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NextGen cell-based immunotherapies in cancer and other immune disorders

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T lymphocyte and other cell therapies have the potential to transform how we treat cancers and other diseases that have few therapeutic options. Here, we review the current progress in engineered T cell therapies and look to the future of what will establish cell therapy as the next pillar of medicine. The tools of synthetic biology along with fundamental knowledge in cell biology and immunology have enabled the development of approaches to engineer cells with enhanced capacity to recognize and treat disease safely and effectively. This along with new modes of engineering cells with CRISPR and strategies to make universal 'off-the-shelf' cell therapies will provide more rapid, flexible, and cheaper translation to the clinic.

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Introduction

It has been over 50 years since the first bone marrow transplant was used successfully to reconstitute the entire blood system to cure cancer [1]. This singular success transformed the field of hematology-oncology and paved the way for a cell therapy revolution including the implementation of antigen-specific T cell therapies to treat a variety of tumors such as melanoma, renal, lung, and multiple other solid tumors [2]. In a secondary wave of innovation, new *in vitro* techniques to efficiently expand neoantigen-specific T cells from tumor tissue have shown efficacy in treating a number of tumors. Recently, the third revolution, enabled by the use of genetic engineering to modify cells with exceptional specificity and almost unlimited flexibility, is poised to dramatically advance modern medicine. Target-specific Chimeric Antigen-specific Receptors (CAR), which combine cell surface tumor antigen specificity of monoclonal antibodies with the signaling machinery of T cells, have led to the development of two FDA-approved cell-based drugs, Yescarta™ and Kymriah™. These novel genetically engineered therapeutics have yielded extraordinary cures of CD19+ lymphomas and for the treatment of multiple blood cancers and myeloma on the horizon. But many challenges in the field remain unresolved including: side effects resulting from cytokine release syndrome (CRS); difficulty in harnessing the technology for solid tumors; and issues of tumor antigen escape, adoptive T cell durability, stability, and exhaustion. In this perspective, we will summarize the advances and opportunities in the field of human adoptive cell therapy (ACT), emphasizing the breath of opportunities using various cell subsets, gene engineering and creative gene editing approaches in TCRαβ cells, although there are ongoing research efforts to develop engineered NK, macrophage and TCRγδ cells as well. Novel synthetic biology approaches will be highlighted that introduce payloads and multi-antigen specificities, regulate receptors and alter epigenetic landscapes that impact T cell functionality, durability, and efficacy in a hostile microenvironment in the cancer, autoimmunity and organ transplant settings. Finally, we peek into the future when highly regulated, universal ACT are developed, not just from T cells but induced Pluripotent Stem (iPS) cells, to treat an array of immune-mediated and other inflammation-associated diseases.

Generation of antigen-specific T cells

The adaptive immune system has evolved to detect small often single-amino acid changes in a foreign protein. For T cells, this is accomplished through the T cell receptor complex (TCR) designed to recognize small peptides presented by major histocompatibility complex (MHC) class I and II. In contrast, B cells use two-chain antibodies to recognize epitopes created by tertiary protein structures. Modern ACT for cancer has hijacked individual

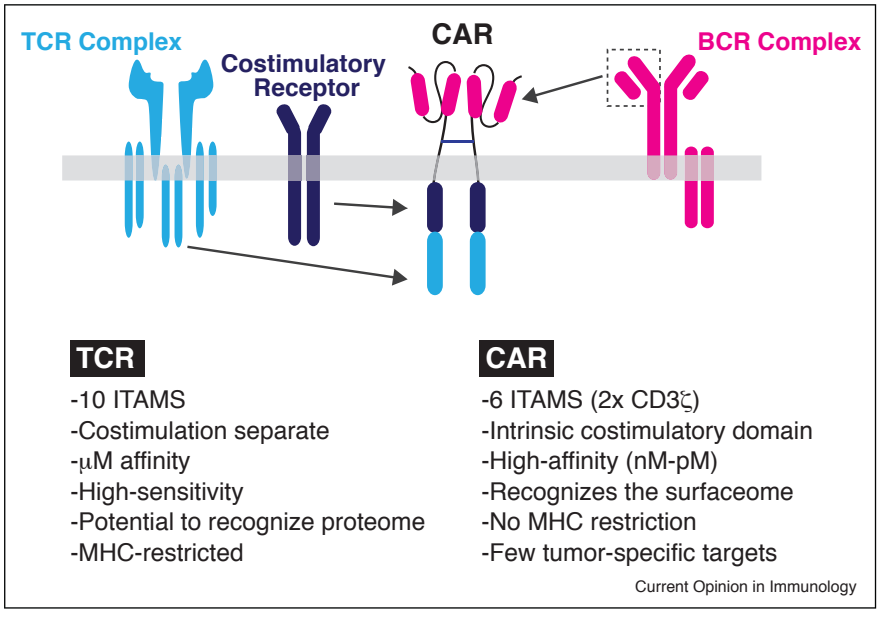
receptors to expand and, in many cases, engineer antigen-specific T cells. Specific TCRαβ chains have been isolated from disease-reactive T cells and introduced into function effector cells to mediate the relevant immunity, be it cytotoxicity (for cancer and infectious diseases) or suppression (in autoimmunity and transplantation). The TCRs have the advantage of recognizing peptides derived from the entire proteome and have evolved high sensitivity to a small number of MHC–peptide complexes on an antigen-presenting cell [3,4]. In contrast, antibodies recognize epitopes expressed on whole proteins, either soluble or on the surface of cells. CARs utilize the antibody recognition structure, fused to costimulatory and TCR signaling domains, to direct T cells to cell surface-displayed whole proteins. CARs are generally less sensitive than TCRs and sometimes exhibit on-target, off-tissue activities. These two modes of targeting and activating T cell therapies are the basis for much of the current approaches to antigen-specific ACT (Figure 1).

TCR versus CAR ACT

Two major strategies have been deployed to treat cancer with T cells with tumor-specific TCRs. One is to isolate, expand, and reinfuse tumor-infiltrating T cells (TILs) from excised tumors. This strategy uses the power of a polyclonal tumor-specific T cell population that can lead to a multi-pronged attack. A second strategy is to sequence the TCRs of TILs and engineer selected receptors into patient-derived T cells for ACT. Unlike TIL therapy, which often has a mixed population that includes many irrelevant T cells thus limiting efficacy, engineered TCR therapies can generate uniformly functional T cells. These neoantigen-specific T cells, which target unique tumor mutations have limited off-target effects [5,6] and, in some cases, the TCRs target shared tumor antigens, often of embryonic origin [7], making the therapeutic strategy even more broadly applicable. The use of antigen-specific TCRs are also being exploited for regulatory T cells (Tregs) therapies as well to treat organ transplant rejection and potentially, autoimmunity [8]. Highly selective TCR-based T cell therapies will continue to evolve and may be superior to CARs in certain therapeutic settings due to the unique properties of the receptor and available targetable antigens.

The efficacy of CARs can be seen in the first two FDA-approved CAR T cell therapies in which both tisagenlecleucel and axicabtagene ciloleucel recognize an extracellularly expressed protein (CD19) to eliminate certain lymphomas. In contrast to TCRs that are capable of being activated by a single peptide-MHC complex [9], CARs require a minimum of ~200–10 000 target molecules to activate [10,11]. The low sensitivity of CARs can be overcome by high-density ligands such that CARs can drive potent anti-tumor T cell responses. However, in some cases, the cells cause toxicity via on-target off-tumor specificity or induction of CRS [12,13]. Despite these flaws, the high affinity, lack of MHC restriction, and modular architecture for rapid engineering CARs have made them a focal point in ACT. Next-generation CARs, reviewed below, have been engineered to address critical problems within

Figure 1



CAR versus TCR. A comparison of T cell receptors (TCRs) and chimeric antigen receptors (CARs) T cell therapy. CARs are composed of a BCR-like monoclonal antibody-based scFv binding domain and an intracellular signaling domain composed of an ITAM containing domain such as CD3ζ and a costimulatory signaling domain.

therapeutic applications such as antigen escape, T cell exhaustion, and the need for universal 'off-the-shelf' CARs.

CARs and TCRs are now being engineered to detect new classes of antigens. For example, TCR-like CARs have been generated that detect intracellular neoantigens in the context of MHC [14]. In addition, both TCRs and CARs are being developed against phosphorylated and citrullinated proteins, fusion proteins, alternative splice variants, and mutations in driver antigens such as KRAS [15,16]. Therefore, investigators at the NCI and companies, such as PACT Pharma, are gearing up to conquer personalized ACT by identifying and generating receptors against neoantigens (especially truncal mutations) [17–19]. In these studies, it will be essential to make sure that these receptors do not have toxic off-tumor effects [20,21]. In the case of TCRs, mispairing with the endogenous chains can lead to receptors with unknown specificity [22], which can be avoided with single-chain alpha-beta TCRs, cysteine bridges and other genetic modifications [23,24]. Lastly, despite the signaling and engineering advantages, TCR therapies are intrinsically HLA-restricted. While this is not a problem in personalized medicine, shared tumor antigens will only be targetable by TCRs within patients of similar genetic backgrounds or on less polymorphic HLA molecules such as HLA-E or HLA-G.

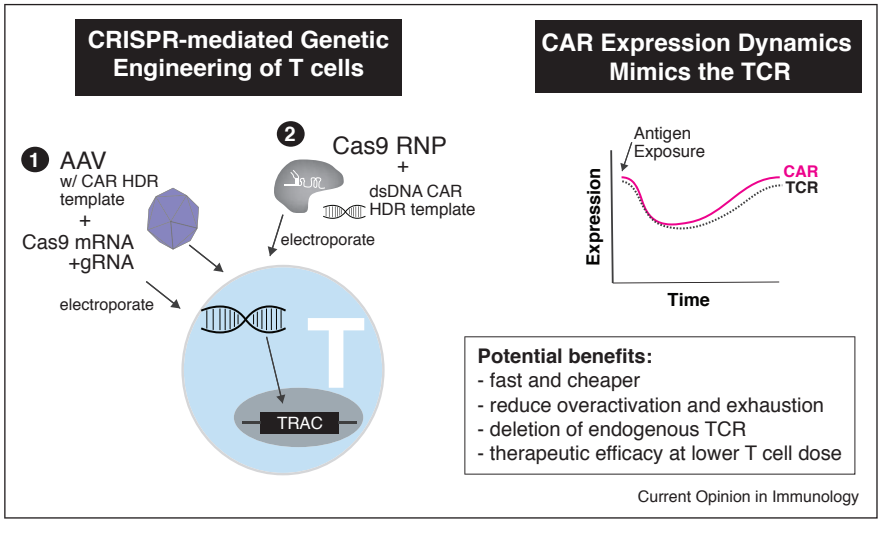
Tackling the challenge of solid tumors

There is growing evidence that controlling T cell activation, specificity, receptor signaling dynamics, and cell communication systems (e.g. cytokines and chemokines) will be essential in the successful adaptation of CARs to treat solid tumors. Many investigators have turned toward engineered regulatory mechanisms to control each aspect of CAR-T cell function.

Controlling CAR expression. As the field has progressed, CAR T cell therapy has challenged researchers and clinicians with the threat of CRS and T cell exhaustion. Regulating CAR T cell activity can be as simple as controlling the longevity of CAR expression. One of the easiest ways to accomplish this is to transiently express the CAR via electroporation of mRNA [25,26]. However, once the CAR expression is lost, there is no way to regain activity and multiple doses will likely be required. Therefore, more sophisticated control of CAR expression dynamics would be ideal. With advances in the use of CRISPR in T cells, groups have achieved high-efficiency integration of CARs into the TCRα locus (TRAC), where CAR expression is controlled by endogenous regulatory elements [27•]. This is beneficial because it reduces the complexity of T cell engineering and mimics the dynamics of TCR expression upon antigen stimulation. The natural regulation of CAR expression, which likely controls the timing and duration of signaling, can reduce exhaustion and improve therapeutic efficacy (Figure 2) [28,29••].

Controlling CAR signaling and activation. Aside from modulating CAR expression, several groups have begun engineering solutions to CRS and T cell exhaustion by directly engineering CAR signaling domains. Feucht *et al.* set out to promote long-term T cell proliferation and persistence by mutation of the CD3ζ immunoreceptor tyrosine-based activation motifs thus dampening CAR signaling [30•]. The company TCR2 has designed a

Figure 2

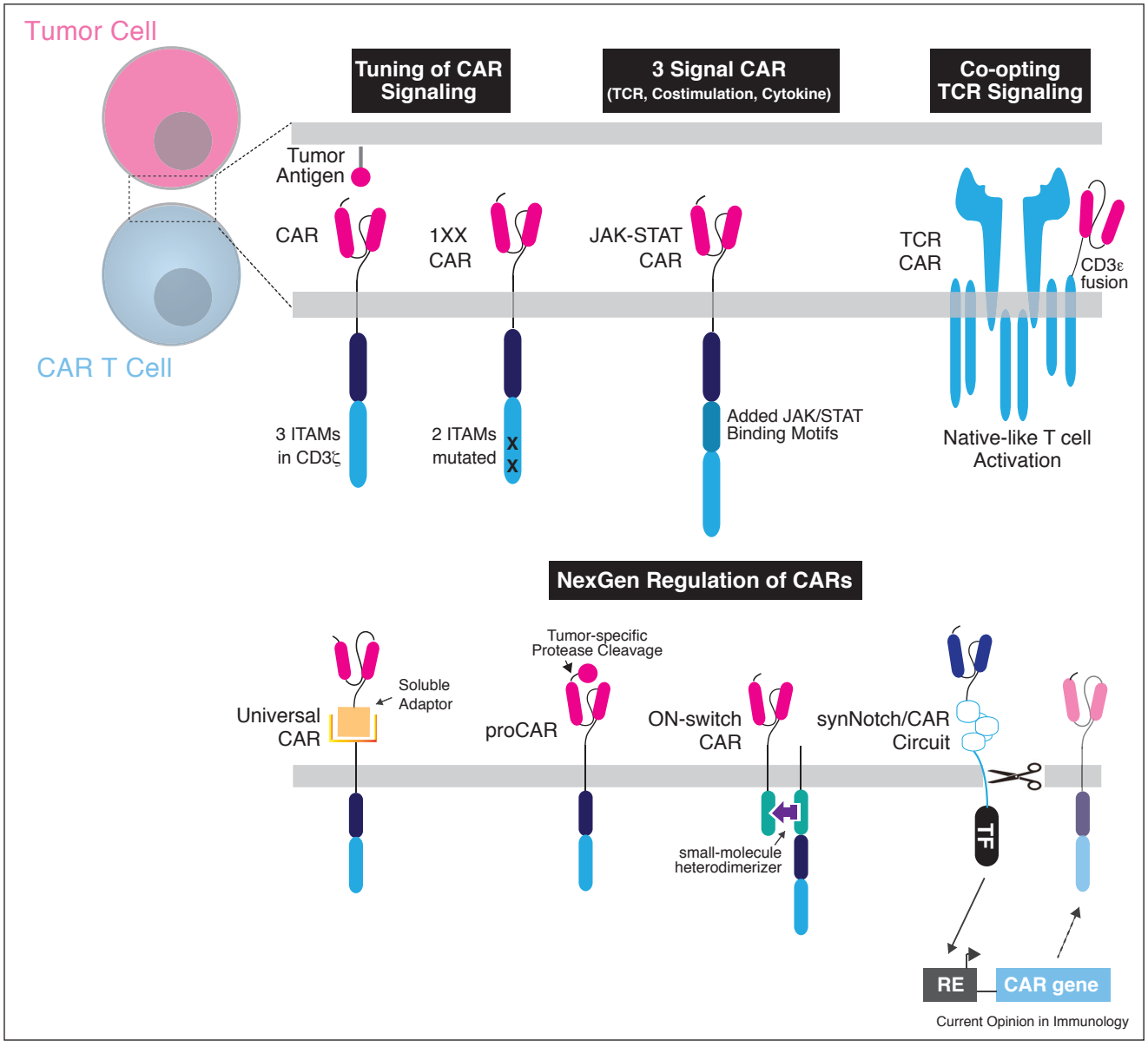


Targeted Insertion of CARs with CRISPR. The entities 1) and 2) demonstrate two recent methods for utilizing CRISPR to genetically engineer T cells for ACT. Insertion of the engineered receptor into the TRAC locus results in a TCR-like expression pattern in response to antigen exposure. These approaches provide a flexible platform to engineer cell therapies with implications beyond CARs.

chimeric scFv–TCR complex that may improve T cell responses due to more natural TCR activation [31]. While many labs have focused on TCR signaling, others enhanced CAR T cell activation and differentiation by integrating cytokine JAK-STAT signaling domains, such that T cells strongly proliferate and are less dependent on their microenvironment (Figure 3) [32••,33]. Although the modularity of CARs is conducive to innovative engineering, it remains unknown how to alter CAR signaling to produce ideal clinical results.

Beyond engineering signaling domains into CARs to shape the T cell response, others have developed new strategies to gate the activity of CARs such that they are activated in a context-dependent manner. Desnoyers *et al.* engineered a system of 'receptor masking' where

Figure 3

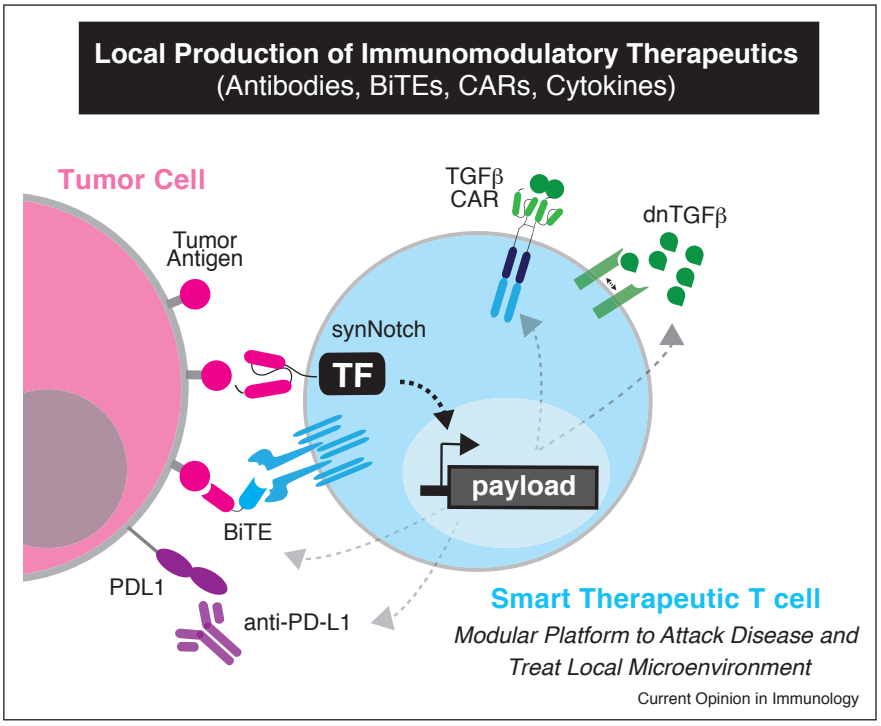


Next-generation CARs for ACT. Overcoming the challenges of CRS and T cell exhaustion with receptor signaling and regulatory modalities. The top panel is a comparison of the most recent CAR designs with altered signaling. From left to right: the classical CAR, the 1XX CAR (with only a singular N terminal CD3ζ ITAM), the JAK-STAT CAR (with additional cytokine signaling domains), and the TCR2 CAR (an scFv CD3ε fusion). The bottom panel is a comparison of the recent CAR designs that allow for dynamic *in vivo* regulation. From left to right: the Universal CAR (with swappable binding elements that allow for titratable and convertible specificity), the proCAR (scFv can only engage with its target only when certain TME-specific proteases are present), the ON-switch CAR (signaling controlled by a small molecule), and the synNotch/CAR circuits (context-specific CAR expression and multi-antigen recognition capabilities).

CAR-target engagement is blocked by a probody: an scFv whose binding is dynamically controlled by matrix metalloproteinases commonly found in the tumor microenvironment (TME) [34]. Roybal *et al.* developed a novel synthetic receptor system called synthetic Notch receptors (synNotch) which, unlike CARs, translate ligand-binding to release of a receptor-tethered transcription factor that regulates a user-defined transcriptional circuit [35,36]. SynNotch receptors can reliably control the expression of CARs such that the CAR is only expressed in the TME, confining T cell activity to the disease site (Figure 3). An added feature of SynNotch/CAR circuits is combinatorial antigen recognition, which improves the specificity of ACTs. Wu *et al.* and later Raj *et al.* took a different approach by controlling CAR activity with small molecule drugs making the strength and duration of CAR activation titratable [37,38]. This synthetic regulation of CARs via their external cues designated a new way of thinking about CAR dynamics and reducing on-target off-tumor toxicities; however, it did not address the growing complication of antigen escape. To address this issue, designs revolving around the dynamic control of CAR specificity and activation once *in vivo* has resulted in solutions such as the convertible CARs of Xyphos, Calibr, and Unum or more recently the SUPRA CAR system [39–42,43•]. These regulation platforms highlight a dynamic, rapidly expanding field of *in vivo* dynamic CAR regulation. (Figure 3).

Controlling the T cell and cellular environments. Investigators have begun to realize how the nature of the T cell type that is modified with the TCR or CAR can control cell fate. For instance, groups have expressed CARs or TCRs in viral-specific T cells (VSTs) and suggest this better exploits the proliferative capability of the T cell with vaccination during treatment [44]. Others have used subsets of purified central memory cells as vaccine studies have suggested that this cell type is more efficient at long-term immunity [45–47]. Conversely, others have focused on modifying the TME, rather than the T cell itself, to alter T cell function. Many of the suppressive mechanisms of the TME (e.g. metabolic control, suppressive cytokines and infiltrating cell types) are now being targeted with ACT to improve the efficacy of the T cell response in the inhospitable environment. Many labs have focused on how CAR T cells can synthetically control the effects of immunosuppressive cytokines such as TGFβ or IL-4. New CAR T cells that sequester immunosuppressive cytokines, activate within

Figure 4



Custom regulation of therapeutic immune cells. The expansive capabilities of locally delivered payload therapeutics to alter the targeted microenvironment. Payload delivery systems such as the synNotch can transcriptionally regulate the expression of natural or non-natural therapeutics that can alter the surrounding microenvironment. Targeted delivery of antibody-based BiTEs and immunotherapies as well as biologics that sequester immunosuppressive cytokines or block their signaling capabilities on the CAR T cell can drastically affect the cell therapy's persistence, proliferation, and continued activation.

their presence, block their signaling, or secrete orthogonal cytokines that confine communication to the engineered T cells are being developed to reduce the impact of the TME [48,49,50•,51,52•]. Recently, multiple versions of antibodies, including nanobodies, have been used to target growth factors to attenuate tumor aggressiveness [53–56], in the form of titratable bispecifics [57–61]. These include the use of bi-specific T-cell engagers (BiTE) that combine specificities for cytokines, cell surface target antigens and checkpoint inhibitors to maximize cell-based therapies [62–66]. CAR-T cells paired with synNotch and granzyme-B systems could soon be used to deliver these therapeutics and attenuate the suppressive capabilities of the TME [36,67,68] (Figure 4).

Cell therapy for the masses — universal ACT

Cell therapies have successfully avoided issues of graft-versus-host disease (GvHD) because the current therapies utilize autologous T cells [69,70]. While this is an FDA-approved pipeline, the treatment could be simplified through the use of 'off-the-shelf' universal cells—therapeutics in which patients would receive allogeneic cells that evade detection by the recipient immune system. Many groups have focused on editing out the TCR to prevent GvHD [71,72] while others, have selectively deleted HLA class I and class II molecules to avoid recognition by host T cells and, thus, prevent rejection by the adaptive immune system of the host. These modifications in combination with CAR engineering may allow allogeneic universal CAR T cells to eradicate tumor cells with similar efficacy to autologous CAR T cells. This approach has been successful clinically in two patients with pediatric B-ALL [71,73].

In fact, there is an incredible rate of progress in genome editing and cellular engineering technologies leading to new approaches to rapidly generate universal T cells for cell therapies. T cells have been reprogrammed into an embryonic-like state to enable unlimited proliferation and production of iPS T cells [74,75]. Clarke *et al.* recently generated FT819 [76], an iPS cell line containing the standard CD19 CAR and a bi-allelic disruption of the TRAC locus [76]. Cooper *et al.* utilized CRISPR/Cas9 to create UCART7, a universal CAR T cell therapy targeting CD7+ T cells with both CD7 and its TRAC locus knocked out, making it fratricide and GvHD resistant [77••]. While these approaches need to be further tested in the lab and clinic, they mark a critical step toward true universal T cell therapies that could be cost-effective, ready for immediate use, and compatible with a wider patient population, particularly those with few therapeutic options.

Conclusion

ACT has now been established as another pillar of medicine along with small molecule drugs and biologics. The promise of cell therapy goes beyond cancer, or even immune diseases such as autoimmunity and organ transplantation. In fact, under the right conditions, 'off-the-shelf' T cells, engineered with novel receptors, may be applied to many applications from tissue repair and regeneration to the elimination of senescent cells in degenerative diseases such as dementias and heart disease. To get there, the use of novel gene editing and deliberate synthetic biology approaches, described above, will be key and the ability to engineer both enhanced therapeutic functionality and control systems into cells will increase safety and efficacy. It should also be noted that the current limitations of cost of goods will need to be addressed using novel manufacturing approaches, virus-free introduction of gene edits and receptors, and better closed automated systems for cell expansion. This is an exciting time in immunology, synthetic biology, and systems biology as we can now envision the wide-range of tools that will be developed to control cells. We are at an inflection point in cell-based immunotherapies, but the field must be thorough in testing this transformative form of therapy to make sure the safety profile matches the therapeutic need.

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KTR is an inventor on patents for synthetic Notch receptors (WO2016138034A1, PRV/2016/62/333,106) and receives licensing fees and royalties. The patents were licensed by Cell Design labs and are now part of Gilead. He was a founding scientist/consultant and stockholder in Cell Design Labs now a Gilead Company. KTR holds stock in Gilead. KTR is also a consultant and stockholder in Xyphos. and a consultant for Nurix and Third Rock Ventures.

JAB is a consultant for Juno, a Celgene company; a stockholder and member of the Board of Directors on Rheos Medicines, a stockholder and member of the Scientific Advisory Boards of Pfizer Center for Therapeutic Innovation, Vir Therapeutics, Arcus Biotherapeutics, Quentis Therapeutics, Solid Biosciences, and Celsius Therapeutics. JAB owns stock in MacroGenics Inc., Vir Therapeutics, Arcus Biotherapeutics, Quentis Therapeutics, Solid Biosciences, Celsius Therapeutics and Kadmon Holdings.

CA has no disclosures to report.

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- •• of outstanding interest

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