Published in final edited form as:
Immunol Rev. 2014 January ; 257(1): 7–13. doi:10.1111/imr.12143.

Engineering T cells for cancer: our synthetic future

Robert H Vonderheide[1] and **Carl H June**[1]
[1]Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Introduction

It is widely accepted that the immune system has evolved cellular and humoral mechanisms that can evoke natural immune responses to tumors (1). However, in most instances, vaccines fail to induce rejection of established tumors (2). Adoptive T-cell transfer, a term coined by Billingham, Brent, and Medawar (3), has the potential to overcome one of the significant limitations associated with vaccine-based strategies, and specifically the requirement to de novo activate and expand a tumor antigen-specific T-cell response in patients, who are often immune compromised. As recounted by Kalos *et al*. in this issue of *Immunological Reviews* (4), Mitchison et al. (e) first reported the targeting of cancer through the adoptive transfer of lymphocytes in rodent models over 50 years ago.

Application of the emerging discipline of synthetic biology to cancer, which combines elements of genetic engineering and molecular biology to create new biological structures with enhanced functionalities (6), is the focus of this volume. In 1989, Eshhar and colleagues (7) reported the first synthetic receptor expressed in lymphocytes. Shortly thereafter, Irving and Weiss (8) reported that a chimeric antigen receptor (CAR) comprised of CD8 and the CD3ζ chain was sufficient to activate T cells. A coalescence of preclinical and clinical data supports the premise that the principles of gene transfer combined with adoptive cellular therapy are poised to overcome the fundamental limitations associated with central and peripheral tolerance and enable the potent and efficient at-will targeting of tumors.

There are many mechanisms that prevent the immune system from eliminating tumors in most patients (9). One major issue is the relatively low affinity of T-cell receptors (TCRs) for self-antigens compared to foreign antigens. In humans, comparative analyses have revealed that the TCRs from T cells that recognize self-tumor antigens have a substantially lower affinity (approximately 1.5 logs) for cognate major histocompatibility complex (MHC):peptide complexes compared to their virus-specific TCRs (10). Adoptive transfer using engineered TCRs and CARs is a promising approach to overcome this obstacle (Fig. 1). The adoptive transfer of T cells with endogenous TCRs is an effective therapy for virally induced tumors. As reviewed by Rooney and colleagues in this issue of *Immunological Reviews* (11), the fraction of cancer caused known to be caused by tumor-associated viruses continues to increase. Because cytomegalovirus (CMV) appears to infect glioblastoma (12), clinical studies are using CMV-specific T cells as a potential therapy (13, 14).

Reviews in this volume of *Immunological Reviews* discuss many of the issues currently facing the field to develop effective cancer therapy with retargeted T cells using synthetic receptors. The individual reviews provide in-depth discussion of the data and status of the

Correspondence to: Carl June 8th Floor, Room 123, 3400 Civic Center Boulevard, Bldg 421, Philadelphia, PA 19104-5156, Tel.: +1 215 573 3269, Fax: +1 215 573 8590, cjune@exchange.upenn.edu.

various approaches that are currently being explored in preclinical and clinical trials. In this introduction, we summarize key elements in each review to achieve a comprehensive overall status of the field of synthetic biology with engineered TCRs.

Tumor-infiltrating lymphocytes

Adoptive transfer of tumor infiltrating lymphocytes (TILs) following harvest from tumor and *ex vivo* expansion was pioneered by group at the National Cancer Institute, under the premise that lymphocytic infiltrates at tumors are enriched for tumor antigen-specific T cells. As reviewed by Hinrichs and Rosenberg (15) in this issue of *Immunological Reviews*, many factors influence the success of this approach, including culture technology and host conditioning with chemotherapy and ionizing radiation. TIL cultures for adoptive transfer typically are generated via short-term *ex vivo* expansion and screening for anti-tumor activity. TIL-based approaches have been primarily evaluated in the setting of melanoma, in part because melanoma biopsies are readily obtainable and in part because melanoma has long been considered to be an 'immunogenic' tumor. TIL therapy has been shown to result in durable tumor regression in a subset of patients with advanced metastatic melanoma (16). As reviewed by Schumacher (17) in this issue of *Immunological Reviews*, the mechanisms of responses of patients treated with TILs are the result of T cells reacting to shared antigens as well as neo-antigens created by tumor specific mutations or by epitopes that are encoded by alternative open reading frames (18, 19). Preliminary data suggest that some T-cell responses against neo-antigens may be of a higher magnitude than T-cell responses against shared self-antigens (20, 21). We believe that the major issue facing the field that prevents the widespread use of TIL therapy has been the infusions of high dose IL-2 and the attendant off target toxicities. A secondary obstacle is the challenging logistics of tumor harvest and TIL culture that has prevented investigators from conducting randomized clinical trials analyzed with intent to treat endpoints.

Chimeric antigen receptors

CARs are modular polypeptides typically consisting of three distinct modules: an extracellular target-binding module, a transmembrane module anchoring the CAR into the cell membrane, and an intracellular signaling module. The extracellular target binding module is usually derived from ScFv determinants isolated from antibodies, linked in a single chain through linker polypeptide sequences. Transmembrane modules are usually derived from molecules involved in T-cell function such as the CD8 and CD4 coreceptor molecules (22). In this issue of *Immunological Reviews*, contributions by Abken (23), Gilham (24), Brenner (25), Kalos (4), and Riddell (26) focus on the status of CARs. The principal advantage of CAR-based strategies is that the target-binding moiety is derived from antibodies with affinities several orders of magnitude higher than TCRs. In addition, because CARs recognize intact cell surface proteins, targeting of target cells is neither MHC restricted nor dependent on processing and effective presentation of target epitopes, and therefore, CAR-based approaches are insensitive to tumor escape mechanisms related to MHC loss variants. At this point, many groups have shown that CAR T cells have potent antitumor effects against a variety of advanced hematologic malignancies of the B-cell lineage. The central issue facing the field is whether the technology can be extended to non-B cell derived malignancies, and in particular, can this strategy work for carcinomas?

There are a number of limitations and challenges, both practical and theoretical, associated with CAR-based strategies. In terms of practical limitations, CAR-based approaches are restricted to the targeting of cell surface determinants to which antibodies can be generated in heterologous species. In addition, since CARs are chimeric molecules composed of distinct combinatorial modules that include unique junctional fragments, there is reasonable

potential for CAR-modified T cells to be targeted by patient humoral and cellular immune responses, which may be clinically silent event or in rare instances can provoke anaphylaxis (27, 28). In terms of theoretical limitations, because CARs are engineered to deliver TCR and costimulation-mediated signals independently from the physiological complex through which natural signaling occurs, it is possible that the signaling cascades initiated through CAR engagement are qualitatively and/or quantitatively distinct from those evoked by native TCR signaling. This could result in adverse effects such as uncontrolled lymphoproliferation, an event which fortunately has not occurred. However, the non-physiologic signaling modules in CARs could also have beneficial effects. An example is that CAR T cells may be less susceptible to regulation, and therefore, may have improved function in the tumor microenvironment (29). In this issue of *Immunological Reviews*, Abken and colleagues (23) describe a clever strategy of targeting the tumor stroma by recruiting innate immunity following the adoptive transfer of CAR T cells engineered to secrete transgenic cytokines such as IL-12.

T-cell receptor engineering

The feasibility of transferring T-cell specificity into primary T cells through transfer of TCR α and β chains was demonstrated almost 20 years ago (30, 31). Tumor-antigen-specific T cells, expanded from both cancer patients and healthy volunteers, have been a primary source for isolating tumor-specific heterodimeric TCRs, and over the years, a large variety of approaches using both peptides and whole antigen have been implemented to expand such T cells. Because of the low frequency of such T cells in peripheral blood, the lack of effective culture and expansion methodologies, and the impact of central tolerance on the repertoire, T cells have only be isolated with considerable difficulty using these approaches; furthermore, such T cells are in general of low affinity and demonstrate weak anti-tumor activity. A number of approaches to overcome these issues and generate more potent tumor antigen-specific T cells have been developed. One recent and promising approach to overcome the issue of the intrinsically low-affinity of TCR to self-antigens has been to enhance the affinity of the TCR isolated from such T cells by mutagenesis of the α and β receptor chains. Recent technological advances have facilitated elegant molecular and rational high-throughput genetic approaches to affinity enhance TCRs (32-34), and such efforts have resulted in the ability to reproducibly generate TCR with substantially higher affinities for target antigens (35). An alternative strategy to enhance TCR affinity follows from observations that enhanced functional avidity and improved recognition of tumor cells following introduction of mutations that reduced *N*-glycosylation on TCR chains (36).

As reviewed by Greenberg (37), Hinrichs (15), and Kalos (4) in this issue, there are promising early results in a variety of tumors treated with T cells expressing TCRs engineered by various approaches. However, there have also been on-target and off-target toxicities with engineered TCRs. In one trial, T cells were engineered to express a TCR generated in HLA-A*0201 transgenic mice (i.e. not subjected to selection by the human immune system) and that recognized an epitope shared between MAGE-A3, –A9, and –A12. Of nine patients treated, five demonstrated objective clinical responses, but three patients demonstrated SAE associated with neural toxicity, including two deaths. Post-mortem analysis revealed rare and previously unrecognized expression of MAGE-A12 in brain tissue (38). Two trials that evaluated the use of affinity enhanced HLA-A*01-restricted and MAGE-A3-specific TCR to target melanoma and myeloma were reported recently. The first treated patient in each of these trials experienced severe cardiac toxicity, and each patient died within 7 days of T-cell infusion (39). Retrospective analysis demonstrated that the affinity enhancement of the TCR resulted in the off target recognition of a related HLA-A*01-restricted epitope from the protein titin expressed in cardiac cells (40). These results

highlight the potency of adoptively transferred T cells with redirected specificity and the need to develop improved methods for pre-clinical screening of engineered TCRs.

A potential toxicity following the introduction of engineered TCRs is the production of mixed dimers comprised of chains from the endogenous TCR with chains from the transgenic TCR (41). As reviewed by Bonini (42) and Cooper (43) in this issue of *Immunological Reviews*, a particularly elegant approach to prevent this complication involves TCR gene editing with zinc finger nucleases. Expression of the endogenous TCR α and β chains can be permanently abrogated using this approach, resulting in improved expression and function of the transgenic TCRs and CARs (44, 45)

Cellular engineering

In addition to receptor engineering, optimizing the effector function of engineered T cells can also increase clinical efficacy. Previous disappointing results with adoptive transfer strategies were due to the use of cell culture approaches that resulted in a population of terminally differentiated effector cells. Recent results with CAR T cells indicate that proliferative capacity of the infused T cells is a predictive biomarker of clinical responses, as reviewed by Kalos in this issue (4). It is now well recognized that stimulation of T cells via their TCR without a second costimulatory signal induces tolerance and more recent CAR-based technologies have focused on overcoming this limitation. Thus, while first generation CARs depended on intracellular transduction of the recognition signal via the CD3ζ chain alone, second and third generation CAR constructs have incorporated costimulatory signaling domains such as those derived from CD27, CD28, CD134, or CD137. In addition, culture systems that provide costimulation by immobilized ligands on beads have improved the function of adoptively transferred T cells (46). Sophisticated artificial antigen-presenting cells that provide arrays of selected costimulatory molecules and cytokines have been developed (47, 48), as reviewed by Hirano herein (49).

A major controversy in the field is defining the optimal cell product for infusion. At issue is whether to purify selected subsets of cells for culture and subsequent genetic engineering or more straightforward, to use bulk cell products that contain mixtures of $CD4^+$ helper, $CD8^+$ cytotoxic, naive, central memory, effector memory, and other subsets. For example, cell culture conditions can be optimized to promote the expansion of T-central memory cells using anti-CD3 and anti-CD28 coated beads with IL-7 and IL-15 (50). As summarized by Fowler in this issue of *Immunological Reviews* (51), the blockade of the mechanistic target of rapamycin (mTOR) during culture has the potential to enhance adoptive therapy approaches. Manipulation of metabolic pathways with rapamycin and other mTOR kinase inhibitors can change the fate and function of adoptively transferred T cells (52). Furthermore, CAR T cells encoding a rapamycin-resistant mutant of mTOR have enhanced antitumor effects in pre-clinical models (53). The factors related to the desired composition of the adoptively transferred cells are reviewed herein by Jensen and Riddell (26). T cells with stem cell-like properties have been described (54, 55); however, it is not yet known if these cells are superior to central memory or naive T cells. In this issue of *Immunological Reviews*, Ghosh, Holland and van den Brink (56) have focused on the development of T-cell-based immunotherapy for use in the context of allogeneic hematopoietic stem cell transplantation. They have reviewed some recent studies on the development ‘off the shelf’ immunotherapies across MHC barriers, highlighting the key milestones in their development and use. In particular, they show that the adoptive transfer of precursor T cells enhances T-cell reconstitution after allogeneic stem cell transplantation (57).

A major issue with clinical adoptive cell transfer therapy is the avoidance of senescent and exhausted states in the infused cells. This issue was not predicted in mouse models because

of substantial differences in telomere biology between the mouse and human immune systems (58). With TIL therapy, the telomere length of the transferred lymphocytes correlates with *in vivo* persistence and tumor regression in melanoma patients receiving cell transfer therapy (59). CD28 costimulation can augment telomerase activity and enhance telomere length during *in vitro* culture (60, 61). One approach to circumvent this issue is the use of hematopoietic stem cells or induced pluripotent stem cells (62, 63), as reviewed by Gschweng, DeOliveira and Kohn (64). Another approach to prevent terminal differentiation during culture is to uncouple cell proliferation from effector differentiation. In this issue of *Immunological Reviews*, Crompton, Sukumar and Restifo review the cellular mechanisms that lead to progressive differentiation during the physiologic immune response and they propose the use of synthetic biology to uncouple proliferation from differentiation (65). A potential safety concern related to the infusion of engineered T cells is virus integration-related insertional mutagenesis and cellular transformation, which has been demonstrated with the genetic engineering of hematopoietic stem cells (HSCs)(66). This issue may also occur with nonviral based integration using Sleeping Beauty, as described by Cooper in this issue of *Immunological Reviews* (43). In patients with congenital and acquired immunodeficiency, genetically modified T cells have been shown to persist after adoptive transfer in humans for more than a decade without adverse effects (67, 68), indicating that the approach to genetically modify mature human T cells is fundamentally safe, at least in part because lentiviral integration sites are not random and do not favor proto-oncogenes (69). Furthermore, unlike B cells, T cells are subject to clonal competition at the TCR level, which may explain the rarity of T-cell leukemia and the relative resistance of T cells to transformation (70).

The development of mechanisms to control the lifespan of the transferred T cells is yet another challenge for the field. Initial approaches attempted to introduce ‘suicide genes’ such as herpes simplex virus thymidine kinase (TK) gene; however, these efforts revealed the strong potential for immunologic rejection based on targeting of TK-derived sequences (170). More recently, an elegant and potentially powerful inducible system based on the use of a modified human caspase-9 fused to a human FK506 binding protein permits conditional dimerization and delivery of apoptotic signals in response to small molecules that can permeate the T-cell plasma membrane is currently being evaluated in clinical trials (72). Approaches to regulate the persistence of engineered T cells are discussed by Dotti, Gottschalk, Savoldo and Brenner (25) and by Jensen and Riddell (26).

Conclusions

The articles in this volume of *Immunological Reviews* highlight two basic gene-transfer approaches that are being pursued to bypass the effects of central and peripheral tolerance on the T-cell repertoire. Clinical data generated principally over the past 5 years suggest that we are at the threshold of a golden era for adoptive T-cell therapy, with a number of recent profound examples of the potency and promise of this approach to target cancer. Recent reports, using CAR T cells with CD137 and CD3ζ signaling domains, which documented long term functional persistence of T cells engineered to target CD19, along with long-lasting clinical remissions and ongoing B-cell aplasia have highlighted the potential for adoptive T-cell transfer to induce a profound long term functional antitumor activity (73, 74). Despite these early successes, a number of fundamental and important questions still remain to be resolved for the broad, reproducible, and effective implementation of this approach to treat cancer beyond B-cell malignancies.

A few common themes emerge from these articles. First, identification of the optimal composition of the transferred cellular product requires clarification. Second, in ongoing clinical studies with CAR engineered cells that target CD19, patients remain disease free

with persisting engineered T cells for more than 3 years post treatment but also with ongoing B-cell aplasia due to targeting of normal CD19-positive B cells, highlighting the practical necessity to eventually ablate engineered cells and enable normal B-cell reconstitution. Therefore, a central issue facing the field is the design and implementation of various approaches to control the fate of adoptively transferred cells. These findings are being translated into the clinic at a rapid pace, and it is likely that engineered T-cell transfer will become established as an effective cancer therapy during the next decade. Finally, a challenge for adoptive T-cell therapy will be the necessity and rationale to combine the therapy with other antitumor therapies. In particular, we will require information to rationally combine with therapeutic vaccination, checkpoint inhibition, agonistic antibodies, small molecule inhibitors of tumors, and the targeting of tumor stroma and neo-vasculature, as discussed by Yee in this issue of *Immunological Reviews* (75).

Acknowledgments

Our work in this area is supported by grants from the NIH (5R01CA120409) and the Leukemia and Lymphoma Society (SCOR grant #7007). CHJ and RHV have patents in the field of adoptive therapy that have been licensed to Novartis Corporation.

References

1. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002; 3:991–998. [PubMed: 12407406]
2. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med. 2004; 10:909–915. [PubMed: 15340416]
3. Billingham RE, Brent L, Medawar PB. Quantitative studies on tissue transplantation immunity. II. The origin, strength and duration of actively and adoptively acquired immunity. Proceedings Royal Soc. 1954; 143:58–80.
4. Ruella M, Kalos M. Adoptive immunotherapy for cancer. Immunol Rev. 2014; 257
5. Mitchison NA. Studies on the immunological response to foreign tumor transplants in the mouse. I. The role of lymph node cells in conferring immunity by adoptive transfer. J Exp Med. 1955; 102:157–177. [PubMed: 13242741]
6. Chen YY, Galloway KE, Smolke CD. Synthetic biology: advancing biological frontiers by building synthetic systems. Genome Biol. 2012; 13:240. [PubMed: 22348749]
7. Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc Natl Acad Sci USA. 1989; 86:10024–10028. [PubMed: 2513569]
8. Irving BA, Weiss A. The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways. Cell. 1991; 64:891–901. [PubMed: 1705867]
9. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011; 480:480–489. [PubMed: 22193102]
10. Aleksic M, et al. Different affinity windows for virus and cancer-specific T-cell receptors: implications for therapeutic strategies. Eur J Immunol. 2012; 42:3174–3179. [PubMed: 22949370]
11. Rooney CM, Leen AM, Vera JF, Heslop HE. T lymphocytes targeting native receptors. Immunol Rev. 2014; 257
12. Sampson JH, Mitchell DA. Is cytomegalovirus a therapeutic target in glioblastoma? Clin Cancer Res. 2011; 17:4619–4621. [PubMed: 21632859]
13. Ghazi A, et al. Generation of polyclonal CMV-specific T cells for the adoptive immunotherapy of glioblastoma. J Immunother. 2012; 35:159–168. [PubMed: 22306904]
14. Crough T, Beagley L, Smith C, Jones L, Walker DG, Khanna R. Ex vivo functional analysis, expansion and adoptive transfer of cytomegalovirus-specific T-cells in patients with glioblastoma multiforme. Immunol Cell Biol. 2012; 90:872–880. [PubMed: 22508289]
15. Hinrichs CS, Rosenberg SA. Exploiting the curative potential of adoptive T-cell therapy for cancer. Immunol Rev. 2014; 257

16. Dudley ME, et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol. 2008; 26:5233–5239. [PubMed: 18809613]
17. Linnemann C, Mezzadra R, Schumacher TNM. TCR repertoires of intratumoral T-cell subsets. Immunol Rev. 2014; 257
18. Andersen RS, et al. Dissection of T-cell antigen specificity in human melanoma. Cancer Res. 2012; 72:1642–1650. [PubMed: 22311675]
19. Robbins PF, et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. Nat Med. 2013; 19:747–752. [PubMed: 23644516]
20. Castle JC, et al. Exploiting the mutanome for tumor vaccination. Cancer Res. 2012; 72:1081–1091. [PubMed: 22237626]
21. Matsushita H, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature. 2012; 482:400–404. [PubMed: 22318521]
22. Sadelain M, Brentjens R, Riviere I. The basic principles of chimeric antigen receptor design. Cancer Discov. 2013; 3:388–398. [PubMed: 23550147]
23. Chmielewski M, Hombach AA, Abken H. Of CARs and TRUCKs: chimeric antigen receptor (CAR) T cells engineered with a n inducible cytokine to modulate the tumor stroma. Immunol Rev. 2014; 257
24. Cheadle EJ, Gornall H, Baldan V, Hanson V, Hawkins RE, Gilham DE. CAR T cells: driving the road from the laboratory to the clinic. Immunol Rev. 2014; 257
25. Dotti G, Gottschalk S, Savoldo B, Brenner MK. Design and development of therapies using chimeric antigen receptor-expressing T cells. Immuno Rev. 2014; 257
26. Jensen MC, Riddell SR. Design and implementation of adoptive therapy with chimeric antigen receptor-modified T cells. Immunol Rev. 2014; 257
27. Lamers C, et al. Immune responses to transgene and retroviral vector in patients treated with ex vivo engineered T cells. Blood. 2011; 117:72–82. [PubMed: 20889925]
28. Maus MV, et al. T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. Cancer Immunol Res. 2013 in press.
29. Pegram HJ, et al. Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. Blood. 2012; 119:4133–4141. [PubMed: 22354001]
30. Clay TM, Custer MC, Sachs J, Hwu P, Rosenberg SA, Nishimura MI. Efficient transfer of a tumor antigen-reactive TCR to human peripheral blood lymphocytes confers anti-tumor reactivity. J Immunol. 1999; 163:507–513. [PubMed: 10384155]
31. Cooper LJ, Kalos M, Lewinsohn DA, Riddell SR, Greenberg PD. Transfer of specificity for human immunodeficiency virus type 1 into primary human T lymphocytes by introduction of T-cell receptor genes. J Virol. 2000; 74:8207–8212. [PubMed: 10933734]
32. Li Y, et al. Directed evolution of human T-cell receptors with picomolar affinities by phage display. Nat Biotechnol. 2005; 23:349–354. [PubMed: 15723046]
33. Chervin AS, Aggen DH, Raseman JM, Kranz DM. Engineering higher affinity T cell receptors using a T cell display system. J Immunol Methods. 2008; 339:175–184. [PubMed: 18854190]
34. Udyavar A, Alli R, Nguyen P, Baker L, Geiger TL. Subtle affinity-enhancing mutations in a myelin oligodendrocyte glycoprotein-specific TCR alter specificity and generate new self-reactivity. J Immunol. 2009; 182:4439–4447. [PubMed: 19299745]
35. Louie KA, et al. Cell-based gene therapy experiments in murine experimental autoimmune encephalomyelitis. Gene Therapy. 2005; 12:1145–1153. [PubMed: 15772685]
36. Kuball J, et al. Increasing functional avidity of TCR-redirected T cells by removing defined N-glycosylation sites in the TCR constant domain. J Exp Med. 2009; 206:463–475. [PubMed: 19171765]
37. Stromnes IM, Schmitt TM, Chapuis AG, Hingorani S, Greenberg PD. Re-adapting T cells for cancer therapy: from mouse models to clinical trials. Immunol Rev. 2014; 257
38. Morgan RA, et al. Cancer Regression and Neurological Toxicity Following Anti-MAGE-A3 TCR Gene Therapy. J Immunother. 2013; 36:133–151. [PubMed: 23377668]

39. Linette GP, et al. Cardiovascular toxicity and titin cross-reactivity of affinity enhanced T cells in myeloma and melanoma. Blood. 2013; 122:863–871. [PubMed: 23770775]
40. Cameron BJ, et al. Identification of a titin-derived HLA-A1-presented peptide as a cross-reactive target for Engineered MAGE A3-directed T cells. Sci Trans Med. 2013; 5:197ra103.
41. Bendle GM, et al. Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy. Nat Med. 2010; 16:565–570. [PubMed: 20400962]
42. Cieri N, Mastaglio S, Oliveira G, Casucci M, Bondanza A, Bonini C. Adoptive immunotherapy with genetically modified lymphocytes in allogeneic stem cell transplantation. Immunol Rev. 2014; 257
43. Sing H, Huls H, Cooper LJN. A new approach to gene therapy using Sleeping Beauty to genetically modify clinical-grade T cells to target CD19. Immunol Rev. 2014; 257
44. Provasi E, et al. Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer. Nat Med. 2012; 18:807–815. [PubMed: 22466705]
45. Torikai H, et al. A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR. Blood. 2012; 119:5697–5705. [PubMed: 22535661]
46. Levine BL, Bernstein W, Craighead N, Lindsten T, Thompson CB, June CH. Effects of CD28 costimulation on long term proliferation of CD4+ T cells in the absence of exogenous feeder cells. J Immunol. 1997; 159:5921–5930. [PubMed: 9550389]
47. Hirano N, et al. Engagement of CD83 ligand induces prolonged expansion of CD8+ T cells and preferential enrichment for antigen specificity. Blood. 2006; 107:1528–1536. [PubMed: 16239433]
48. Suhoski MM, et al. Engineering artificial antigen-presenting cells to express a diverse array of co-stimulatory molecules. Mol Ther. 2007; 15:981–988. [PubMed: 17375070]
49. Butler MO, Hirano N. Human cell-based artificial antigen-presenting cells for cancer immunotherapy. Immunol Rev. 2014; 257
50. Kaneko S, et al. IL-7 and IL-15 allow the generation of suicide gene-modified alloreactive self-renewing central memory human T lymphocytes. Blood. 2009; 113:1006–1015. [PubMed: 18978209]
51. Fowler DH. Rapamycin-resistant effector T-cell therapy. Immunol Rev. 2014; 257
52. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. Immunity. 2013; 38:633–643. [PubMed: 23601682]
53. Huye LE, et al. Combining mTor inhibitors with rapamycin-resistant T cells: a two-pronged approach to tumor elimination. Mol Ther. 2011; 19:2239–2248. [PubMed: 21878902]
54. Lugli E, et al. Superior T memory stem cell persistence supports long-lived T cell memory. J Clin Invest. 2013; 123:594–599. [PubMed: 23281401]
55. Gattinoni L, et al. A human memory T cell subset with stem cell-like properties. Nat Med. 2011; 17:1290–1297. [PubMed: 21926977]
56. Ghosh A, Holland AM, van den Brink MRM. Genetically engineered donor T cells to optimize graft-versus-tumor effects across MHC barriers. Immunol Rev. 2014; 257
57. Zakrzewski JL, et al. Adoptive transfer of T-cell precursors enhances T-cell reconstitution after allogeneic hematopoietic stem cell transplantation. Nat Med. 2006; 12:1039–1047. [PubMed: 16936725]
58. Weng NP. Aging of the immune system: How much can the adaptive immune system adapt? Immunity. 2006; 24:495–499. [PubMed: 16713964]
59. Zhou J, Shen X, Huang J, Hodes RJ, Rosenberg SA, Robbins PF. Telomere length of transferred lymphocytes correlates with in vivo persistence and tumor regression in melanoma patients receiving cell transfer therapy. J Immunol. 2005; 175:7046–7052. [PubMed: 16272366]
60. Weng NP, Levine BL, June CH, Hodes RJ. Regulation of telomerase RNA template expression in human T lymphocyte development and activation. J Immunol. 1997; 158:3215–3220. [PubMed: 9120276]

61. Weng NP, Palmer LD, Levine BL, Lane HC, June CH, Hodes RJ. Tales of tails: regulation of telomere length and telomerase activity during lymphocyte development, differentiation, activation, and aging. Immunol Rev. 1997; 160:43–54. [PubMed: 9476664]
62. Giannoni F, et al. Allelic exclusion and peripheral reconstitution by TCR transgenic T cells arising from transduced human hematopoietic stem/progenitor cells. Mol Ther. 2013; 21:1044–1054. [PubMed: 23380815]
63. Themeli M, et al. Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy. Nat Biotechnol. 2013; 31:928–933. [PubMed: 23934177]
64. Gschweng W, De Oliveira S, Kohn DB. Hematopoietic stem cells for cancer immunotherapy. Immunol Rev. 2014; 257
65. Crompton JG, Sukumar M, Restifo N. Uncoupling T-cell expansion from effector differentiation in cell-based immunotherapy. Immunol Rev. 2014; 257
66. Hacein-Bey-Abina S, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest. 2008; 118:3132–3142. [PubMed: 18688285]
67. Muul LM, et al. Persistence and expression of the adenosine deaminase gene for 12 years and immune reaction to gene transfer components: long-term results of the first clinical gene therapy trial. Blood. 2003; 101:2563–2569. [PubMed: 12456496]
68. Scholler J, et al. Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. Sci Trans Med. 2012; 4:132Ra153.
69. Bushman FD. Retroviral integration and human gene therapy. J Clin Invest. 2007; 117:2083–2086. [PubMed: 17671645]
70. Newrzela S, et al. T-cell receptor diversity prevents T-cell lymphoma development. Leukemia. 2012; 26:2499–2507. [PubMed: 22643706]
71. Marktel S, et al. Immunologic potential of donor lymphocytes expressing a suicide gene for early immune reconstitution after hematopoietic T-cell-depleted stem cell transplantation. Blood. 2003; 101:1290–1298. [PubMed: 12393508]
72. Di Stasi A, et al. Inducible apoptosis as a safety switch for adoptive cell therapy. N Engl J Med. 2011; 365:1673–1683. [PubMed: 22047558]
73. Kalos M, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Trans Med. 2011; 3:95ra73.
74. Grupp SA, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med. 2013; 368:1509–1518. [PubMed: 23527958]
75. Yee C. The use of endogenous T cells for adoptive transfer. Immunol Rev. 2014; 257

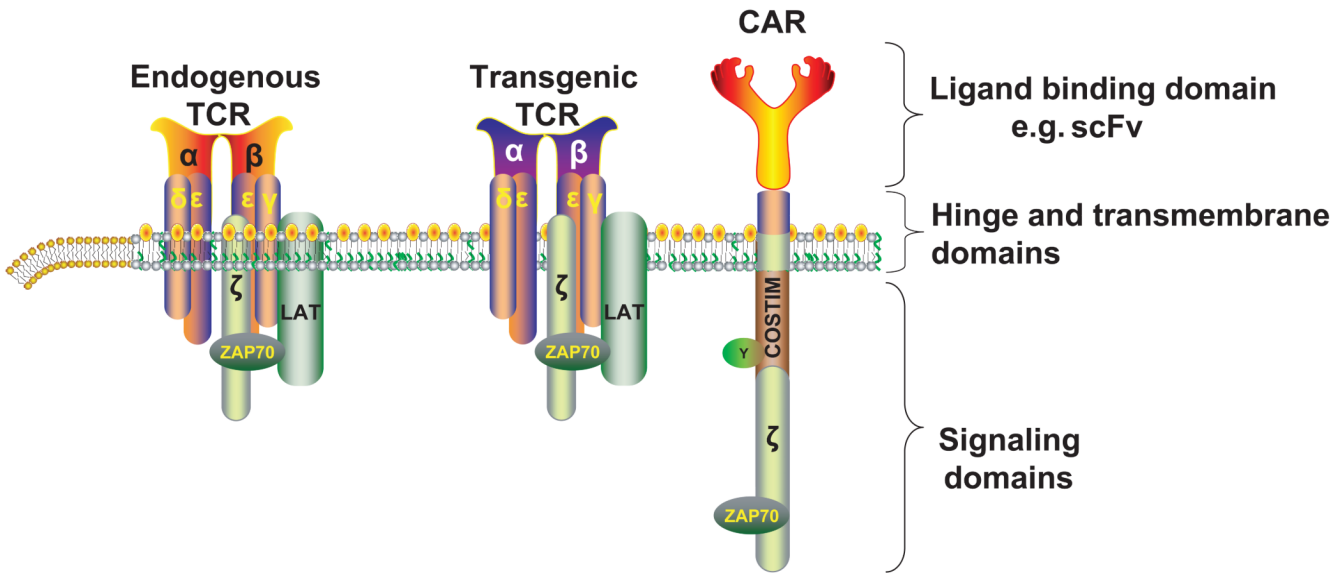

Fig. 1.

T cells can be engineered to have retargeted specificity for tumors. Bispecific T cells are created by introduction of genes that encode T-cell receptors (TCRs) and chimeric antigen receptors (CARs) of desired specificity and affinities for tumors. CARs target surface antigens in an MHC-independent fashion. The T cells retain expression of the endogenous TCR, unless this is knocked down by various approaches. Abbreviations are as follows: Costim, cosignaling domain such as CD28 or 4-1BB; LAT, linker for activation of T cells; scFv, single-chain variable fragment; ZAP70, ζ chain associated protein kinase 70 kDa.