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Driving CAR-Based T-Cell Therapy to Success

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Abstract

T-cells that have been genetically modified, activated, and propagated ex vivo can be infused to control tumor progression in patients who are refractory to conventional treatments. Early-phase clinical trials demonstrate that the tumor-associated antigen (TAA) CD19 can be therapeutically engaged through the enforced expression of a chimeric antigen receptor (CAR) on clinical-grade T-cells. Advances in vector design, the architecture of the CAR molecule especially as associated with T-cell co-stimulatory pathways, and understanding of the tumor microenvironment, play significant roles in the successful treatment of medically fragile patients. However, some recipients of CAR^+ T-cells demonstrate incomplete responses. Understanding the potential for treatment failure provides a pathway to improve the potency of adoptive transfer of CAR^+ T-cells. High throughput single-cell analyses to understand the complexity of the inoculum coupled with animal models may provide insight into the therapeutic potential of genetically modified T-cells. This review focusses on recent advances regarding the human application of C19-specific CAR^+ T-cells and explores how their success for hematologic cancers can provide a framework for investigational treatment of solid tumor malignancies.

Keywords

B-cell malignancies; Chimeric antigen receptor; Gene therapy; T-cell therapy

INTRODUCTION

The clinical utility of T cells genetically modified to redirect specificity depends on the interplay between the design of an introduced chimeric antigen receptor (CAR), the cell type as template for bioengineering, and the condition and conditioning of the recipient. Most trials enrolling patients with B-cell malignancies to receive genetically modified T cells

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Conflict of Interest

Dr. Bipulendu Jena, Dr. Judy S Moyes, and Dr. Helen Huls each declare no potential conflicts of interest relevant to this article.

Human and Animal Rights and Informed Consent

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employ a second-generation CAR that upon docking with cell-surface CD19 coordinates an activation signal through chimeric CD3-ζ with CD28 or CD137. It is generally accepted that co-signaling through a CD19-specific CAR is required to achieve competent T-cell activation, defined at a minimum as proliferation, killing, and cytokine production. Indeed, when a first-generation CAR (that activates through chimeric CD3-ζ) was compared to a second-generation CAR (that activates through chimeric CD3-ζ and CD28) in a competitive repopulation experiment, there was a survival advantage for the CD19-specific T cells expressing the advanced design (1). These encouraging clinical data targeting $CD19^+$ leukemias and lymphomas provide a foundation for developing CARs with alternative specificities and designs. While a CAR can bind to a tumor-associated antigen (TAA) independent of HLA there is uncertainty whether one CAR species will be sufficient to encompass the variability in tumor bioburden and type between recipients. To add to the complexity of CAR design(s) that pre-dispose to a therapeutic effect, there are data supporting the preferential use of T-cell subsets, especially those that avoid terminal differentiation, as preferred templates for genetic reprogramming. Furthermore, other lymphocyte populations, such as NK cells and invariant NKT cells may be appealing alternatives to T cells. The candidate recipient and their tumor will also influence the therapeutic effect. For example, T cells expressing the same CD19-specific CAR vary in ability to control and perhaps eliminate acute versus chronic leukemias. This may be accounted for by differences in pre-infusion chemotherapy, damage to T-cell function due to tumor or from iatrogenic causes, or impact of tumor on T-cell mediated killing. Thus, while much progress has been made in recent years demonstrating the promise of CAR^+ T cells, the premise as to why these T cells function (and will continue to function) within and between patients remains to be fully elucidated.

Test-Driving CARs

CAR, as a fusion protein, is expressed on primary T cells through synthetic expression vectors derived from lentivirus, gamma retrovirus, or DNA transposons. Stable and sustained expression of the CAR payload enables genetically modified, clinical-grade T cells to dock with and destroy target cells expressing the TAAs. Table 1 summarizes the common constructs currently in use in clinical trials in the USA. The CAR design is one of the variables that impact the therapeutic potential of the infusion product. The structure of a prototypical CAR can be divided into (at least) three distinct parts: (i) an scFv derived from a TAA-specific monoclonal antibody (mAb) that mediates recognition of tumor, (ii) extracellular scaffold which links scFv to the transmembrane and cytosolic signaling domains, and (iii) co-stimulatory molecules that sustain proliferation and activation of gene modified T cells. CARs in clinical trials activate T cells after binding with TAAs via phosphorylation of multiple immunoreceptor tyrosine-based activation motifs (ITAMs) in chimeric CD3-ζ to provide “signal 1”. However, to prevent anergy and provide a fully-competent T-cell activation signal, additional T-cell co-stimulation (“signal 2”) is likely required, such as mediated by chimeric CD28, 4-1BB, OX-40, ICOS, as included within the second generation CARs. Many studies and reviews are published reflecting the translational appeal of co-stimulation through advances in design in the endodomain (1–5). Here, we provide additional thoughts regarding constructing these immunoreceptors with

respect to the extracellular domain. The affinity of the scFv to the TAA (6), the specificity of the scFv to distinguish a tumor from normal tissue (7), and the adaption of bi-specific antibodies where epitope specificities could be adjusted through rational design (8), are attributes that might be considered when advancing CAR constructs. The infusion of T cells expressing a CAR targeting one TAA, such as CD19, may render the patient at risk for disease progression due to the emergence of escape variants in which tumor lacks expression of the TAA (9). This underlines the need to identify additional TAAs, such as CD22 (10) which may be targeted by genetically modified T cells, likely in addition to CD19. Even low levels of tissue expression of TAA on normal tissues can be recognized and targeted by CAR^+ T cells leading to deleterious toxicity (11, 12). It remains uncertain whether the co-expression of a "suicide gene" [e.g., inducible Caspase9 (13)] with CAR to conditionally ablate infused T cells in the event of serious toxicity will be clinically appealing. Alternatively, desired transient expression of CARs through electro-transfer of in vitro-transcribed mRNA species into T cells may be used to define a therapeutic potential of T cells targeting TAA with broad tissue distribution (14). Regulating affinity of the scFv, and thus the functional affinity of the derived CAR, may help build immunoreceptors that avoid on-target toxicity. Currently, there is a paucity of TAA that segregate to tumor versus vital normal cells. Thus, investigators have developed immunotherapies based on the understanding that most, if not all, tumor cells are identified by the aberrant expression of more than one TAA, rather than the expression of a unique and abnormal TAA. The unwelcomed potential to recognize a TAA on healthy cells may be reduced by distributing the T-cell activation event between two CAR species. In this instance, two TAAs on a tumor cell can be synchronously recognized by the genetically modified T-cell in which signal 1 and signal 2 are separated between two CAR types with specificity for each of the TAAs (15) (16). The clinical deployment of this approach will depend on these genetically modified T cells failing to recognize normal cells that express one of the two TAAs. A single CAR molecule may be designed that has two specificities. A bi-specific CAR has been developed that uses a glycine-serine tandem repeat to flexibly join two different scFvs to target Her2 and CD19 (7). In addition to the design and composition of the scFv, it is probable that the extracellular domain may impact the immunobiology of the assembled CAR. Three recent clinical trials reporting therapeutic success infusing autologous CD19-specific CAR^+ T cells vary in the composition of the scaffold used to append the scFv from the cell surface. The University of Pennsylvania (UPENN) (17), Memorial Sloan-Kettering Cancer Center (MSKCC) (18), and National Cancer Institute (NCI) (19) employ either CD8 extracellular and transmembrane domain or a small portion of CD28 extracellular and transmembrane domains. Other clinical trials harness the hinge and CH_2 and CH_3 (Fc) constant regions from modified human immunoglobulin sequences (Table 1). These scaffolds are derived from IgG_1 (20) or IgG_4 (21, 22). Changes are typically made to these stalks to reduce the potential for binding to Fc receptors (FcR), such as by replacing specific amino acids in the IgG_1 Fc spacer with IgG_2 sequence to reduce binding to Fc RI (23). Alternately, the hinge and Fc sequence from IgG_4 instead of IgG_1 will likely help reduce unintended FcR binding (24, 25). Glycosylation may also impact recognition by FcR. For example, O-linked carbohydrates in all CH_2 domains may impact interaction with FcR (26). To help address an effect of the length of the scaffold, a ROR1-specific second-generation CAR with reduced spacer length was shown to impart improved T-cell effector function and

tumor recognition of CLL (6). Thus, each facet of the CAR, from the scFv region at the amino terminus to the CD3-ζ signaling motif and the carboxyl terminus, will inform on the therapeutic potential of the genetically modified T cells. It is readily apparent that one CAR design will not be sufficient to achieve clinical success for patients with multiple types of tumor. The multitude of clinical trials targeting CD19 provides an opportunity for investigators to compare and contrast CAR species with respect to anti-tumor effect. Such data have not yet led to firm conclusions regarding a favored CAR design to target CD19 and provide only initial thoughts about the construction of CARs to target solid tumors.

Super CARs

Factors that influence the in vivo fate of clinical-grade CAR^+ T cells can be broadly divided into three categories: (i) the composition of the infused product (T cells and their sub-types); (ii) the tumor, its distribution, and its microenvironment; and (iii) the recipient. The complexity and heterogeneity of tumors pose a challenge to applying one principle of CAR design in one population of T cells as a precision tool for multiple patients with a given cancer type. Thus, investigators have strived to infuse a heterogeneous population of CAR^+ T cells containing a multitude of cells with individual therapeutic effect. This approach relies on the ability of genetically modified T cells not only to kill cancer cells in response to CAR binding the TAA, but that the same T cells are activated for sustained proliferation. Thus, a sub-population of T cells can emerge after infusion which swells in number and can engage in serial killing to eliminate a large bioburden of tumor (27–29). The proliferative potential of adoptively transferred T cells not only depends on the CAR design and potential to deliver a fully-competent activation signal, but also on factors impacting from outside the T cell. These include competition for scarce resources, such as certain cytokines that signal through the common-chain receptor, and immunosuppression mediated by regulatory cells. Lymphodepletion may favor the survival and indeed the proliferation of administered T cells by liberating pro-survival cytokines and eliminating suppressor cells. The in vivo propagation of CD19-specific CAR^+ T cells appears to predict therapeutic success. Thus, measuring the number and persistence of administered T cells can be used to guide treatment decisions. Quantitative measurement of the presence of circulating CAR^+ T cells in peripheral blood and cerebral spinal fluid has been achieved by Q-PCR using CAR-specific probes (30) and identifying CAR on surface of T cells by flow cytometry assays (31). The clonality, and thus preferential survival of a subset of administered T cells, can be assessed by sequencing CDR3 regions unique to the endogenous TCR in the infused and recovered T cells. Additional correlative data are emerging which inform on the ability of subsets of infused T cells to mediate and complete an anti-tumor effect. For example, upregulation of checkpoints has been observed on CAR^+ T cells in some patients (32). Additional correlative data will be needed to determine the optimal CAR design that can sustain an anti-tumor effect in patients with B cell malignancies. The two popular second-generation CD19-specific CAR designs currently associated with in vivo persistence activate autologous T cells via CD137 and CD3-ζ or CD28 and CD3-ζ. Initial studies demonstrate that both CAR species can exhibit superior anti-tumor responses in recipients with acute in contrast to chronic B-cell leukemias (18, 33). It appears that patients with B-lineage acute lymphoblastic leukemia receiving CARs with CD137/CD3-ζ enter into a state

of remission that does not require additional therapeutic intervention, whereas patients receiving CARs with CD28/CD3-ζ are being referred for consolidation with allogeneic hematopoietic stem-cell transplantation. The relative merit of these two CAR designs will become apparent as additional patients are infused and the follow-up time is lengthened. In addition to targeting B cell leukemias, studies reported that CD19-specific CAR^+ T cells can also target lymphomatous masses (19, 32). Head-to-head comparisons using competitive repopulations experiments infusing more than one type of genetically modified T cell would help to determine a CAR design that can impart improved T-cell persistence and anti-tumor effect. One such trial is underway funded under the NCI's special translational research acceleration projects (STRAPs) to compare the CARs derived from UPENN and MSKCC.

Future CARs

The ability to manipulate patient and/or donor derived cells ex vivo provides an opportunity to choose the type of T cells to manipulate and infuse for gene therapy. Subsets of T cells are favored for adoptive cell therapies which retain plasticity to self-replicate and thus proliferate in vivo. Several clinically-appealing T-cell types for adoptive transfer have been proposed using flow cytometry to identify the desired sub-populations. In addition to naïve and central memory T cells, one attractive subpopulation of T cells that has recently emerged, characterized as $CD45RA^+CCR7^+CD62L^+$ $CD95^{neg}IL2R\beta^{neg}$appears to have stem cell-like qualities (34) (35). Such T cells may be preferentially propagated ex vivo by cross-linking CD3 (signal 1) and CD28 (signal 2) in the presence of recombinant human soluble IL-7 and IL-15 (signal 3) to generate T cells that preserve a stem cell-like phenotype (36, 37). The a priori identification of desired T-cell subset(s), and propagating those cells for human application, may also require dedicated infrastructure such as selection using paramagnetic beads and/or sorting in compliance with current good manufacturing practice. Regarding the latter, new technology is emerging that combines fluorescence-activated cell sorting with micro-electromechanical systems which eliminates issues associated with aerosolization and cross-contamination. However, manufacturing a homogenous cell population may not be possible or perhaps even desirable. For a complex mixture containing sub-sets of T cells with favorable in vivo immunological functions and desirable effect on tumor immunity may preferentially proliferate to benefit the recipient. Other lymphocyte populations may also impact the effector function of infused T cells. For example, restoring type I NKT cells may enhance the anti-tumor effect (38). Eliminating cells and immunosuppressive factors may also benefit the immunobiology of CAR^+ T cells. For example, the presence of IL-10, TGFβ and VEGF, up-regulation of T-cell suppressive molecules such as IDO and arginase, the contaminating presence of myeloid-derived suppressor cells and regulatory T cells can dampen the ability of CAR^+ T cells to undertake effector functions (39). In addition to extrinsic negative influence, the CAR^+ T cells possess endogenous mechanisms that may self-limit an anti-tumor effect. Up-regulation of programmed death 1 (PD-1) on infused genetically modified cells suggests that CAR-mediated immunotherapy is subjected to pressures from immune regulatory mechanisms (32). Genetic engineering has been used to circumvent check-point blockade such as by expressing a hybrid of CTLA4-CD28, where truncated CTLA4 was fused to CD28 signaling domain to compete with endogenous CTLA4 (40). Similarly, a fusion of PD-1 to CD28

enabled genetically modified T cells to receive a positive costimulation when PD-1 engaged with programmed death ligand 1 (PD-L1) on tumor cells (41, 42). In aggregate, the ability of CAR^+ T cells to realize their full in vivo therapeutic potential will depend of identifying and thus infusing T cells that can recycle effector functions in a hostile tumor microenvironment.

CARs for Humans

A beneficial aspect of immunotherapy for CD19 is that expression of this TAA is limited to B cells. Indeed, CD19-specific T cells cannot at present distinguish between CD19 on normal versus malignant B cells. However, the loss of humoral immunity is currently an acceptable long-term toxicity to recipients with intractable B-cell leukemias and lymphomas. We do note that there has been one death of a patient after infusion of CD19-specific T cells due to opportunistic viral infection (43). Thus, targeting CD19 appears to be a safe harbor for advancing new approaches to gene and immunotherapy as we have demonstrated infusing T cells that, for the first time, were modified with a transposon/transposase system (44, 45). The synchronous activation and proliferation of T cells by a resident large bioburden of $CD19^+$ tumor cells often leads to supra-physiological release of cytokines and is associated on most occasions with systemic side effects including fever, hypotension, and changes in mental status. These can be managed with supportive care, including stabilization in the intensive care setting, if needed, the judicious application of blockade of IL-6 receptor (infusing tocilizumab) (9) and, if necessary, systemic dosing of corticosteroids. Nevertheless, it would be preferable to avoid chronic and acute toxicities associated with CD19-specific T-cell therapy. This may be achieved through (i) targeting TAAs, such as receptor tyrosine kinase-like orphan receptor 1 (ROR1), (46, 47) that appears to have a pattern of expression restricted to a subset of malignant and not-normal B cells and (ii) the timely administration of CD19-specific CAR^+ T cells to eliminate minimal residual disease rather than treat high tumor burdens present at frank relapse. The clinical toxicity data associated with targeting CD19 do not justify the co-expression of a “suicide” gene with the CAR to conditionally ablate T cells applied in human trials. However, the ability to eliminate an infused product may be needed as (i) T cells are selected or engineered for long-lived persistence (such as by co-expression of cytokine mutein with CAR), (ii) with the deployment of advanced (e.g. third-generation) CARs that are capable of triggering and sustaining multiple signaling pathways, and (iii) upon the human application of CAR^+ T cells that target one or more TAAs that are expressed on vital normal structures. Indeed, the identification of safe TAAs for targeting is emerging as a limitation to the field of CAR-based immunotherapies (48). This dearth of suitable TAAs will undermine the development and implementation of clinical trials especially targeting solid tumors. Nevertheless, some investigators have been able to proceed with testing CAR^+ T cells to target such malignancies. From these data, the ganglioside GD_2 has emerged as an attractive TAA (49). However, caution is warranted when selecting TAA(s) on solid tumors as infusing a large number of HER2-specific T cells expressing a third-generation CAR led to immediate pulmonary toxicity and the death of the first recipient (11), and targeting CAIX led to unacceptable side effects from destruction of healthy cells (12). In aggregate, the tolerance for risk by administrators, the fortitude of investigators skilled in clinical translation, and bravery of patients has enabled a select group of not-for-profit academic centers to advance

the field of CAR-based T-cell therapies. This has led to the genesis of a new investment opportunity by for-profit biomedical concerns that will be needed if early single-institution results are to be repeated in multicenter trials powered for efficacy.

CARs: Back to the Future

What is needed, but is not yet available, are pre-clinical model systems that predict clinical success and absence of toxicity for CARs prior to their human application. For example, the lack of a functioning immune system in immunocompromised mice used for in vivo modeling of human T cells, and the use of scFv sequences derived from mouse mAbs, undermines our understanding of the CAR with respect to efficacy and adverse events. Comparative oncology and the judicious use of large animals such as non-human primates and companion canines may provide a pathway to the clinic (50). However, these models are resource intensive and not amenable to high throughput. In vitro evidence obtained from multiplexed single cell genomics studies reveals the heterogeneity of cell populations within the T-cell inoculum. Indeed, T cells with identical immunoreceptors may undergo completely different patterns of activation and expansion during the processes of gene transfer and culturing. To understand the complexity of a given manufactured product, we are undertaking correlative studies that measure massive numbers of specific and serial killing events mediated by individual T cells (51, 52) and also undertaking an analysis of hundreds of genes expressed in a single T cell using a robotic microplate platform from Fluidigm (53). Such assessments of single-cell CAR^+ T cells can then be aggregated to inform on the bulk population and avoid problems associated with measuring the average effector function of populations of T cells. Such studies are expected to reveal subsets of T cells and CAR designs with preferred anti-tumor effects.

Conclusion

T cells can be genetically modified ex vivo to overcome immune tolerance by the expression of a CAR to and target cell-surface TAA in vivo. The therapeutic potential of a given CAR^+ T cell in the inoculum is difficult to predict. Thus, recipients receive millions of T cells engineered to contain at least a subset that can sustain proliferation and participate in serial killing after infusion. It is not yet possible to identify, let alone control, all the variables impacting the therapeutic success of CAR^+ T cells. As a result, data from iterative clinical trials will be needed to assess the anti-tumor effect of populations of genetically modified, activated, and propagated T cells. Correlative studies associated with the human application of genetically modified T cells will then inform on current and future modifications of CAR designs, cellular template, and trials. Therefore, efforts to lower the barriers to distribution such as streamlining regulatory compliance as well as reducing costs of vector production and T-cell manufacture will help immunologists translate CAR^+ T cells into immunotherapies. Any one patient can expect to benefit from a given T-cell infusion, but the hope for the field is that we can build precision immunotherapy from CAR^+ T cells that are predicted to have therapeutic success for treatment of multiple hematologic malignancies and solid tumors across patient population.

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References

• Important article

•• Very important article

1. Savoldo B, Ramos CA, Liu E, Mims MP, Keating MJ, Carrum G, et al. CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. J Clin Invest. 2011; 121:1822–1826. [PubMed: 21540550] •• Results published from this clinical trial showed that second generation CD19-28z CAR perform better in terms of in vivo expansion and persistence than first generation CD19z-CAR in a controlled repopulation experiment.
2. Jena B, Dotti G, Cooper LJ. Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. Blood. 2010; 116:1035–1044. [PubMed: 20439624]
3. Suhoski MM, Golovina TN, Aqui NA, Tai VC, Varela-Rohena A, Milone MC, et al. Engineering artificial antigen-presenting cells to express a diverse array of co-stimulatory molecules. Molecular Therapy. 2007; 15:981–988. [PubMed: 17375070]
4. Milone MC, Fish JD, Carpenito C, Carroll RG, Binder GK, Teachey D, et al. Chimeric Receptors Containing CD137 Signal Transduction Domains Mediate Enhanced Survival of T Cells and Increased Antileukemic Efficacy In Vivo. Molecular Therapy. 2009; 17:1453–1464. [PubMed: 19384291]
5. Hombach A, Wieczarkowiecz A, Marquardt T, Heuser C, Usai L, Pohl C, et al. Tumor-specific T cell activation by recombinant immunoreceptors: CD3 zeta signaling and CD28 costimulation are simultaneously required for efficient IL-2 secretion and can ge integrated into one combined CD28/CD3 zeta signaling receptor molecule. Journal of Immunology. 2001; 167:6123–6131.
6. Hudecek M, Lupo-Stanghellini M-T, Kosasih PL, Sommermeyer D, Jensen MC, Rader C, et al. Receptor Affinity and Extracellular Domain Modifications Affect Tumor Recognition by ROR1-Specific Chimeric Antigen Receptor T Cells. Clinical Cancer Research. 2013; 19:3153–3164. [PubMed: 23620405]
7. Grada Z, Hegde M, Byrd T, Shaffer DR, Ghazi A, Brawley VS, et al. TanCAR: A Novel Bispecific Chimeric Antigen Receptor for Cancer Immunotherapy. Molecular therapy Nucleic acids. 2013; 2:e105-e. [PubMed: 23839099]
8. Choi BD, Gedeon PC, Kuan C-T, Sanchez-Perez L, Archer GE, Bigner DD, et al. Rational design and generation of recombinant control reagents for bispecific antibodies through CDR mutagenesis. Journal of Immunological Methods. 2013; 395:14–20. [PubMed: 23806556]
9. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric Antigen Receptor-Modified T Cells for Acute Lymphoid Leukemia. New England Journal of Medicine. 2013; 368:1509–1518. [PubMed: 23527958] • First report to describe antigen escape and emergence of $CD19^{neg}$ variants in response to CAR-mediated T-cell therapy in a clinical trial.
10. Haso W, Lee DW, Shah NN, Stetler-Stevenson M, Yuan CM, Pastan IH, et al. Anti-CD22-chimeric antigen receptors targeting B-cell precursor acute lymphoblastic leukemia. Blood. 2013; 121:1165–1174. [PubMed: 23243285]
11. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case Report of a Serious Adverse Event Following the Administration of T Cells Transduced With a Chimeric Antigen Receptor Recognizing ERBB2. Molecular Therapy. 2010; 18:843–851. [PubMed: 20179677] • Case report of a serious adverse event attributed to the use of a third generation CAR that caused cytokine storm resulting in the death of a patient.

12. Lamers CHJ, Sleijfer S, van Steenbergen S, van Elzakker P, van Krimpen B, Groot C, et al. Treatment of Metastatic Renal Cell Carcinoma With CAIX CAR-engineered T cells: Clinical Evaluation and Management of On-target Toxicity. Molecular Therapy. 2013; 21:904–912. [PubMed: 23423337]
13. Di Stasi A, Tey S-K, Dotti G, Fujita Y, Kennedy-Nasser A, Martinez C, et al. Inducible Apoptosis as a Safety Switch for Adoptive Cell Therapy. New England Journal of Medicine. 2011; 365:1673–1683. [PubMed: 22047558]
14. Barrett DM, Zhao Y, Liu X, Jiang S, Carpenito C, Kalos M, et al. Treatment of Advanced Leukemia in Mice with mRNA Engineered T Cells. Human Gene Therapy. 2011; 22:1575–1586. [PubMed: 21838572]
15. Kloss CC, Condomines M, Cartellieri M, Bachmann M, Sadelain M. Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells. Nature Biotechnology. 2013; 31:71-+. •• An elegant report that showed CARs could be engineered to target tumors positive for two different antigens.
16. Lanitis E, Poussin M, Klattenhoff AW, Song D, Sandaltzopoulos R, June CH, et al. Chimeric Antigen Receptor T cells with dissociated signaling domains exhibit focused antitumor activity with reduced potential for toxicity in vivo. Cancer Immunology Research. 2013; 1:43–53.
17. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric Antigen Receptor-Modified T Cells in Chronic Lymphoid Leukemia. N Engl J Med. 2011 •• In a clinical trial second generation CD19+CARs were shown to cause complete regression of tumors and that CAR modified T cells could persist for a long time and become serial tumor killers.
18. Brentjens R, Yeh R, Bernal Y, Riviere I, Sadelain M. Treatment of chronic lymphocytic leukemia with genetically targeted autologous T cells: case report of an unforeseen adverse event in a phase I clinical trial. Mol Ther. 2010; 18:666–668. [PubMed: 20357779]
19. Kochenderfer JN, Wilson WH, Janik JE, Dudley ME, Stetler-Stevenson M, Feldman SA, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Blood. 2010; 116:4099–4102. [PubMed: 20668228]
20. Vera J, Savoldo B, Vigouroux S, Biagi E, Pule M, Rossig C, et al. T lymphocytes redirected against the kappa light chain of human immunoglobulin efficiently kill mature B lymphocyte-derived malignant cells. Blood. 2006; 108:3890–3897. [PubMed: 16926291]
21. Cooper LJ, Topp MS, Serrano LM, Gonzalez S, Chang WC, Naranjo A, et al. T-cell clones can be rendered specific for CD19: toward the selective augmentation of the graft-versus-B-lineage leukemia effect. Blood. 2003; 101:1637–1644. [PubMed: 12393484]
22. Jensen MC, Popplewell L, Cooper LJ, DiGiusto D, Kalos M, Ostberg JR, et al. Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans. Biol Blood Marrow Transplant. 2010; 16:1245–1256. [PubMed: 20304086]
23. Hombach A, Hombach AA, Abken H. Adoptive immunotherapy with genetically engineered T cells: modification of the IgG1 Fc 'spacer' domain in the extracellular moiety of chimeric antigen receptors avoids 'off-target' activation and unintended initiation of an innate immune response. Gene Therapy. 2010; 17:1206–1213. [PubMed: 20555360]
24. Armour KL, Clark MR, Hadley AG, Williamson LM. Recombinant human IgG molecules lacking Fc gamma receptor I binding and monocyte triggering activities. European Journal of Immunology. 1999; 29:2613–2624. [PubMed: 10458776]
25. Reddy MP, Kinney CAS, Chaikin MA, Payne A, Fishman-Lobell J, Tsui P, et al. Elimination of Fc receptor-dependent effector functions of a modified IgG4 monoclonal antibody to human CD4. Journal of Immunology. 2000; 164:1925–1933.
26. Jefferis R. Glycosylation as a strategy to improve antibody-based therapeutics. Nature Reviews Drug Discovery. 2009; 8:226–234.
27. Davila ML, Brentjens R, Wang X, Riviere I, Sadelain M. How do CARs work? Early insights from recent clinical studies targeting CD19. Oncoimmunology. 2012; 1:1577–1583. [PubMed: 23264903]

28. Kalos M, June CH. Adoptive T cell transfer for cancer immunotherapy in the era of synthetic biology. Immunity. 2013; 39:49–60. [PubMed: 23890063]

29. Kochenderfer JN, Rosenberg SA. Treating B-cell cancer with T cells expressing anti-CD19 chimeric antigen receptors. Nature Reviews Clinical Oncology. 2013; 10:267–276.

30. Maiti SN, Huls H, Singh H, Dawson M, Figliola M, Olivares S, et al. Sleeping Beauty System to Redirect T-cell Specificity for Human Applications. Journal of Immunotherapy. 2013; 36:112–123. [PubMed: 23377665]

31. Jena B, Maiti S, Huls H, Singh H, Lee DA, Champlin RE, et al. Chimeric Antigen Receptor (CAR)-Specific Monoclonal Antibody to Detect CD19-Specific T Cells in Clinical Trials. Plos One. 2013:8.

32. Kochenderfer JN, Dudley ME, Carpentor RO, Kassim SH. Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. Blood. 2013 On line edition.

33. Davila ML, Kloss CC, Gunset G, Sadelain M. CD19 CAR-Targeted T Cells Induce Long-Term Remission and B Cell Aplasia in an Immunocompetent Mouse Model of B Cell Acute Lymphoblastic Leukemia. Plos One. 2013:8.

34. Restifo NP, Gattinoni L. Lineage relationship of effector and memory T cells. Current opinion in immunology. 2013; 25:556–563. [PubMed: 24148236]

35. Gattinoni L, Klebanoff CA, Restifo NP. Paths to stemness: building the ultimate antitumour T cell. Nature Reviews Cancer. 2012; 12:671–684.

36. Cieri N, Camisa B, Cocchiarella F, Forcato M, Oliveira G, Provasi E, et al. IL-7 and IL-15 instruct the generation of human memory stem T cells from naive precursors. Blood. 2013; 121:573–584. [PubMed: 23160470] • This study showed that a memory stem-cell like CD8+ T cell population could be generated from naïve precursor cells in vitro and this has translational appeal.

37. Gattinoni L, Restifo NP. Moving T memory stem cells to the clinic. Blood. 2013; 121:567–568. [PubMed: 23349370]

38. Izhak L, Ambrosino E, Kato S, Parish ST, O'Konek JJ, Weber H, et al. Delicate Balance among Three Types of T Cells in Concurrent Regulation of Tumor Immunity. Cancer Research. 2013; 73:1514–1523. [PubMed: 23319803]

39. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011; 480:480–489. [PubMed: 22193102]

40. Shin JH, Park HB, Oh YM, Lim DP, Lee JE, Seo HH, et al. Positive conversion of negative signaling of CTLA4 potentiates antitumor efficacy of adoptive T-cell therapy in murine tumor models. Blood. 2012; 119:5678–5687. [PubMed: 22538857]

41. Prosser ME, Brown CE, Shami AF, Forman SJ, Jensen MC. Tumor PD-L1 co-stimulates primary human CD8(+) cytotoxic T cells modified to express a PD1:CD28 chimeric receptor. Molecular Immunology. 2012; 51:263–272. [PubMed: 22503210]

42. Ankri C, Shamalov K, Horovitz-Fried M, Mauer S, Cohen CJ. Human T Cells Engineered To Express a Programmed Death 1/28 Costimulatory Retargeting Molecule Display Enhanced Antitumor Activity. Journal of Immunology. 2013; 191:4121–4129.

43. Kochenderfer JN, Dudley ME, Feldman SA, Wilson WH, Spaner DE, Maric I, et al. B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. Blood. 2012; 119:2709–2720. [PubMed: 22160384]

44. Hackett PB, Largaespada DA, Cooper LJ. A transposon and transposase system for human application. Mol Ther. 2010; 18:674–683. [PubMed: 20104209]

45. Singh H, Manuri PR, Olivares S, Dara N, Dawson MJ, Huls H, et al. Redirecting specificity of T-cell populations for CD19 using the Sleeping Beauty system. Cancer Res. 2008; 68:2961–2971. [PubMed: 18413766] • Non-viral vectors like the transposon based Sleeping Beauty system were shown as alternatives to commonly used viral vectors such as gammaretrovirus or lentivirus for genetic modification of T cells.

46. Fukuda T, Chen L, Endo T, Tang L, Lu D, Castro JE, et al. Antisera induced by infusions of autologous Ad-CD154-leukemia B cells identify ROR1 as an oncofetal,antigen and receptor for

Wnt5a. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:3047–3052. [PubMed: 18287027]

47. Zhang S, Chen L, Wang-Rodriguez J, Zhang L, Cui B, Frankel W, et al. The Onco-Embryonic Antigen ROR1 Is Expressed by a Variety of Human Cancers. American Journal of Pathology. 2012; 181:1903–1910. [PubMed: 23041612]
48. Hinrichs CS, Restifo NP. Reassessing target antigens for adoptive T-cell therapy. Nature Biotechnology. 2013; 31:999–1008.
49. Pule MA, Savoldo B, Myers GD, Rossig C, Russell HV, Dotti G, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nature Medicine. 2008; 14:1264–1270.
50. O'Connor CM, Sheppard S, Hartline CA, Huls H, Johnson M, Palla SL, et al. Adoptive T-cell therapy improves treatment of canine non-Hodgkin lymphoma post chemotherapy. Scientific Reports. 2012:2.
51. Liadi I, Roszik J, Romain G, Cooper LJN, Varadarajan N. Quantitative high-throughput single-cell cytotoxicity assay for T cells. Journal of visualized experiments : JoVE. 2013:e50058-e. [PubMed: 23407457]
52. Varadarajan N, Kwon DS, Law KM, Ogunniyi AO, Anahtar MN, Richter JM, et al. Rapid, efficient functional characterization and recovery of HIV-specific human CD8(+) T cells using microengraving. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109:3885–3890. [PubMed: 22355106]
53. Dominguez MH, Chattopadhyay PK, Ma S, Lamoreaux L, McDavid A, Finak G, et al. Highly multiplexed quantitation of gene expression on single cells. Journal of Immunological Methods. 2013; 391:133–145. [PubMed: 23500781]

Table 1

Various constructs currently in use in clinical trials involving CAR-based T-cell therapy.

Institute	$CD19^+$ Disease	Clinical Trial.gov	Gene Transfer Method	CAR Scaffold	scFv Clone (derived from murine monoclonal antibodies)	CAR Signaling
U Penn	CLL/ALL	NCT01747486 NCT01626495	Lentivirus	CD8α	FMC63	CD137 and CD3-z
NCI	Leukemia, Lymphom a, ALL	NCT00924326 NCT01593696 NCT01087294	Retrovirus	CD28 TM	FMC63	CD28 and CD3-z
Fred Hutchinson/UWCC	LCL/CLL /MCL	NCT01475058	Lentivirus	IgG_4 Fc	FMC63	CD28 and CD3z
MSKCC	CLL and B-ALL	NCT01430390 NCT00466531 NCT01416974 NCT01044069	Retrovirus	CD8α	SJ25C1	CD28-CD3z and CD137-CD3z
BCM	B-NHL or CLL/ALL	NCT00840853 NCT01316146 NCT01853631 NCT00881920	Retrovirus	IgG_1-CH_2CH_3	FMC63	CD28 and CD3-zeta
MDACC	B-ALL, CLL, other B-cell malignancies	NCT00968760 NCT01497184 NCT01362452 NCT01653717	Transposon	IgG_4	FMC63	CD28-CD3z and CD137-CD3z
COH	Non-Hodgkin Lymphoma	NCT01815749	Lentivirus	IgG_4	FMC63	CD28-CD3 zeta

Abbreviation: U PENN: University of Pennsylvania, UWCC: University of Washington Cancer Consortium NCI: National Cancer Institute, MSKCC: Memorial Sloan-Kettering Cancer Center, MDACC: MD Anderson Cancer Center, COH: City of Hope Medical Center