ non-antibody receptors, and even ligands for another receptor.³² The scFvs used are most commonly derived from murine monoclonal antibodies, although humanized scFvs have also been used with the goal of reducing the immunogenicity of the ectodomain. Additionally, the length of the hinge region between the scFv and the TM domain can affect the degree of T-cell activation upon antigen recognition. The TM domain itself is usually derived from the TCR- ζ , CD4, CD8, or CD28 molecules, and the choice of TM domain can also have a significant effect on CAR-T effector function and stability.²⁰

PRODUCTION OF CAR T-CELLS

Most clinical trials of CAR-Ts published to date have utilized unselected patient-derived (autologous) peripheral blood mononuclear cells, which are activated using anti-CD3 (OKT3) and anti-CD28 monoclonal antibodies (either in soluble form, immobilized, or coated to the surface of beads)³⁰ and engineered to express the CAR transgene using viral vectors. CAR-Ts are then expanded *in vitro* with recombinant cytokines.³⁰ There is significant variability in these processes between institutions, however, and the optimization of various steps involved in CAR-T production remains an area of active investigation.

Source of T-cells: allogeneic versus autologous CARs. Most reported trials of CAR-T therapy for B-cell malignancies have utilized autologous lymphocytes that have been expanded *ex vivo*, transduced to express a CAR transgene, and then reinfused into the patient. In the setting of prior allogeneic HSCT, several trials have also evaluated the use of allogeneic, donor-derived CD19-specific CAR-Ts in patients who have experienced, or are at high risk for experiencing, relapse of their disease.³³⁻³⁶ Although there are significant differences between these studies, a recent trial evaluating allogeneic CD19-specific CAR-Ts for adults with relapsed B-ALL following allogeneic HSCT demonstrated a minimal residual disease (MRD)-negative complete remission (CR) in 4 of 5 treated patients.³⁴ In addition to this approach, there has also been substantial interest in generating allogeneic CAR-Ts which lack expression of their native TCR, thereby allowing their infusion into unrelated patients without the risk of inducing graft-versus-host disease. As discussed below, the generation of so-called “universal CARs” has recently been demonstrated via the use of several novel gene-editing techniques, and the first clinical application of “universal” CD19-specific CAR-Ts was recently reported in pediatric B-ALL.^{37,38}

Gene transfer techniques. Most CAR-T trials to date have used either lentiviral or γ -retroviral vectors to deliver the CAR transgene into the genome of activated T-cells. Each vector allows for efficient gene delivery and contains a promoter leading to constitutive CAR expression. Although γ -retroviral vectors, when compared with lentiviral vectors, have been shown to be associated with an increased risk of insertional mutagenesis in hematopoietic stem cells,³⁹ experience to date does not suggest any clinically-relevant differences between CAR-Ts produced via transduction by γ -retroviral versus lentiviral vectors, and long-term follow-up of patients treated with γ -retrovirally transduced CARs supports the safety of

this technique.²³ Nonviral gene transfer techniques such as the Sleeping Beauty^{35,40} and PiggyBac⁴¹ transposon/transposase systems are also being evaluated; however, the efficiency of DNA integration associated with these methods is generally low. Other studies have used electroporation of naked plasmid DNA; however, the efficiency of this technique is even lower.⁴² It is also possible to transiently express a CAR via electroporation of mRNA, which may prove to be a valuable tool to more safely investigate CAR-Ts specific for novel target antigens. Each of the above gene transfer techniques involves random insertion of the CAR transgene into the genome, and variegated transgene expression via transcriptional silencing and vector copy number variation⁴³ has the potential to contribute to constitutive signaling and T-cell exhaustion, which could decrease CAR-T persistence *in vivo*.⁴⁴

Limitations of the above approaches have driven substantial interest in applying gene-editing tools such as prokaryotic type II CRISPR (clustered regularly interspaced short palindromic repeats) Cas9 (CRISPR-associated protein 9) to the generation of CAR-Ts.⁴⁵ Two separate groups have recently demonstrated the use of gene-editing tools to insert a CD19-specific CAR transgene directly into the native TCR α -chain locus, thereby simultaneously “knocking in” a CAR transgene and knocking out the endogenous TCR.^{44,46} Eyquem et al.⁴⁴ accomplished this using CRISPR/Cas9 gene-editing techniques, and MacLeod et al.⁴⁶ used an engineered homing endonuclease and an adeno-associated virus template to target a CD19-specific CAR into the TCR α -chain locus. A potential benefit of this approach is the ability to place the CAR transgene under the control of endogenous regulatory elements, thereby potentially reducing tonic signaling and associated T-cell differentiation and exhaustion.⁴⁴ To this end, Eyquem et al. demonstrated that gene-edited cells had lower but more uniform levels of CAR expression, and that these cells significantly outperformed conventional retrovirally transduced CARs in a mouse model of B-ALL. It remains to be seen whether such approaches will offer substantial clinical advantages as compared with non-targeted CAR insertion in T-lymphocytes.

Ex vivo expansion and CAR T-cell subsets. Following transduction, CAR-Ts are typically provided intermittent cytokine stimulation with the goals of both driving T-cell expansion and influencing the final composition of T-cell subsets. The differentiation status of adoptively transferred T-cells can have a profound impact on their clinical activity and persistence *in vivo*. Because unsorted peripheral blood mononuclear cells are usually transduced, both CD4⁺ and CD8⁺ CAR-Ts

are simultaneously generated. Although CD8⁺ effector T-cells generally exhibit greater antitumor cytotoxicity in vitro, CD4⁺ cells may play a role in vivo in promoting the persistence of CD8⁺ T-cells. In addition, various studies have demonstrated that the adoptive transfer of less-differentiated T-cells leads to greater persistence and antitumor efficacy in vivo.⁴⁷⁻⁵⁰ The production of CAR-Ts for clinical use has traditionally involved T-cell expansion via stimulation with recombinant IL-2.³⁰ The cytokines IL-7 and IL-15 have subsequently been demonstrated, by our group and others, to increase the frequency of CD8⁺CD45RA⁺CCR7⁺ T-cells, a phenotype corresponding to memory stem T-cells that, when compared with more differentiated T-cell subsets, was shown to correlate with enhanced in vivo persistence and antitumor activity.^{51,52} Because ex vivo expansion techniques remain an active area of investigation, there is significant heterogeneity in such techniques between studies, and consequently, in the composition of T-cell subsets found in the CAR-T products administered to patients thus far.

In an approach that differs from most other trials to date, investigators at the Fred Hutchinson Cancer Research Center (FHCRC)/Seattle Children's are evaluating the utility of selecting for and purifying different T-cell subsets before transduction rather than using cytokine stimulation to drive the differentiation of unsorted T-cells.⁵³⁻⁵⁵ In two ongoing trials, the investigators selected CD4⁺ T-cells by positive immunomagnetic selection, selected CD8⁺ central memory T-cell cells by depleting CD4⁺, CD14⁺, and CD45RA⁺ cells, and then selected for CD62L⁺ cells from the remaining fraction. They transduced the purified CD4⁺ T-cells and CD8⁺ central memory T-cell cells separately, expanded them in vitro, and mixed them at a specific ratio before infusing the T-cell product into patients. Although this approach seems feasible, it remains to be seen whether such techniques would be broadly applicable and if they would offer significant advantages in terms of safety and efficacy compared with the use of unselected T-cells.⁵⁵

ADMINISTRATION OF CAR T-CELLS

CAR-Ts are generally administered following lymphodepletion, which has been shown to improve both CAR-T persistence and antitumor activity.^{55,56} The administration of either total body irradiation or preparative chemotherapy regimens was initially demonstrated to improve antitumor responses in melanoma patients treated with autologous tumor-infiltrating lymphocytes and IL-2, and this was shown to correlate with increasing circulating levels of

homeostatic cytokines such as IL-7 and IL-15.⁵⁶ Lymphodepletion is thought to directly affect the survival, persistence, and proliferation of adoptively transferred T-lymphocytes primarily via elimination of cellular elements that compete for endogenous homeostatic cytokines and by minimizing immune-mediated CAR-T rejection.^{55,56} The limited antitumor efficacy and CAR-T persistence seen in some early trials may be at least partially explained by the lack of adequate lymphodepletion.^{33,57} Most patients on recent trials have been treated with lymphodepleting chemotherapy. Although the combination of chemotherapy agents has often been left to the investigator's choice, the most common regimens have included cyclophosphamide with or without fludarabine.

The importance of adequate lymphodepletion is further emphasized by a trial conducted at FHCRC in which second-generation CD19-specific CAR-Ts were administered to adult subjects with B-ALL. The initial 13 patients received lymphodepletion with cyclophosphamide alone. Although 83% of these patients achieved a CR, 70% of them relapsed at a median of 66 days after CAR-T infusion, and this was associated with the emergence of transgene-specific cytotoxic CD8⁺ T-cell-mediated immune responses.^{55,58} The addition of fludarabine to the lymphodepletion regimen for the subsequent 17 patients enrolled into the study produced durable responses, with 71% of these patients remaining in CR at a median follow-up of 300 days.⁵⁵ In a study conducted at the National Cancer Institute (NCI) of CD19-specific CAR-Ts for pediatric patients with relapsed/refractory B-ALL, Lee et al. also reported superior outcomes among patients who received lymphodepletion with fludarabine plus cyclophosphamide, compared with those receiving alternative lymphodepletion regimens (median overall survival 13.3 months versus 5.5 months, respectively).⁵⁹

TOXICITIES OF CAR T-CELLS

Despite significant clinical responses across multiple clinical trials, the widespread use of CAR-T therapy has thus far been prevented by both observed and theoretical toxicities, including cytokine release syndrome (CRS), neurotoxicity, and on-target off-tumor effects.⁶⁰

Cytokine release syndrome and neurotoxicity. Cytokine release syndrome is the most common and often the most severe toxicity of CAR-T therapy in patients with lymphoid malignancies, and is characterized by increased levels of multiple pro-inflammatory cytokines, including IL-6, interferon-gamma, tumor necrosis factor, IL-2, IL-2-receptor- α , IL-8, and IL-10.⁶⁰ CRS can affect multiple organs, and can occur anywhere from hours to more than 7 days following

CAR-T infusion. Mild cases of CRS produce flu-like symptoms; however, more severe cases may lead to hypotension, vascular leak, respiratory failure due to pulmonary edema and/or pneumonitis, renal insufficiency, and neurologic complications including delirium, hallucinations, ataxia, aphasia, and seizures.⁶⁰ Multiple CRS grading systems have been proposed,⁶¹⁻⁶³ complicating the direct comparison of CRS cases between trials. Of note, the Common Terminology Criteria for Adverse Events, Version 4.0⁶⁴ criteria for grading CRS is applicable only to infusion reactions caused by monoclonal antibodies, and does not apply to adoptive cellular immunotherapy.⁶⁰ Brudno and Kochenderfer have recently published guidelines for managing patients with CRS and other toxicities associated with CAR-Ts.⁶⁰ Tocilizumab, a monoclonal antibody targeting the interleukin-6 receptor (IL-6R), is recommended for the initial treatment of CRS, with corticosteroids used for cases refractory to tocilizumab, and dexamethasone is specifically recommended for grade ≥ 3 neurologic toxicities.⁶⁰ Corticosteroids may impair CAR-T expansion and persistence, and are thus avoided in milder cases of CRS.^{63,65} Although not yet evaluated in clinical trials, recent preclinical studies have suggested a potential benefit of the Bruton's tyrosine kinase inhibitor, ibrutinib, and the JAK/STAT pathway inhibitor, ruxolitinib, in reducing CAR-T-mediated cytokine release and preventing CRS.^{66,67} Despite the use of tocilizumab and corticosteroids, severe CRS has led to several deaths in clinical trials of CD19-specific CAR-Ts, and 5 deaths due to unexpected severe neurologic toxicity recently prompted the discontinuation of a large phase II clinical trial in adult subjects with relapsed/refractory B-ALL.^{55,68-70} Although neurotoxicity is frequently seen in the context of CRS, neurologic complications due to CD19-specific CAR-Ts have also occurred independently of other CRS symptoms. In a recent analysis of CRS in 39 pediatric subjects treated for B-ALL at the Children's Hospital of Philadelphia (CHOP), grade ≥ 3 CRS was reported in 46% and encephalopathy was reported in 33% of patients, with encephalopathy most often occurring later in the course of CRS and after fevers had resolved.⁷¹ The pathogenesis of the serious neurologic complications seen with certain CAR-T products remains unclear.^{60,65} Severe encephalopathy is unfortunately unresponsive to steroids in most cases and thus predictive models⁷² and alternative treatment strategies are needed.

On-target toxicities. The widespread applicability of CAR-T therapy and its extension beyond B-cell malignancies has in large part been limited by the

paucity of known antigens that are truly specific to tumor cells. Expression of target antigens on normal tissues can lead to severe toxicity in the setting of CAR-T therapy, as evidenced by early experience in trials among patients with solid tumors.^{15,73} The comparative success of CD19-specific CAR-Ts is largely explained by the tolerability of associated on-target toxicities. CD19 is an extracellular glycoprotein whose expression is limited to normal B-cells and malignant B-cell clones, and aplasia of normal B-cells resulting from successful treatment with CD19-specific CAR-Ts can be effectively managed with the use of intravenous immunoglobulin infusions. Similarly, in a trial of CAR-Ts targeting the κ -light chain of human immunoglobulins, on-target toxicities led to the elimination of normal κ -expressing (but not λ -expressing) B-cells.⁷⁴ Given the rapidly expanding field of CAR-T therapies, and ongoing clinical trials evaluating novel antigenic targets for both hematologic malignancies and solid tumors,⁷⁵ on-target toxicities will continue to be a major concern. As discussed below, there has consequently been significant interest in the incorporation of various safety mechanisms into CAR designs with the goal of mitigating any observed toxicities.

Safety strategies. Given the potential for life-threatening, and often unpredictable toxicities associated with CAR-T therapy, a compelling strategy to improve the safety of such therapy has been the addition of "elimination" or "suicide" genes into CAR constructs.

The herpes simplex virus thymidine kinase (HSV-tk) was the first such transgene to be evaluated in the context of cellular therapy.⁷⁶ HSV-tk-expressing lymphocytes can be killed by administration of the pro-drug, ganciclovir, leading to accumulation of toxic metabolites and disruption of DNA synthesis in dividing cells. While this approach has been evaluated clinically in the context of HSCT,⁷⁷ disadvantages include the slow onset of action (due to effects only on dividing cells), transgene immunogenicity limiting the in vivo persistence of HSV-tk-expressing cells,⁷⁸ and the potential interference with alternative clinical use of ganciclovir.

One more promising approach has been the inclusion of an inducible caspase-9 (iC9) safety switch, which is based on a modified human caspase-9 gene fused to the human FK506 binding protein. When incorporated into a CAR, dimerization of iC9, and subsequent apoptosis of the expressing cells, can be induced by a chemical inducer of dimerization known as AP1903/Rimiducid (Bellicum Pharmaceuticals). In a humanized mouse model, our group recently demonstrated the

ability of AP1903 to eliminate iC9-containing CD19-specific CAR-Ts in a dose-dependent manner, thus allowing the selective containment of CAR-T expansion when clinically appropriate (eg, mild CRS) or the complete deletion of the CAR-Ts to allow B-cell reconstitution.⁷⁹ The iC9 system has been evaluated clinically in the setting of allogeneic HSCT.⁸⁰ There are currently several registered clinical trials evaluating the iC9 gene in the context of CAR-T therapy for solid tumors⁸¹⁻⁸³ and hematologic malignancies,⁸⁴⁻⁸⁶ including a trial of an iC9-containing CD19-directed CAR for the treatment of relapsed/refractory acute lymphoblastic leukemia that our group will be opening at the University of North Carolina.⁸⁶

Another strategy to improve the safety of CAR-Ts has been the incorporation of a truncated form of the epidermal growth factor receptor (EGFRt), with the goal of allowing CAR-Ts to be eliminated in case of toxicity via administration of the anti-EGFR monoclonal antibody, cetuximab.⁸⁷ The EGFRt domain was included in a recent CD19-directed CAR-T product evaluated in clinical trials at the FHCRC/Seattle Children's Hospital;^{53,55} however, the administration of cetuximab was not reported despite the occurrence of 2 treatment-related deaths due to severe CRS and irreversible neurologic toxicity, respectively.⁵⁵

CLINICAL TRIALS OF CAR T-CELLS FOR B-CELL MALIGNANCIES

Early clinical trial experience. Although initial clinical trials of CAR-Ts focused on patients with solid tumors,¹⁷ the most impressive clinical results to date have occurred in the treatment of B-cell malignancies. As noted above, this is in large part due the ideal candidacy of CD19 as a target for CAR-Ts. The initial experience with CAR-Ts in B-cell malignancies came from a trial conducted in 2008 by investigators at the City of Hope and FHCRC in which a first-generation CD20-specific CAR was evaluated in 7 patients with relapsed or refractory follicular lymphoma (FL).¹⁸ Although efficacy was limited (1 patient achieved a partial response [PR] and no patient developed any significant B-cell aplasia), there were also no grade ≥ 3 adverse events reported and CAR-Ts persisted up to 5–9 weeks in 4 of 7 patients. The potential toxicity of CAR-Ts was highlighted early, however, by a report from the Memorial Sloan Kettering Cancer Center (MSKCC) describing the death, due to symptoms consistent with CRS, of a patient with chronic lymphocytic leukemia (CLL) who was treated with cyclophosphamide followed by infusion of CD19-CD28- ζ second-generation CAR-Ts.⁸⁸ In 2010, Jensen et al. reported their experience in treating 2 patients

with DLBCL with first-generation CD20-specific CAR-Ts and 2 patients with refractory FL with CD19-specific CAR-Ts. As with most trials of first-generation CAR-Ts, in vivo persistence was poor (1–7 days). Although this may be at least partially explained by antitransgene immune responses observed in 2 patients, it is unknown whether this activity was directed at the CAR or an included hygromycin resistance gene.¹⁹ In 2010, Kochenderfer et al. at the NCI published an early report of success in which a patient with FL was treated with a CD19-specific CD28- ζ -containing second-generation CAR-T product following lymphodepleting chemotherapy with cyclophosphamide and fludarabine. The authors demonstrated CAR-T persistence until 27 weeks following initial infusion, which was associated with a PR of the patient's lymphoma and, most notably, the development of isolated B-cell aplasia (used as a surrogate marker for CD19 CAR-T persistence and cytotoxic activity) lasting until 39 weeks.³⁶ It was not until 2011, however, that the enormous potential of CAR-T therapy was initially demonstrated by the preliminary results of a landmark trial from the University of Pennsylvania (UPenn).^{89,90} In this trial, patients with advanced chemotherapy-resistant CLL were treated, following lymphodepleting chemotherapy, with T-cells lentivirally transduced with a second-generation 4-1BB- ζ -containing CAR.^{89,90} The authors demonstrated significant (>1000 fold) in vivo CAR-T expansion, B-cell aplasia, and tumor lysis syndrome, and reported that 2 of the initial 3 patients achieved a CR lasting >10 months. Mature trial results published in 2015 showed an overall response rate (ORR) of 57%, with 4 of 14 patients achieving a CR that was negative for MRD, and a median progression-free survival of 7 months.⁶² Although the earliest successes were reported in NHL and CLL, the most impressive results with CAR-T therapy to date have been seen with second-generation CD19-specific CAR-Ts for relapsed and refractory B-ALL for both children and adults, initially demonstrated in trials at CHOP^{91,92} and the University of Pennsylvania.^{63,92} Significant efficacy has also been demonstrated in DLBCL and indolent B-cell lymphomas.⁹³

Clinical trials of CD19-specific CAR T-cells. Clinical trials of CD19-specific CAR-Ts are summarized in detail in [Tables I and II](#). Outcomes have been reported for over 350 patients with B-ALL and over 200 patients with NHL/CLL. There is significant heterogeneity between these trials, including differences in the CAR design, T-cell subsets, lymphodepletion regimens, autologous vs allogeneic T-cells, and the dose of

Table I. CAR-T clinical trials with reported efficacy data: B-ALL

Year	Academic institution (Industry)	Antigen	Treated population	scFv/Costim. Domain	Vector	Lymphodepletion	CAR-T dose range	CRS/Neurologic toxicity	Outcomes
2013	BCM ³³	CD19 + virus-specific	Adult B-ALL, n = 4	FMC63/CD28	RV	None, history of allo-HSCT 3mo–12 mo before infusion	3.8×10^7 – 9.7×10^7 /kg (allogeneic)	No Gr ≥ 3 toxicities reported	ORR: 3/4; CR: 1/4 (3 mo); Continued CR: 2/4 (2+ to 8+ mo)
2015	CHOP ^{92,94-96} (Novartis)	CD19	Pediatric B-ALL, n = 60	FMC63/4-1BB	LV	IC	1.1×10^6 – 1.7×10^7 /kg	Any Gr CRS: 52/59; sCRS: 16/59; Neurotoxicity NR (Any Gr neurotoxicity in 13/30 pts in initial combined analysis of pediatric/adult trials)	CR: 56/60, MRD(–) in 51/56; 12 mo RFS: 60%; 24 mo RFS: 53%; 12 mo OS: 79%; 24 mo OS: 61%
2015	CPLAGH, Beijing, China ⁵⁷	CD19	Adult B-ALL, n = 9	FMC63/4-1BB	LV	None (n = 7/9), Cy-MOAD (n = 2/9)	3×10^6 – 1×10^7 /kg	Any Gr CRS: 4/9; Gr ≥ 3 CRS: 3/9	ORR: 4/9; CR: 2/9 (9 wks, 38+ wks), MRD(–) in 2/2; PR: 2/9; 18 wk OS: 5/9
2016	CHOP/Global Multicenter ⁹⁷ (Novartis)	CD19	Pediatric B-ALL, n = 34	FMC63/4-1BB	LV	IC (FluCy in majority)	0.2×10^6 – 4×10^6 /kg (median 2.9×10^6)	Any Gr CRS: 28/34; Gr 3 CRS: 7/34; Gr 4 CRS: 8/34; Gr 3–4 neurotoxicity: 7/34	CR/CRi: 24/29, MRD(–) in 24/24; 6 mo OS: 89%; 6 mo RFS: 60%
2016	CHOP/US Multicenter ⁹⁸ (Novartis)	CD19	Pediatric B-ALL, n = 29	FMC63/4-1BB	LV	FluCy	2.0 – 5.0×10^6 /kg (≤ 50 kg); 1.0 – 2.5×10^8 (> 50 kg)	Gr 3–4 CRS: 11/29; Gr 3 neurotoxicity: 1; Gr 4–5 neurotoxicity: 0	CR/CRi: 20/29, MRD(–) in 18/20; 6 mo RFS: 66.4%; 6 mo OS: 75.7%
2016	CHOP ^{99,100} (Novartis)	CD19	Pediatric B-ALL, n = 30 ¹⁰⁰ (11/30 prior murine CAR-Ts)	HuCART19/4-1BB (humanized)	LV	FluCy	Not reported	Gr 3 CRS: 3/30; Gr 4 CRS: 1/30; Any Gr encephalopathy: 5; Any Gr seizure: 4	CR: 26/30 (7/11 with prior murine CAR-T therapy), MRD(–) in 19/19 without prior CAR-T therapy, 5/7 with prior CAR-T therapy
2016	FHCRC ^{54,55,58} (Juno)	CD19	Adult r/r B-ALL, n = 30	FMC63/4-1BB (EGFRt)	LV	Cy (n = 13/30) FluCy (n = 17/30)	2×10^5 – 2×10^7 /kg; defined 1:1 CD4⁺:CD8⁺ ratio	CRS: 25/30; sCRS: 7/30; one pt died due to CRS ; Gr 3–4 neurotoxicity: 14/30; Gr 5 neurotoxicity: 1/30 (Gr ≥ 3 neurotoxicity in 6/6 who received CAR-Ts dose $\geq 2 \times 10^6$ /kg)	CR: 28/30 (17/17 FluCy, 11/13 Cy), MRD(–) in 16/17 FluCy, 10/11 Cy; Alive in CR: 12/17 FluCy (median f/u 300 d), 1/13 Cy (f/u 600 + d)

2016	GIMI/Multicenter, China ¹⁰¹	CD19	B-ALL, n = 102 (47 adult, 55 pediatric)	<i>CD19scFv/CD28-4-1BB-CD27 (iCasp9)</i>	LV	FluCy (n = 54), Cy (n = 17), other (n = 29), none (n = 2)	$1.05 \times 10^6/\text{kg}$ (average)	Gr 0–1 CRS: 73/102; Gr 3–4 CRS: 3/17 (BM blasts > 80%), 8/38 (BM blasts $\geq 50\%$); Neurotoxicity NR.	CR: 91.3% (<50% BM blasts, n = 69), 75.8% ($\geq 50\%$ BM blasts, n = 33); median OS: 485 d (<50% BM blasts), 317 d ($\geq 50\%$ BM blasts)
2016	HYLDH, Langfang, China ¹⁰²	CD19	B-ALL, n = 47 (ages 2–68)	<i>CD19scFv/4-1BB</i>	LV	FluCy (n = 46; Flu $30 \text{ mg}/\text{m}^2 \times 3$, Cy $250 \text{ mg}/\text{m}^2 \times 3$)	5×10^3 – $1.4 \times 10^6/\text{kg}$ (median $1.0 \times 10^5/\text{kg}$)	Median CRS grade 2 (range 1–5); Gr ≥ 3 CRS and Gr 5 CRS NR; neurotoxicity NR.	CR/CRi: 31/35, MRD(–) in 29/35 (15 remained in CR with median f/u 197 d)
2016	MDACC ³⁵ (Ziopharm/Intrexon)	CD19	Adult B-ALL undergoing allo-HSCT, n = 17	FMC63/CD28	SB	/C (following allo-HSCT)	1×10^6 – $1 \times 10^8/\text{m}^2$	No CRS or neurotoxicity reported; Acute GVHD: 3/17	Continued CR: 9/17 (median duration 6 mo at last follow-up); Died in CR: 1/17
2016	MSKCC ^{63,103-106} (Juno)	CD19	Adult r/r B-ALL, n = 51 ¹⁰³ (20/51 with baseline MRD)	SJ25C1/CD28	RV	Cy or FluCy	1×10^6 – $3 \times 10^6/\text{kg}$	sCRS: 14/51 (13/31 baseline morphologic dz, 1/20 baseline MRD); Gr 3–4 neurotoxicity: 15/51 (11/31 baseline morphologic dz, 4/20 baseline MRD); Note: development of this CAR-T product was discontinued after 5 deaths due to cerebral edema in a multicenter phase II trial. ^{69,70,107}	CR: 41/50 (CR in 23/30 with baseline morphologic dz, 18/20 with baseline MRD), MRD(–) in 33/39 CRs (no MRD testing in 2 pts); 15/33 with MRD(–) CR relapsed, 9/33 MRD(–) CR dz free 12+ mo; 6 mo OS: 73% (baseline MRD), 57% (baseline morphologic dz), 92% (pts attaining MRD(–) CR)
2016	Multicenter ¹⁰⁸ (Kite)	CD19	B-ALL, n = 5 (3 adult, 2 pediatric)	FMC63/CD28	RV	FluCy (Flu $25 \text{ mg}/\text{m}^2 \times 3$, Cy $900 \text{ mg}/\text{m}^2$)	1×10^6 vs $2 \times 10^6/\text{kg}$ (target)	Any Gr CRS: 5/5; No Gr ≥ 3 CRS; Gr ≥ 3 neurotoxicity: 3/5 (3/3 adult pts); tocilizumab +/- corticosteroids +/- siltuximab (5/5)	CR: 5/5, MRD(–) in 5/5 (duration NR)

(Continued)

Table I. (Continued)

Year	Academic institution (Industry)	Antigen	Treated population	scFv/Costim. Domain	Vector	Lymphodepletion	CAR-T dose range	CRS/Neurologic toxicity	Outcomes
2016	NCI ³⁴	CD19	Adult B-ALL relapsed after allo-HSCT, n = 5	FMC63/CD28	RV	None	4.2×10^6 – 7.0×10^6 /kg allogeneic	Gr 3–4 sinus tachycardia: 3/5; Gr 3–4 hypotension: 4/5; Neurotoxicity NR	CR: 4/5; MRD(–) in 4/4
2016	NCI ^{59,91}	CD19	Pediatric r/r B-ALL, n = 51	FMC63/CD28	RV	FluCy (low-dose n = 36, high-dose n = 8), FLAG (n = 6), ifos/etop (n = 2)	1×10^6 – 3×10^6 /kg	Gr 3–4 CRS: 7/51; Gr 1–2 seizures: 2/51; Gr 3 neurotoxicity: 3/51	CR: 31/51 (18/21 < 25% BM blasts, 13/32 > 25% BM blasts, 29/44 FluCy, 2/8 other LD), MRD(–) in 28/31, median RFS 18 mo for MRD(–); median OS 13.3 mo (FluCy) vs 5.5 mo (other LD)
2016	NCI ¹⁰⁹ (Juno)	CD22	Pediatric B-ALL, n = 9 (7/9 with prior CD19 CAR therapy)	m971/4-1BB	LV	FluCy (Flu 25 mg/m ² × 3, Cy 900 mg/m ² × 1)	3×10^5 vs 1×10^6 /kg	Gr 1–2 CRS: 6/9; Neurotoxicity NR	CR: 4/9, MRD(–) in 4/4; 3/3 pts treated at higher dose are in sustained remission at 3 mo
2016	Seattle Children's ⁵³ (Juno)	CD19	Pediatric B-ALL, n = 43	FMC63/4-1BB (EGFRt)	LV	Cy (n = 27), FluCy (n = 14), CE (n = 1), Flu (n = 1)	5×10^5 – 4×10^6 /kg defined 1:1 CD4⁺:CD8⁺ ratio	CRS: 40/43; sCRS: 10/43 (severity correlated to dose level, but not dz burden; no toxic deaths); Any Gr neurotoxicity: 21/43; Severe neurotoxicity: 10/43	CR: 40/43, MRD(–) in 40/40; 12 mo OS: 69.5%; Median CAR-T persistence: 6.4 mo (>15% CD19 ⁺ BM cells), 1.7 mo (<15% CD19 ⁺ BM cells); Loss of CAR-T persistence associated with CD19 ⁺ relapse, HR 34

2016	UPenn ^{68,92} (Novartis)	CD19	Adult r/r B-ALL, n = 30	FMC63/4-1BB	LV	IC (Cy-based)	5×10^7 vs 5×10^8	Gr ≥ 3 CRS: 22/30; Gr 5 CRS: 3/30 (each received 5×10^8 CAR-Ts in single infusion, each received tocilizumab + corticosteroids); Gr ≥ 3 neurotoxicity: 1/30 (Gr 3 encephalopathy)	CR: 15/27; PR: 1/27
2017	University College London ^{37,38} (Cellestis)	CD19	Pediatric B-ALL, n = 2	4g7/4-1BB	LV	Flu, Cy, Alemtuzumab (anti-CD52)	4.0–4.6 $\times 10^6$ /kg “universal” (allogeneic, TCR⁻/CD52⁻ via TALEN gene-editing)	No CRS or neurotoxicity reported; Gr 2 skin GVHD in 1/2.	CR: 2/2 (duration 12+ and 18+ mo), both MRD(–)

Abbreviations: ALL, acute lymphoblastic lymphoma; *allo-HSCT*, allogeneic hematopoietic stem cell transplantation; *auto-HSCT*, autologous hematopoietic stem cell transplant; BCM, Baylor College of Medicine; CAR-T, chimeric antigen receptor T-cell; CE, cyclophosphamide + etoposide; *costim.*, costimulatory; CPLAGH, Chinese PLA General Hospital; CHOP, Children’s Hospital of Philadelphia; CR, complete response or complete remission; CRI, complete remission with incomplete count recovery; Cy, cyclophosphamide; dz, disease; etop, etoposide; FLAG, fludarabine + cytarabine + granulocyte colony-stimulating factor; Flu, fludarabine; FluCy, fludarabine + cyclophosphamide; f/u, follow-up; GIMI, Shenzhen Genoimmune Medical Institute (Shenzhen, China); Gr, grade; HYLH, Hebei Yanda Lu Daopei Hospital; IC, investigator’s choice; ifos, ifosfamide; LV, lentivirus; MDACC, MD Anderson Cancer Center; mo, months; MSKCC, Memorial Sloan Kettering Cancer Center; MRD, minimal residual disease; MRD(–), negative evaluation for minimal residual disease; NCI, National Cancer Institute; NE, not evaluable; NR, not reported; ORR, overall response rate (PR or better); OS, overall survival; PR, partial response; pt, patient; r/r, relapsed and/or refractory; RFS, relapse-free survival; RV, retrovirus; SB, Sleeping Beauty; scFv, single-chain variable fragment; sCRS, severe CRS (requiring ICU management, treatment with tocilizumab and/or corticosteroids); TALEN, transcription activator-like effector nuclease; UPenn, University of Pennsylvania.

Allogeneic CAR-Ts are derived from patient’s donor for prior allo-HSCT (except University College London study). Year refers to year most recent results were reported.

Table II. CAR-T clinical trials with reported efficacy data: B-cell NHL and CLL

Year	Academic institution (Industry)	Antigen	Treated Population	scFv/Costim. Domain	Vector	Lympho-depletion	CAR-T dose range	CRS/Neurologic toxicity	Outcomes
2012	NCI ¹¹⁰	CD19	Adults: B-NHL or CLL, n = 8 (4 CLL, 3 FL, 1 SMZL)	FMC63/CD28	RV	FluCy (Flu 25 mg/m ² × 5, Cy 60 mg/kg × 2)	3.0 × 10 ⁶ –3.0 × 10 ⁷ /kg	CRS not graded; Gr ≥ 3 hypotension (n = 2), Gr ≥ 3 hypoxemia (n = 2), Gr ≥ 3 acute renal failure (n = 3)	ORR: 6/7 (1/8 NE); CR: 1/7 (CLL, 15+ mo); PR: 5/7 (CLL, n = 2; FL, n = 2; sMZL, n = 1)
2013	BCM ³³	CD19 + virus-specific	Adults: relapsed CLL after allo-HSCT, n = 4	FMC63/CD28	RV	None	6.8 × 10 ⁷ –1.13 × 10 ⁸ /kg (allogeneic)	No Gr ≥ 3 toxicities reported	ORR: 1/4; PR: 1/4 (duration 8 wk); SD: 1/4 (duration 15+ mo)
2013	NCI ³⁶	CD19	Adults: persistent B-NHL or CLL after allo-HSCT, n = 15 (4 CLL, 2 DLBCL, 4 MCL)	FMC63/CD28	RV	None	4.0 × 10 ⁵ –7.8 × 10 ⁶ /kg (allogeneic)	Gr ≥ 3 fever: 2/15; Gr ≥ 3 hypotension: 2/15; Gr ≥ 3 hypoxia: 1/15	ORR: 2/10; CR: 1/10 (CLL, duration 9+ mo); PR: 1 (MCL, duration 3+ mo)
2015	MSKCC ¹¹¹	CD19	Adults: poor-risk aggressive r/r B-NHL, n = 8	SJ25C1 /CD28	RV	Following HDT-ASCT with BEAM conditioning	5 × 10 ⁶ /kg (n = 7) vs 1 × 10 ⁷ /kg (n = 1)	CRS: 4/8; sCRS: 1/8; Tocilizumab: 4/8	ORR: 5/8; CR: 5/8 (duration 10+ to 18+ mo)
2015	NCI ⁹³ (Kite)	CD19	Adults: B-NHL or CLL, n = 15 (4 CLL, 9 chemo-refractory DLBCL, 2 indolent B-NHL)	FMC63/CD28	RV	FluCy (Flu 25 mg/m ² × 5, Cy 60 vs 120 mg/kg × 1)	1.0 × 10 ⁶ –5.0 × 10 ⁶ /kg	CRS not graded; Gr ≥ 3 hypotension: 4/15, Gr ≥ 3 hypoxia: 2/15, Gr ≥ 3 neurologic toxicity: 6/15; Tocilizumab: 2/15	ORR: 11/13 (2 NE); CR: 4/7 DLBCL (duration 6 to 22+ mo), 3/4 CLL (duration 14 to 23+ mo), 1/2 indolent B-NHL (duration 11+ mo)

2015	UPenn ⁶²	CD19	Adults: r/r CLL, n = 14	FMC63/4-1BB	LV	IC (Bend, n = 6; FluCy, n = 3; PenCy, n = 5)	1.4×10^7 – 1.1×10^9 (flat dose)	Any Gr CRS (UPenn ⁶²): 9/14; Gr 3 CRS: 2/14; Gr 4 CRS: 4/14; Gr ≥ 3 neurotoxicity (Gr 4 confusion) in 1/14, concurrent with CRS; Tocilizumab: 4; Corticosteroids: 2	ORR: 8/14; CR: 4/ 14, MRD(–) in 4/4; PR: 4/14; Median PFS: 7 mo; Median OS: 29 mo; 18 mo OS: 71%
2016	BCM ²⁹	CD19	Adults: r/r DLBCL, n = 6	FMC63/CD28 + FMC63/CD28- 4-1BB	RV	FluCy (Flu $30 \text{ mg/m}^2 \times 5$, Cy 500 $\text{mg/m}^2 \times 3$)	$1\text{--}20 \times 10^6/\text{m}^2$ (both 2nd and 3rd gen CAR-Ts simultaneously)	Gr 2 CRS: 2/6; No neurotoxicity reported	ORR: 4/5; CR: 2/5 (1 NE), longest 9+ mo; continued CR: 1/5; PR 1/5; 3rd gen CAR-Ts had mean 23- fold (1.1- to 109- fold) higher expansion, longer persistence
2016	BCM ⁷⁴	κ-light chain	Adults: r/r B-NHL/CLL, n = 16 (7 NHL, 2 CLL/SLL, 7 MM)	CRL-1758/CD28	RV	Cy 12.5 mg/kg (1/7 NHL, 4/ 7 MM); none 9/16	1.7×10^7 – $1.9 \times 10^8/\text{m}^2$	No CRS or neurotoxicity observed. Gr 3 lymphopenia in one pt. No other Gr ≥ 3 treatment-related toxicity.	ORR: 3/9; CR: 2/9 (both DLBCL, one relapsed after 6 wks, other with CR 32+ mo); PR: 1/9
2016	CPLAGH, Beijing, China ^{112,113}	CD20	Adults: r/r B-NHL, n = 11	AY160760.1/4-1BB	LV	IC (none in 4/11)	4.1×10^6 – $1.5 \times 10^7/\text{kg}$	No CRS or neurotoxicity observed. No Gr ≥ 3 toxicities observed other than hypokalemia (n = 1), herpes zoster (n = 1)	ORR: 9/11; CR: 6/11 (duration 5 to 27+ mo); PR: 3/11; median PFS: 6 mo; pt with longest CR had 2nd CAR-T infusion at 16 mo

(Continued)

Table II. (Continued)

Year	Academic institution (Industry)	Antigen	Treated Population	scFv/Costim. Domain	Vector	Lympho-depletion	CAR-T dose range	CRS/Neurologic toxicity	Outcomes
2016	FHCRC ¹¹⁴ (Juno)	CD19	Adults: refractory CLL, n = 18	FMC63/4-1BB (EGFRt)	LV	FluCy (n = 15), Flu (n = 2), Cy (n = 1)	2×10^5 – 2×10^7 /kg; defined 1:1 CD4⁺:CD8⁺ ratio	Any Gr CRS: 17/17; Gr 3–4 CRS: 4/17; Gr ≥ 3 neurotoxicity: 4/17. Tocilizumab + corticosteroids: 3/17	ORR: 13/17; CR 5/17, MRD(–) in 4/4 (1 NE), none relapsed with median f/u 8.4 mo; PR 8/17
2016	FHCRC ^{54,115,116} (Juno)	CD19	Adults: B-NHL, n = 32 (21 DLBCL, 5 FL, 4 MCL)	FMC63/4-1BB (EGFRt)	LV	Cy, CE, or FluCy	2×10^5 – 2×10^7 /kg; defined 1:1 CD4⁺:CD8⁺ ratio	sCRS: 4/32 (all cases in pts treated with FluCy and $\geq 1 \times 10^6$ CAR-Ts/kg); tocilizumab: 3; Gr ≥ 3 neurotoxicity: 9/32 (7/9 had received FluCy, 8/9 had received $\geq 1 \times 10^6$ CAR-Ts/kg)	ORR: 19/30; CR: 10/30 (8/21 DLBCL, 2/5 FL, 0/4 MCL); 9/10 CRs in pts who received FluCy; CAR expansion and persistence better with FluCy
2016	MDACC ³⁵ (Ziopharm/ Intrexon)	CD19	Adults: undergoing autologous (n = 7) or allogeneic (n = 2) HSCT for B-NHL (4 DLBCL, 3 FL, 1 MCL), 1 nHL	FMC63/CD28	SB	Following HSCT (preparative regimen /C)	1×10^7 – 5×10^9 /m ² (autologous or allogeneic)	No CRS or neurotoxicity reported.	Continued CR: 6/7 autologous (median duration 26 mo at last follow-up), 0/2 allogeneic
2016	MSKCC ¹¹⁷	CD19	Adults: r/r CLL, n = 11	SJ25C1/CD28	RV	FluCy (n = 3), Cy (n = 6), Bend (n = 2); + ibrutinib (n = 5)	1×10^7 /kg (median)	sCRS: 2/11 (2/5 ibrutinib, 0/6 non-ibrutinib)	ORR: 5/11 (4/5 ibrutinib, 1/6 non-ibrutinib); CR 2, MRD(–) in 2/2 (duration 16+ and 50+ mo); continued CR: 1/11; PR: 2/11

2016	Multicenter (Kite) ¹¹⁸	CD19	Adults: B-NHL, n = 6 (3 PMBCL, 3 transformed FL)	FMC63/CD28	RV	FluCy (Flu 30 mg/ m ² × 3, Cy 500 mg/ m ² × 3)	2 × 10 ⁶ /kg (target)	Any Gr CRS: 6/6; No Gr ≥ 3 CRS; Any Gr neurotoxicity: 4/6; Gr 3–4 neurotoxicity: 2/6	ORR: 6/6; CR: 6/6 (median f/u 3.2 mo)
2016	Multicenter ¹¹⁹ (Juno)	CD19	Adults: r/r B-NHL, n = 14 (13 DLBCL, 1 MCL)	FMC63/4-1BB	LV	FluCy (Flu 30 mg/m ² , Cy 300 mg/m ²)	5 × 10 ⁷ /kg	Gr 1–2 CRS: 3 (grading system NR) Gr ≥ 3 CRS: 0 Gr ≥ 3 neurotoxicity: 2 (1 Gr 4 encephalopathy, 1 Gr 4 seizure)	DLBCL (n = 13, 2 NE) – ORR: 9/11; CR: 8/11 (7/8 remained in CR, follow-up duration NR); PR: 1/11. MCL – ORR: 0/1
2016	NCI ³⁴	CD19	Adults: B-NHL or CLL, relapsed following allo- HSCT, n = 15 (5 CLL, 5 DLBCL, 5 MCL)	FMC63/CD28	RV	Following allogeneic HCT	4 × 10 ⁵ –8.2 × 10 ⁶ /kg	CRS not graded; Gr ≥ 3 fever: 4/15; Gr ≥ 3 tachycardia: 3/15; Gr ≥ 3 hypotension 3/15; Gr ≥ 3 headache 1/15	ORR: 4/15; CR: 2/15 (1/5 CLL with duration 30+ mo, 1/5 DLBCL with duration 6+ mo)
2016	NCI ¹²⁰ (Kite)	CD19	Adults: B-NHL, n = 22 (19 DLBCL, 2 FL, 1 MCL)	FMC63/CD28	RV	FluCy (Flu 30 mg/m ² × 3, Cy 300 vs 500 mg/m ² × 3)	1.0 × 10 ⁶ – 6.0 × 10 ⁶ /kg	Gr ≥ 3 Neurotoxicity 12/22 (confusion, dysphagia, encephalopathy, gait disturbances); Gr ≥ 3 hypotension 4/22	ORR: 16/22; CR: 12/22; (9/19 DLBCL with duration 7–20 mo, 2/2 FL, 1/1 MCL); PR: 4/19 DLBCL
2016	NCI ¹²¹	CD19	Adults: B-NHL, n = 9 (4 DLBCL, 2 FL, 1 Burkitt, 1 unclassified)	HuCAR19/CD28 (fully human)	LV	FluCy (Flu 30 mg/m ² × 3, Cy 300 mg/m ² × 3)	6.6 × 10 ⁵ – 2.0 × 10 ⁶ /kg	Any Gr CRS (NCI ⁶¹): 9/8 (1 NE); Gr 3–4 CRS: 3/8	ORR: 6/8 (1 NE); CR: 2/7 (duration 2+ mo)
2016	Upenn ^{122,123}	CD19	Adults: r/r CLL, n = 35	FMC63/4-1BB	LV	IC	5 × 10 ⁷ vs 5 × 10 ⁸ (phase I); 5 × 10 ⁸ (phase II)	Any Gr CRS (Upenn ⁶²): 19/35; Gr 3–4 CRS: 7/35; Tocilizumab: 4; CAR-T dose not associated with CRS development or severity	ORR: 13/30 (4/13 low-dose, 9/17 high-dose); CR: 7/30 (1/13 low-dose, 6/17 high-dose; duration 5 to 34+ mo); PR: 3/17

(Continued)

Table II. (Continued)

Year	Academic institution (Industry)	Antigen	Treated Population	scFv/Costim. Domain	Vector	Lympho-depletion	CAR-T dose range	CRS/Neurologic toxicity	Outcomes
2016	Upenn ¹²⁴⁻¹²⁷	CD19	Adults: r/r NHL, n = 27 (13 DLBCL, ¹²⁵ 14 FL ¹²⁶)	FMC63/4-1BB	LV	IC	DLBCL – 3.10 × 10 ⁶ –6.75 × 10 ⁶ /kg. FL – 3.08 × 10 ⁶ –8.87 × 10 ⁶ /kg	DLBCL – Any Gr CRS (Upenn ⁶²): 9/13; Gr ≥ 3 CRS: 1/13; Gr ≥ 3 Neurotoxicity: 1/13. FL – Any Gr CRS: 6/14 Gr ≥ 3 CRS: 2; Gr 5 encephalitis (possibly treatment-related)	DLBCL – ORR: 7/13; CR: 6/13, none relapsed; Median PFS: 5.8 mo FL – ORR: 11/14; CR 9/14; 11 mo PFS: 77%
2017	Multicenter ^{128,129} (Kite)	CD19	Adults: refractory DLBCL, n = 7	FMC63/CD28	RV	FluCy (Flu 30 mg/m ² , Cy 500 mg/m ²)	1.1 × 10 ⁶ –2.0 × 10 ⁶ /kg (target: 2.0 × 10 ⁶)	Any Gr CRS (NCI ⁶¹): 6/7; Gr ≥ 3 CRS: 1/7; Gr ≥ 3 neurotoxicity: 4/7; Gr 4 CRS, Gr 4 neurotoxicity, and Gr 5 intracranial hemorrhage in one pt (death deemed unrelated to CAR-Ts); Tocilizumab: 6/7; Corticosteroids: 4/7	ORR: 5/7; CR: 4/7 (3 ongoing with duration 12+ mo)

Abbreviations: *allo-HSCT*, allogeneic hematopoietic stem cell transplantation; *auto-HSCT*, autologous hematopoietic stem cell transplant; *B-NHL*, B-cell non-Hodgkin lymphoma; *BCM*, Baylor College of Medicine; *BEAM*, carmustine, etoposide, cytarabine, and melphalan; *Bend*, bendamustine; *CAR-T*, chimeric antigen receptor T-cell; *CE*, cyclophosphamide + etoposide; *chemo*, chemotherapy; *costim.*, costimulatory; *CPLAGH*, Chinese PLA General Hospital; *CHOP*, Children's Hospital of Philadelphia; *CLL*, chronic lymphocytic leukemia; *CR*, complete response or complete remission; *Cy*, cyclophosphamide; *DLBCL*, diffuse large B-cell lymphoma; *dz*, disease; *FL*, follicular lymphoma; *Flu*, fludarabine; *FluCy*, fludarabine + cyclophosphamide; *f/u*, follow-up; *gen*, generation; *Gr*, grade; *HDT-ASCT*, high-dose therapy and autologous stem cell transplant; *HSCT*, hematopoietic stem cell transplantation; *IC*, investigator's choice; *ifos*, ifosfamide; *LV*, lentivirus; *MCL*, mantle cell lymphoma; *MDACC*, MD Anderson Cancer Center; *mo*, months; *MSKCC*, Memorial Sloan Kettering Cancer Center; *MRD*, minimal residual disease; *MRD(–)*, negative evaluation for minimal residual disease; *NCI*, National Cancer Institute; *NE*, not evaluable; *NR*, not reported; *nHL*, nodular Hodgkin Lymphoma; *NHL*, non-Hodgkin lymphoma; *NR*, not reported; *ORR*, overall response rate (PR or better); *OS*, overall survival; *pen*, pentostatin; *PMBCL*, Primary Mediastinal B-Cell Lymphoma; *PR*, partial response; *pt*, patient; *r/r*, relapsed and/or refractory; *RFS*, relapse-free survival; *RV*, retrovirus; *SB*, Sleeping Beauty; *scFv*, single-chain variable fragment; *sCRS*, severe CRS (requiring ICU management, treatment with tocilizumab and/or corticosteroids); *sMZL*, splenic marginal zone lymphoma; *UPenn*, University of Pennsylvania.

Allogeneic CAR-Ts are derived from patient's donor for prior allo-HSCT. Year refers to year most recent results were reported.

CAR-Ts administered. Rates of CR in larger trials have ranged from 50% to over 90%. Considering all pediatric and adult patients in aggregate who were treated with CD19-specific CAR-Ts in the United States, we estimate the combined rate of CR or continued CR to be close to 80%, with a rate of MRD-negative CR of over 60%. Efficacy for B-NHL and CLL is somewhat lower, with an estimated ORR around 60% across reported trials.

Toxicity rates have varied significantly across studies, likely reflecting the differences in the various factors described above. The median rate of severe or grade ≥ 3 CRS is around 25% based on available data, and has ranged from less than 15%⁵⁹ to over 70%⁶⁸ (although differences may be partly explained by variation in grading systems). At least 10 treatment-related deaths have occurred, predominantly among subjects with B-ALL.

To date, MSKCC, FHCRC, and UPenn have reported on the largest number of adult B-ALL patients treated with CD19-specific CAR-Ts. At the 2016 annual meeting of the American Society of Clinical Oncology (ASCO), investigators from MSKCC presented an update of their experience treating 51 adult patients with relapsed or refractory CD19⁺ B-ALL with second-generation CD19-specific CAR-Ts containing the CD28 costimulatory domain.¹⁰³ Sixty-one percent of patients had morphologic disease at the time of T-cell infusion, with the remainder having evidence of MRD. The median age for patients in the morphologic disease cohort was 40 and the median age in the minimal disease cohort was 53. Median follow-up was 8.5 months (range 1–54 months) and 17 of 50 evaluable patients had ≥ 1 year of follow up. Patients with morphologic disease had received a median of 3 previous lines of therapy, whereas those in the minimal disease cohort had received a median of 2 previous lines of therapy. This included prior blinatumomab in 12 of 51 patients and prior allogeneic HSCT in 18 of 51 patients. Of 51 patients, 15 were Philadelphia chromosome positive (Ph+). The CR rate was 77% among patients with baseline morphologic disease and 90% among patients with baseline minimal disease. Patients attaining CR were found to be MRD-negative in 33 of 39 cases where MRD-assessment was available. Fifteen of 33 patients attaining an MRD-negative CR relapsed, whereas 9 of 33 remained disease free with >1 year follow up. Severe CRS was seen in 44% of patients in the morphologic disease cohort and none of the patients in the minimal disease cohort. Grade 3/4 neurotoxicity occurred in 40% of patients with morphologic disease versus 14% of patients with minimal disease. A multicenter phase II trial of this CAR-T product was opened in 2015; however, this trial was recently terminated after

5 patients died due to treatment-related cerebral edema.^{69,70,107} These results have not yet been formally reported and the cause of the increased toxicity on this trial remains unclear.

Investigators from UPenn also presented an update of their trial of a 4-1BB-containing second-generation CD19-specific CAR-T product at the 2016 ASCO annual meeting.⁶⁸ Thirty patients with relapsed or refractory B-ALL, with a median age of approximately 44, were treated in 4-dosing cohorts. At least 9 patients had earlier received blinatumomab and at least 9 patients had undergone earlier allogeneic HSCT. Grade ≥ 3 CRS was reported in 22/30 patients, including 3 deaths due to severe CRS (each had received the highest dose of CAR-Ts in a single infusion). Of note, grade ≥ 3 neurotoxicity was only reported in 1 of 30 patients.

The group at the FHCRC published the results of a phase I/II trial of second-generation 4-1BB-containing CD19-specific CAR-Ts in 30 adults (median age 40) with relapsed or refractory B-ALL.⁵⁵ Patients had received a median of 3 previous lines of chemotherapy and 11 of 30 patients had relapsed following allogeneic HSCT. Before CAR-T infusion, patients were treated with a cyclophosphamide-based lymphodepletion regimen with or without the addition of fludarabine. As discussed above, the investigators in this trial selected for CD4⁺ and CD8⁺ cells, transduced each subset separately, and then treated patients with a defined 1:1 ratio of CD4⁺:CD8⁺ CAR-Ts. Complete remissions were seen in 28 of 30 patients, and 26 of these patients were negative for MRD. The addition of fludarabine was associated with an improvement in both CAR-T persistence and disease-free survival with 12 of 17 such patients reported to be alive and in CR (median follow-up 300 days) compared with 1 of 13 patients who received lymphodepletion with cyclophosphamide alone (follow-up > 600 days). Severe CRS was reported in 7 of 30 patients and grade ≥ 3 neurotoxicity occurred in 15 of 30 patients. One patient died due to severe CRS and another due to irreversible neurotoxicity.

For pediatric ALL, the most extensive experience of CD19-specific CAR-T therapy has come out of CHOP, Seattle Children's, and the NCI. At the 2016 ASCO annual meeting, investigators from CHOP presented an update on their largest trial to date of CD19-specific CAR-Ts for pediatric ALL, reporting outcomes for 60 patients aged 1.7 to 24 (median 11) years. Of 60 patients, 39 had already undergone allogeneic HSCT, 44 had detectable disease at baseline, and 16 were in an MRD-negative remission. Severe CRS was reported in approximately 27% of patients. Of 60 patients, 56 attained a CR and 51 of those patients were found to be MRD negative. The 2-year relapse-free survival and overall survival were 53% and 61%, respectively.

At the 2016 American Society of Hematology annual meeting, investigators from the NCI presented the outcomes of 51 pediatric patients with ALL who were treated with a second-generation CD19-specific CAR-T product containing the CD28 endodomain.⁵⁹ Eleven patients had primary refractory ALL, 5 had Ph+ disease, and 6 had CNS disease. Grade 3–4 CRS was seen in 7 of 51 patients and grade 3 neurotoxicity was seen in 3 of 51 patients. Of 51 patients, 31 attained a CR and 28 were found to be negative for MRD. The use of fludarabine/cyclophosphamide-based lymphodepletion in 36 of 51 patients was associated with significantly better outcomes, with a median overall survival of 13.3 months compared with 5.5 months among patients receiving other lymphodepletion regimens.

Also at the 2016 American Society of Hematology annual meeting, investigators from Seattle Children's reported outcomes for 43 pediatric patients with B-ALL who were treated with CD19-specific CAR-Ts.⁵³ Similar to the adult-ALL trial at FHCRC discussed above, patients were infused with a defined composition of CD4⁺:CD8⁺ CAR-Ts. Severe CRS and severe neurotoxicity were both reported in 10 of 43 patients, with no reported deaths due to treatment-related toxicity. Of 43 patients, 40 attained a CR and all were MRD negative. The 12-month overall survival was 70%.

Despite the impressive efficacy seen in trials of CD19-specific CAR-Ts to date, the significant rate of CRS and/or neurotoxicity, coupled with our lack of ability to accurately predict and effectively manage such toxicities, underscores the need for further optimization before the wider application of CAR-T therapies. Incorporating safety mechanisms into the CAR will likely prove to be an immediate and important component of this optimization. Alternatively, if proven to be scalable and highly reproducible, methodologies allowing the production of more uniform CAR-T products⁵⁵ and gene-editing techniques that lead to more consistent CAR transgene expression^{44,46} may represent valuable future approaches to better standardize therapeutic potential, while minimizing collateral toxicities.

Beyond CD19-specific CAR T-cells for B-cell malignancies. Although CD19 is highly expressed in B-cell leukemias and NHLs, many patients do not respond to CD19-directed therapy, and in certain patients who do initially respond, CD19-negative relapse has also been described.¹³⁰ Additional targets are under investigation for the treatment of B-cell malignancies, including CD20, CD22, tyrosine kinase-like orphan receptor 1 (ROR1), and the κ -light chain of human immunoglobulins.

Shah et al.¹⁰⁹ recently reported preliminary results of a trial conducted at the NCI in which autologous CD22-specific CAR-Ts were infused following lymphodepletion with fludarabine and cyclophosphamide in 9 pediatric subjects with B-ALL. Of these patients, 7 had relapsed following previous CD19-specific CAR-Ts and 6 were shown to have CD19^{dim/neg} tumor cells. Treatment-related toxicities included grade 3 diarrhea in 1 patient and grade ≤ 2 CRS in 7 patients. Of 9 patients, 7 obtained an MRD-negative CR and 3 were in a sustained CR at 3 months.

A clinical trial was previously conducted at BCM evaluating CAR-Ts targeting the κ -light chain of human immunoglobulins for the treatment of relapsed or refractory κ -expressing NHL, CLL, or multiple myeloma (MM).⁷⁴ A total of 16 patients were treated, including 7 with NHL, 2 with CLL, and 7 with MM. Patients received either no lymphodepletion or limited lymphodepletion consisting of cyclophosphamide 12.5 mg/kg. There were no grade ≥ 3 toxicities attributable to the CAR-Ts; however, in patients with MM (who had higher baseline B-cell counts), there was evidence of a decrease in normal circulating κ -expressing B-cells. Of the patients with NHL/CLL, the ORR was 3 of 9 and 2 patients with DLBCL attained a CR (1 relapsed after 6 weeks and the other remained in CR at a follow-up of 32 months).

Third-generation CD20-specific CAR-Ts including both the CD28 and 4-1BB costimulatory domains have previously been evaluated by Till et al. at the FHCRC. Three patients with B-cell NHL were treated. One patient developed grade 3 hypoxemia potentially related to CAR-Ts. Two patients without evaluable disease at baseline remained progression free for 12 and 24 months and a third patient attained a PR before relapsing 12 months following CAR-T administration. More recently, a second-generation CD20-specific CAR-T product containing the CD28 costimulatory domain was evaluated in a clinical trial at the Chinese PLA General Hospital. Eleven patients with relapsed or refractory B-NHL were treated. No CRS or neurotoxicity were observed. The ORR was 9 of 11 (82%), with 6 patients achieving CR and a median progression-free survival of 6 months (see Table II).^{112,113}

ROR1 is another proposed target for the treatment of B-cell malignancies. ROR1 is principally expressed during embryonic development after which its expression is thought to be negligible in normal tissues. ROR1 is aberrantly expressed by many malignancies, however,¹³¹ and preclinical studies of ROR1-specific CAR-Ts have shown promise.⁹² Clinical trials are currently open at the FHCRC (for ROR1-positive CLL, ALL, and mantle cell lymphoma)¹³² and at the M.D. Anderson Cancer Center (MDACC; for CLL).¹³³

NOVEL APPROACHES TO CAR T-CELL DESIGN

In addition to the evaluation of novel targets for CAR-Ts, many innovative approaches are being developed to both improve antitumor activity and to reduce toxicities. Examples include the development of CARs engineered to secrete proinflammatory cytokines to protect them from inhibitory signals in the tumor microenvironment, the inclusion of a cytokine-receptor in addition to the CAR to provide cytokine-mediated growth stimulation to the engineered T-cells, and the inclusion of natural killer cell receptors in engineered T-cells to allow better discrimination between tumor cells and nonmalignant cells.^{134,135} Although a comprehensive discussion is beyond the scope of this review, several promising strategies are highlighted below.

Improving CAR T-cell specificity via dual antigen recognition. Given the challenges in identifying antigens that are truly specific to a given tumor, there has been significant interest in developing CAR-Ts that would require the recognition of multiple antigens in combination to exploit their full potential, thereby allowing better discrimination between tumor cells and normal tissue.¹³⁶⁻¹³⁹ One such approach has been the expression of two CARs on the same cell, each with a distinct scFv, and both with incomplete signaling domains such that simultaneous engagement of the two CARs would be required for optimal T-cell activation.¹³⁹ Another approach has been the development of inhibitory CARs (iCARs) which incorporate the signaling domains of immunoinhibitory receptors (cytotoxic T-lymphocyte-associated protein 4 or programmed cell death protein 1) with the goal of dampening T-cell activation in the presence of an antigen expressed on normal tissues, while allowing full T-cell activation via a separate CAR when the same cell encounters a different, tumor-associated antigen.¹³⁷ Roybal et al.¹³⁸ recently described an innovative combinatorially activated T-cell circuit, which they termed a “Two Antigen AND-Gate Circuit,” whereby activation of a synthetic Notch receptor for one antigen induces the expression of a CAR targeted toward a second antigen. Although none of these techniques have yet been evaluated in humans, further development of clinically-feasible techniques to improve CAR-T specificity will likely be an important prerequisite to expanding the applicability of such therapies.

Strategies to prevent antigen escape. Whereas concern for off-target toxicities is driving efforts to improve CAR-T specificity, this has been somewhat less of a concern thus far in the development of CAR-Ts for B-cell malignancies. Perhaps more relevant to B-cell malignancies, is the occurrence of disease relapses due

to antigen escape. Despite the successes of CD19-directed CAR-Ts for B-ALL, CD19-negative relapse has been reported in several trials.^{55,59,91,94} Several mechanisms have been observed, including the development of mutations in CD19, expression of alternative splice variants of CD19, and complete loss of CD19-expression, which was reported in a trial among 2 patients who developed a relapse due to myeloid lineage switch, both harboring a rearrangement of the mixed lineage leukemia gene.^{130,140} One strategy for B-ALL has been to target alternative surface antigens other than CD19, and as mentioned above, a trial recently investigated the use of CD22-directed CAR-Ts for relapsed pediatric B-ALL.¹⁰⁹ Although not yet evaluated in clinical trials, there has also been substantial interest in the possibility of targeting two or more antigens simultaneously, which could potentially be accomplished by pooling CAR-T products, expressing multiple CARs in the same cells, or designing CARs that include two scFvs in tandem.¹⁴⁰

Universal CARs. Technical or economic challenges associated with the production of autologous cellular products may also limit the widespread clinical applicability of CAR-Ts. Inadequate leukapheresis, failure of CAR-T manufacturing, and rapid progression of the disease while CAR-Ts are being generated may all cause severe limitations for the routine use of CAR-Ts. Banked, third-party CAR-Ts have been evaluated in the context of concurrent or prior HSCT, with CAR-Ts derived from the patient’s donor lymphocytes; however, many of the same challenges remain with donor-derived CAR-Ts, and such therapies are only potentially applicable to a minority of patients. The heterogeneity of both autologous and donor-derived allogeneic CAR-Ts also presents several challenges, including unpredictable efficacy and toxicity. In an attempt to develop “universal” CAR-Ts that could be produced in a standardized fashion from T-cells derived from one or more healthy unmatched donors, with subsequent administration to multiple patients, several groups have developed gene-editing techniques such as prokaryotic type II CRISPR-Cas9, transcription activator-like effector nucleases, and zinc-finger nucleases to eliminate endogenous $\alpha\beta$ -TCR expression in T-cells to remove the risk of inducing graft-versus-host disease.¹⁴¹⁻¹⁴⁴ Investigators at the University College of London demonstrated the possibility of using transcription activator-like effector nuclease gene-editing techniques to produce T-cells deficient in expression of both the endogenous $\alpha\beta$ -TCR and CD52 (for the purposes of inducing recipient immunosuppression by

using the anti-CD52 monoclonal antibody, alemtuzumab), with subsequent lentiviral transduction to express a CD19-specific CAR.¹⁴¹ Initial clinical assessment of this “off the shelf” CAR-T product in 2 infants with relapsed/refractory CD19⁺ B-ALL produced ongoing molecular remission in both patients (see Table I)^{37,38} and larger clinical trials are underway to further validate this approach.^{145,146}

CONCLUSIONS

The field of cancer immunotherapy has seen remarkable advances over the past decade, leading to long-term remissions in diseases that would otherwise be rapidly fatal. The theoretical benefit of redirecting the immune system to target cancer cells has long been appreciated, but it was not until the development of immune checkpoint inhibitors that this potential became a reality for large numbers of patients. As is clear from the clinical trials discussed above, CAR-Ts have given us the ability to further unleash the power of cell-mediated immunity against cancer cells, now in a highly-targeted manner that has not previously been possible. This has led to unprecedented successes in the treatment of B-ALL and other B-cell malignancies, and we remain hopeful that similar successes will soon be replicated in other hematologic malignancies and in solid tumors. Combination with other immunotherapeutics is also likely to further maximize the potential of CAR-Ts.¹⁴⁷ While the power of CAR-Ts is clear, it is also clear that we require a better understanding of how these artificially created receptors interact with the complex signaling networks of the immune system. Further elucidation of the biology of CAR-Ts and their downstream effects will allow us to minimize unpredictable toxicities and improve their therapeutic potential.

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