REVIEW

The future of engineered immune cell therapies

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Immune cells are being engineered to recognize and respond to disease states, acting as a "living drug" when transferred into patients. Therapies based on engineered immune cells are now a clinical reality, with multiple engineered T cell therapies approved for treatment of hematologic malignancies. Ongoing preclinical and clinical studies are testing diverse strategies to modify the fate and function of immune cells for applications in cancer, infectious disease, and beyond. Here, we discuss current progress in treating human disease with immune cell therapeutics, emerging strategies for immune cell engineering, and challenges facing the field, with a particular emphasis on the treatment of cancer, where the most effort has been applied to date.

The first uses of living immune cells as a therapy were demonstrated in the late 1980s, when tumor-infiltrating T cells isolated from cancer patients were used to treat metastatic melanoma. The early promise of these clinical trials fueled efforts exploring immune cell therapy (ICT) using diverse immune cell types and applying ICT to additional cancers and pathologies ranging from infectious disease to autoimmunity (*1*). However, taking the case of T cells as an example, a number of challenges quickly become evident: Isolation and preparation of large numbers of functional tumor-specific T cells are difficult in many types of cancer, natural T cells can lose function over time in the face of high tumor antigen burden (termed exhaustion), and tumors evolve diverse means to suppress attack by native lymphocytes (*1*). Such issues motivated the field early on to explore engineering of immune cells (*2*–*4*). Approaches to immune cell engineering include pharmacologic manipulation and genetic modification, which can be performed either ex vivo prior to infusion of the cell therapy or directly in the body. Genetic engineering has played a critical role in the development of clinically effective ICTs, with several important categories of modifications (Fig. 1A): (i) engineering of antigen receptors, including transgenic T cell receptors (TCRs) and synthetic antigen receptors termed chimeric antigen receptors (CARs); (ii) genetic modification of intracellular pathways that modulate natural properties such as metabolism, survival, and proliferation; and (iii) introduction of accessory genes that provide new functions to immune cells. ICTs are being developed based on T cells, macrophages, natural killer (NK) cells, and dendritic cells, derived from autologous patient-derived cells or "off the shelf" sources such as engineered cell lines or induced pluripotent stem cell (iPSC)–derived products (*1*, *5*). In parallel, important advances in immunobiology over the past 30 years have enabled this field, such as the discovery of key pathways mediating immune cell killing and dysfunction, definition of mechanisms underlying immunosuppression, and determination of factors that control successful immune cell engraftment (*3*). The first US Food and Drug Administration (FDA)–approved immune cell therapy product, a cell-based cancer vaccine (Provenge), was licensed in the US in 2010. Over the past 5 years, six CAR T cell therapies for hematologic malignancies and an engineered thymus tissue therapy for treatment of congenital athymia immunodeficiency have been approved in the US, and ICTs with tumor-infiltrating lymphocytes, as well as T cells engineered to express defined T cell receptors, are on the verge of approval. Furthermore, thousands of clinical trials of immune cell therapies in diverse diseases are currently underway, and the pace of discovery preclinically and in humans continues to accelerate.

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Clinical progress with engineered immune cell therapies

The first approved "gene therapy" in the US was a form of CAR T cell therapy (Kymriah, a CD19-targeting CAR T cell, approved in 2017). CARs are synthetic receptors composed of an antibody-like extracellular domain fused to a transmembrane domain and T cell activation and costimulatory domains. CAR expression endows T cells with specificity to a target antigen in a major histocompatibility complex (MHC)–unrestricted fashion, effectively initiating cytotoxicity, cytokine production, proliferation, and, in some cases, long-term memory formation (*2*). First-generation CARs had a single intracellular domain, composed of the CD3ζ chain or other signaling domains such as Fcγ; however, early trials showed that such CAR T cells had limited clinical impact (*6*), attributed to relatively short persistence or engraftment of the modified cells. Second-generation CARs included a costimulatory signaling domain derived from either CD28 or 4-1BB, and these CAR T cells directed to the CD19 antigen were shown to be effective in early trials in patients with B cell lymphomas and leukemias (*7*).

Over the ensuing decade, the clinical development of CAR T cells targeting CD19 progressed rapidly, with FDA approvals based on single-arm phase 2 clinical trials in different types of B cell malignancies using four different CD19-directed CAR T cell products (*7*). Tantalizingly, all these CD19-directed CAR T cell products result in durable remissions ("cures") in ~40% of patients with refractory or relapsed disease (*1*), and recent studies evaluating them in earlier lines of therapy have shown promise compared to standard high-dose chemotherapy. In addition, two CAR T cell products targeting the B cell maturation antigen (BCMA) have been tested and approved in multiple myeloma (*8*, *9*). Both of these BCMA-directed CAR T cell therapies resulted in high response rates in patients who progressed after multiple prior lines of therapy, though unlike in lymphoma, long-term "cures" have remained elusive.

In parallel to the development of CAR T cells, ICTs based on T cells transduced with transgenic T cell receptors (TCR T cells) have been pursued. TCR-T are generated by the identification of native or engineered TCRs that recognize peptides presented in the cleft of MHC molecules. Thus, in contrast to CAR T cells, TCR T cells can recognize peptides derived from mutated or overexpressed intracellular proteins, widening the space of potential antigen targets. TCR-T trials are at an early stage, but promising objective response rates have been seen with TCRs targeting tumor-associated antigens in melanoma and HPV antigens in HPV^+ epithelial cancers (*10*, *11*). CAR– and TCR–T cell therapies have complementary strengths and weaknesses—TCR T cells appear to have a lower prevalence of systemic toxicity (e.g., cytokine release syndrome) (*12*), and TCRs have high sensitivity, with the capacity to recognize a single ligand on a target cell (*13*). However, TCRs are restricted by requiring a specific MHC molecule that must be matched with the patient's MHC repertoire, limiting the number of patients that can be treated with any individual TCR product. Whether CAR– or TCR–T cell approaches will be more effective in addressing solid tumors remains to be determined.

T cell therapies have also entered clinical testing for diseases beyond cancer, though many of these studies are in very early stages. Preclinical studies indicated that the B cell

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A

APPROACHES	EXAMPLE FUNCTIONS	MAJOR ATTRIBUTES
Receptor engineering	Logic Switch	Safety Specificity
Genome engineering	Knockout Knockin	Efficacy Consistency
Payload coengineering	Deliver therapeutic proteins	Efficacy

B

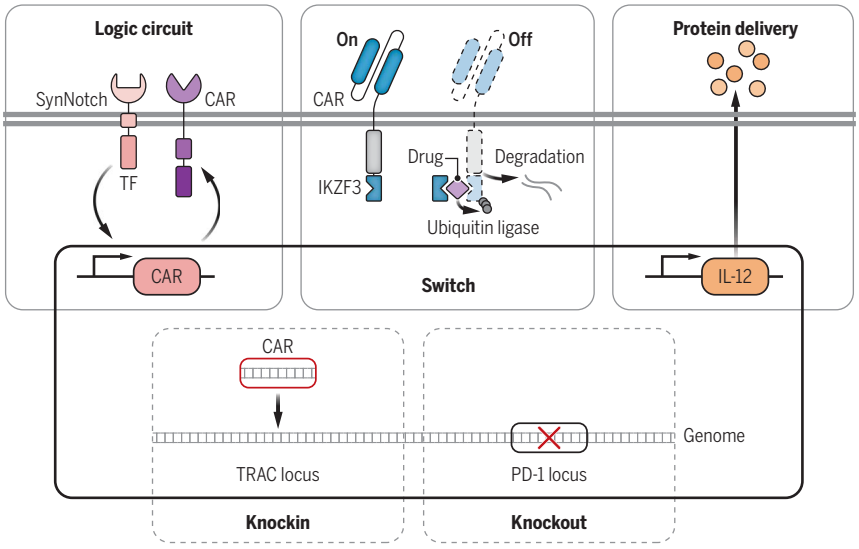

Fig. 1. Ex vivo immune cell engineering. (**A**) Major ex vivo immune cell engineering approaches and their key attributes. (**B**) Example systems of various engineering approaches. Logic circuit: Synthetic notch (SynNotch) receptor is composed of an extracellular binding domain, a notch core transmembrane domain, and a transcription factor (TF). The binding of the extracellular binding domain to a target antigen on another cell leads to the cleavage of the TF, which subsequently induces the expression of a CAR. The CAR is designed to bind to another antigen on the same target cell, forming an IF-THEN AND logic. Switch: A lenalidomide regulatable CAR is composed of a conventional CAR fused to a degron IKZF3. The binding of IKZF3 to lenalidomide recruits the endogenous ubiquitin ligase, leading to CAR degradation. Therapeutic protein delivery: Immune cells can be engineered to locally produce and secrete therapeutic proteins. Knockin: The specific integration of the CAR sequence into the T cell receptor α constant (TRAC) locus enables more uniformed CAR expression and better CAR T cell efficacy. Knockout: PD-1 is an inhibitory receptor that can limit immune cell therapy efficacy. PD-1 knockout can potentially lead to more potent immune cell therapy.

aplasia induced by CD19-targeting CARs could effectively treat murine models of B cell–mediated autoimmune disease (*14*), and an exploratory study that treated a patient with treatment-refractory lupus with a CD19 CAR T cell product led to a rapid drop in systemic autoantibody levels and disease remission (*15*). As an alternative approach to addressing autoimmunity, CARs created by replacing the antibody domain with an autoantigen enabled engineered T cells to eliminate autoreactive B cells in models of the skin disease pemphigus vulgaris (*16*); a phase 1 trial of this concept in patients is currently underway. Engineered regulatory T cells (T_{regs}) are also being developed for treatment of autoimmunity, transplant tolerance, and graft-versus-host disease (*3*). Examples include ongoing clinical trials of T_{regs} expressing a CAR in which the antibody domain is replaced by the class I MHC molecule human leukocyte antigen HLA-A2, with the goal of suppressing rejection of HLA-A2$^+$ kidney and liver transplants (*17*). Further cell engineering to stabilize the T_{reg} phenotype may be important for these approaches. In infectious disease, CARs generated with an HIV-1 broadly neutralizing antibody as the binding domain were recently tested in HIV$^+$ subjects and were shown to be safe, delayed viral rebound up to 10 weeks following temporary suspension of antiretroviral therapy, and temporarily reduced the viral reservoir (*18*). Further refinements of this therapy may provide an approach toward a functional cure of HIV.

Therapies based on other immune cells are also now entering clinical testing. In a first phase 1 trial, MHC-mismatched NK cells transduced with an anti-CD19 CAR, interleukin-15 (IL-15; a key cytokine to maintain NK cell survival in vivo), and a suicide gene (enabling deletion of the cells by a small-molecule drug in case of safety issues) were administered to 11 lymphoma and leukemia patients, leading to eight objective responses and seven complete remissions (*19*). Because these cells were prepared from third-party cord blood, these findings are a promising step toward an "off the shelf" ICT. Macrophages transduced with a CAR were recently demonstrated to phagocytose tumor cells and remodel the tumor microenvironment, polarizing bystander macrophages to an antitumor phenotype and recruiting T cells to treated tumors (*20*). A first-in-humans clinical trial of this concept is currently underway.

At the macro level, the field has grown substantially: There are now hundreds of companies developing new types of engineered T cells, using CARs, TCRs, and various new synthetic antigen receptors, and additional functionalities to enhance T cell functions. Currently, there are over 1000 clinical trials of just "CAR T cells" listed on clinicaltrials.gov. However, important biologic challenges have also been identified. Fundamentally, discovery and testing of tumor-specific targetable antigens remains a notable barrier, which may not be completely overcome by engineering strategies. Even in leukemias and lymphomas initially susceptible to CAR T cell treatment, loss or down-regulation of antigens targeted by the CAR T cells can lead to relapse. Beyond hematologic malignancies, small clinical studies have reported complete responses in patients with carcinomas (*11*) or brain cancers (*21*) treated with TCR- or CAR-engineered T cells, but, in general, responses to ICT in common solid tumors have been poor. Several barriers have been identified (Fig. 2), including inefficient tumor infiltration, heterogeneity of antigen expression, poor functional persistence or exhaustion of cells, and diverse mechanisms of immunosuppression including metabolic inhibitors (adenosine), checkpoint molecules (PD-L1), suppressive cytokines such as transforming growth factor–β (TGF-β) or IL-10, and various suppressive cell types (cancer-associated fibroblasts, myeloid-derived suppressor cells, and regulatory T cells) (*4*, *5*). Toxicity of immune cell therapies is a particular concern, especially with efforts to engineer amplified immune cell effector functions (*22*). Finally, although autologous T cells have a clear biological advantage in terms of safety and potential for long-term engraftment, there is considerable interest in developing a more "drug-like" model, using off-the-shelf, allogeneic immune cells to enable greater control of the input cell product, faster delivery to patients, and reduced cost of manufacturing. Ongoing efforts in ex vivo and in vivo cell engineering are underway to address each of

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these challenges. Here we highlight key examples of progress and challenges in the field.

Ex vivo cell engineering

Inspired by the complexity of biological networks, sophisticated engineered immune cells capable of sensing and logically responding to diverse stimuli with enhanced efficacy have been developed. From the clinical perspective, therapeutic immune cells are primarily designed with three main objectives: to improve (i) target specificity, (ii) efficacy, and (iii) safety. Regulatory, manufacturing, and commercial (e.g., cost) challenges are also beginning to influence cell therapies. From the ex vivo engineering standpoint, the clinical cell design objectives are typically achieved through three genetic and molecular engineering approaches, such as (i) receptor engineering, (ii) host cell genome engineering, and (iii) therapeutic payload coengineering (Fig. 1, A and B). These design objectives, which typically lead to a larger DNA footprint (more than the DNA size limit of a lentiviral vector, ~6 to 7 kilo–base pairs), demand innovation in gene delivery and manufacturing to ensure their successful clinical translation.

Antigen receptor engineering

Immune cell surface receptors are tolerant to many types of modifications and protein engineering, which has enabled design of receptor circuits with new sense-and-respond phenotypes to improve targeting specificity and safety or address antigen escape (*23*). One of the most-studied receptors in immune cell therapy is the CAR, which has been engineered to fine-tune signaling, introduce remote controls, and implement logic computation circuits. CARs incorporate both TCR and costimulatory receptor signaling domains that control T cell proliferation, effector function, and metabolic fitness (*24*, *25*); genetic screens are being pursued to identify optimal signaling domains for these synthetic receptors (*26*). Logic CAR circuits, in particular, underscore the engineering potential of CARs: The identity of target cells (e.g., cancer cells) is best classified by multiple antigens, and T cell therapies targeting a single marker have resulted in fatalities when the targeted antigen is also expressed by healthy tissues (*27*). Receptors that can sense multiple antigens and perform combinatorial logic operations can effectively discriminate between healthy and cancer cells, thus minimizing "on-target/off-tumor" effects (*28*). Such logic circuits can also be combined with receptors that sense factors in the tissue microenvironment rather than cell surface antigens, to aid in "decoding" tissue location of the engineered cell (*29*). Currently, three receptor platforms have demonstrated up to three input logic operations (*23*). These complex logic operations are very difficult, if not impossible, to achieve with other therapeutic modalities, such as small molecules or engineered proteins, thus highlighting the potential that cell therapy has to offer.

As stated above, an advantage of CARs over TCRs is their ability to target any surface antigen independent of patients' MHC haplotype. However, compared to TCRs, CARs tend to have a lower antigen sensitivity. In an effort to marry the strengths of CARs and TCRs, recently hybrid receptor designs have been described. TruCs (T cell receptor fusion constructs) link antibody domains to different components of the TCR complex and showed a similar antigen sensitivity to that of CARs while lowering inflammatory cytokine production (*30*). STARs [synthetic TCR and antigen receptors (*31*)] and HITs [HLA-independent TCRs (*32*)] are synthetic receptors with antibody binding domains fused to the native TCR receptor constant regions. These new receptors have shown pronounced in vivo activity and antigen sensitivity.

Although many new antigen receptor and advanced logic receptors are still undergoing preclinical testing, CARs designed to mitigate relapse owing to antigen escape have already been evaluated in initial clinical trials. These studies showed that OR gate CARs (CARs that can be triggered by binding to one of two different target antigens) were safe, but relapses were still observed in a proportion of patients owing to poor CAR T cell persistence or unequal potency of the two CARs employed (*33*–*35*). Some AND-gate CAR systems (CARs that are only activated when two different target antigens are engaged simultaneously) have demonstrated high specificity in multiple preclinical tumor models (*36*), and these systems are primed for testing in clinical trials. Several companies have also demonstrated promising preclinical results with NIMPLY CAR circuits (CARs activated by the presence of one or more antigens in the absence of a third antigen) and are preparing for trials against solid and blood tumors.

Therapeutic payload coengineering

In addition to expressing an antigen-specific receptor, engineering immune cells to express therapeutic payloads provides an additional dimension for modulating cell function. When combined with CAR expression, they are sometimes referred to as "armored" CARs or TRUCKs (T cells redirected toward universal cytokine killing) (*37*). Some of the most promising therapeutic payloads are secreted factors such as cytokines (e.g., IL-12, IL-15), therapeutic antibodies (e.g., anti-PD-L1), or enzymes that can remodel the tumor microenvironment or activate prodrugs (*37*, *38*). As such, ICTs can also serve as a living drug delivery device. Examples include NK cells engineered to express IL-15, a critical cytokine for NK cell survival (*19*), and myeloid cells transduced to express IL-12, which can counter the immunosuppressive gene signature found in solid tumors (*39*). Though potent, many of these factors have substantial side effects that require careful regulation for safe deployment. One approach

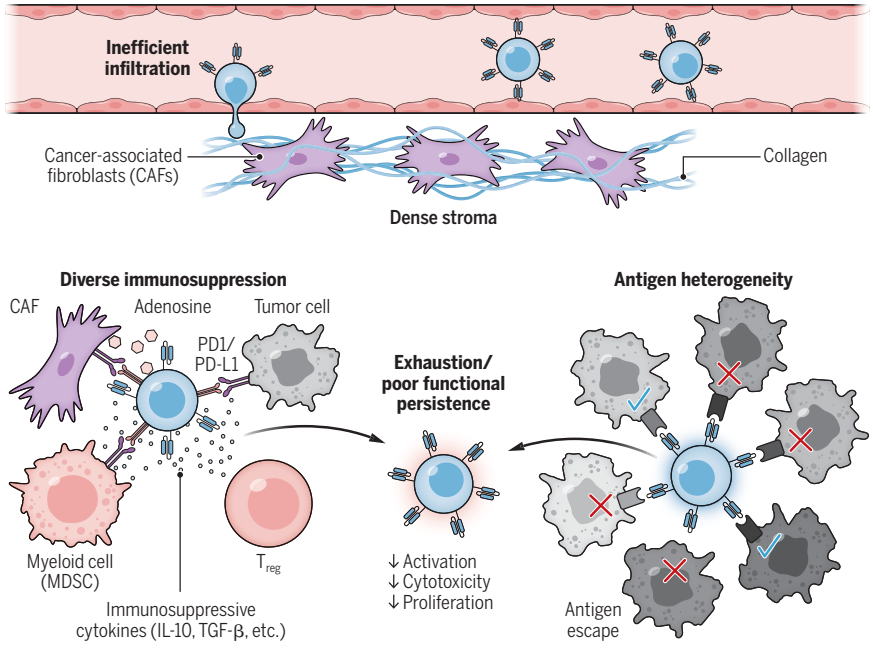

Fig. 2. Barriers to the effectiveness of engineered T cells in solid tumors. Several barriers to eradication of solid tumors by immune cell therapies have been identified, including inefficient migration of T cells from the blood to reach viable tumor cells (limited in some cases by dense tumor extracellular matrix), many mechanisms of tumor-induced immune suppression, loss of T cell function due to persistent engagement with antigen (exhaustion), and tumor escape due to antigenic heterogeneity.

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is to engineer soluble factors such as cytokines to be expressed on the plasma membrane, to restrict their dissemination and focus signaling on the donor cell (*40*). Another strategy is to design inducible gene switches to tune the strength and timing of accessory payload production. Many mammalian gene switches have been developed, but most are incompatible with ICTs because they either are derived from nonhuman origins, have a large DNA footprint, or use inducers that are not clinically approved or have poor or undefined pharmacokinetic properties; a promising exception is a lenalidomide-based switch (*41*). A flexible platform, such as those based on human zinc-finger proteins (*42*) and using clinically approved drug inducers, could help to optimize immune cell therapy.

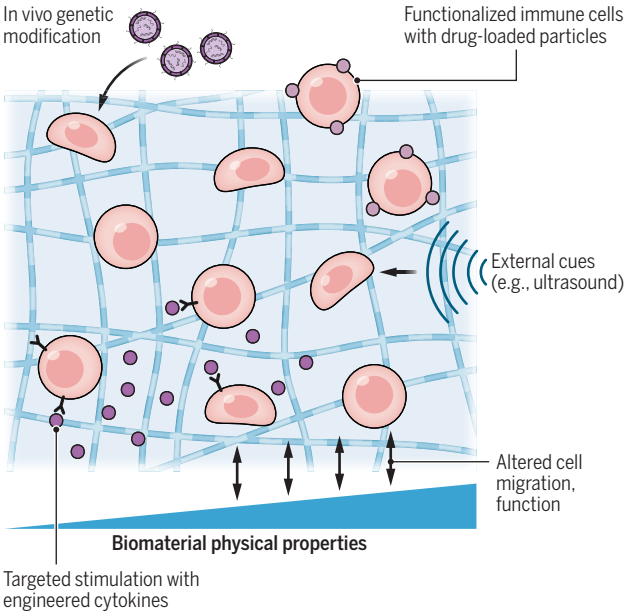

Fig. 3. In vivo immune cell engineering approaches. Cell transfer can be aided by biomaterial delivery vehicles that localize immune cells to specific anatomic sites and provide biochemical cues (e.g., cytokines) or mechanical properties that promote desirable cell phenotypes. Transferred cells can also be manipulated through externally applied signals such as ultrasound or magnetic fields. Alternatively, host or transferred immune cells may be genetically altered in situ, through viral or nonviral strategies, bypassing ex vivo cell manipulation and transfer.

Genome engineering

Beyond expression of tumor-specific receptors and immunomodulatory factors, recent work has demonstrated the potential of engineering the immune cell genome to enhance cell therapy safety and efficacy. Several genes, such as checkpoint receptors (e.g., PD-1), are known to inhibit ICTs. Furthermore, the TCR and MHC from donor cells can cause graft-versus-host disease or rejection if the immune cell source is allogenic. Genetic disruption of these genes in immune cells has enhanced their ability to combat tumors and prevent immunogenicity related to the use of allogeneic cell sources in preclinical models. Moreover, many knockout screens have been performed on immune cells to identify genes (*43*), such as RASA2 RAS guanosine triphosphatase–activating protein (*44*), or RNA helicase Dhx37 (*45*), which, when deleted, promote T cell persistence and activity against cancer. We are now waiting to see which of these genetic perturbations have the most impact in the clinic.

Currently, genetic modification of immune cells for clinical application utilizes engineered viruses, which randomly integrate payload DNA into the genome of donor cells, presenting the possibility of causing unregulated cell growth and cancer. Targeted integration at defined genomic locations is an attractive alternative approach to ensure the generation of safe and consistent ICTs. Furthermore, genome editing enzymes such as CRISPR-Cas can be introduced by electroporation as a ribonucleoprotein (RNP) complex with high efficiency, removing the possibility that the genome editing enzyme will persist for a long duration. Moreover, integrating CARs directly into the native genetic locus of the TCR complex can improve CAR T cells' efficacy, probably because of the more favorable expression dynamics afforded by the TCR promoter (*46*). In a recent clinical trial with eight patients, T cells with a CD19 CAR integrated into the PD-1 locus using CRISPR-Cas have demonstrated an 87% complete remission rate with mild CRS in some patients and no immune effector cell–associated neurotoxicity syndrome (*47*). This result underscores the potential of using CRISPR-Cas to manufacture CAR T cell products with precise genome placement of the cargo DNA.

One of the major limitations in targeted integration is the need to deliver a sequence template prescribing the insertion site. The most efficient approach to deliver such DNA is by using adeno-associated virus (AAV). However, AAV is expensive and complicated to manufacture. Codelivery of double-stranded DNA templates with the CRISPR-Cas RNP is toxic to primary cells. Recently, a method using single-stranded DNA achieved 62% knock-in efficiency with a high yield (more than a billion cells) (*48*). Although these new genome editing technologies can provide precise manipulation of the immune cell genome, they can have off-target effects or exhibit errors in genome rearrangement; more research is needed to precisely determine the risk of these editing errors.

Immune cell manufacturing

Challenges in immune cell therapy manufacturing are well documented (*49*), which has hampered their clinical and commercial potential. Beyond the logistics around implementation, such as maintenance of chain of custody, one of the biggest challenges in ICT production is the use of viral transduction to deliver DNA payloads, a complex process with a high failure rate. To overcome this challenge, nonviral approaches to gene delivery in primary human immune cells, such as mRNA transfection or transposon-based genome engineering tools, are being developed (*47*, *50*). Cell source variability is another challenge, especially when using patient-derived cells. The medical history or the stage of the patient's disease can result in T cell dysfunction and alter immune cell composition, leading to suboptimal T cell products during the manufacturing process (*51*). Many resources have been devoted to developing allogeneic "off-the-shelf" cell products, either through alternative cell types (e.g., NK cells, iPSC–derived immune cells) or via genome editing tools to disrupt proteins that lead to allogeneic rejection (e.g., β_2 microglobulin) and graft-versus-host disease (e.g., endogenous TCRs) (*37*). However, these approaches have their own particular sets of challenges. For instance, NK cells are less amenable to viral transduction than T cells, and the regulatory issues related to genome editing specificity have yet to be resolved.

In vivo cell engineering

Upon adoptive transfer, engineered immune cells face a number of challenges in finding and destroying their target, including surviving for a sufficient time frame, homing to the appropriate anatomic site and engaging the target cell, and maintaining an appropriate phenotype to allow cancer clearance. Both passive and active barriers in the host are responsible for these challenges, and a variety of technologies are under development to overcome and enable efficient and safe immune cell therapy. These technologies include materials used to place transferred immune cells at the desired site, targeting of transferred cells by externally controlled cues, and direct genetic manipulation in the body (Fig. 3).

Targeted stimulation of transferred cells

Achieving an appropriate balance of therapeutic efficacy and safety is increasingly being pursued with strategies providing targeted, in vivo stimulation of the transferred cells. These approaches often involve pharmacologic manipulation to allow remote, temporal control over transcriptional activity, or alterations of

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the cells' microenvironment to achieve greater spatial control over the activity of transferred and host immune cells. For example, the favorable bioavailability and pharmacokinetics of FDA-approved small-molecule drugs are being exploited to control genetic on/off switches with rapid, reversible dynamics to tune the functionality of engineered T cells. This allows (for example) tight control over the activity of CAR T cell therapy while reducing exhaustion in preclinical studies and also provides a safety switch allowing cell activity to be turned off at toxicity onset (*52*). Activating factors such as cytokines and costimulatory ligands play a critical role in the function of immune cells, but systemic delivery of these potent, pleiotropic molecules often results in side effects, which has motivated several strategies to deliver these cues in a controlled manner to ICTs. For example, cytokines are being packaged into nanoparticles and microparticles that can be adhered to immune cells before transfer, or targeted to immune cells in vivo by metabolic labeling, to enable cytokine activity to be localized to the desired therapeutic site with minimal systemic exposure (*53*, *54*). Cytokine receptors have also been engineered to allow donor T cells (but not native endogenous cells) to respond specifically to engineered cytokine drugs administered in tandem, or to engineer distinctive intracellular signals in response to cytokine stimulation (*55*). Engineered T cells can also be stimulated specifically with vaccines that activate their synthetic antigen receptor, promoting cell expansion, survival, and effector functions (*56*, *57*). Extracellular vesicles, lipid bilayer–enclosed particles derived from cells, provide a potent intracellular communication pathway and are also being engineered to elicit specific immune responses (*58*). The physical microenvironment of transferred cells is also being manipulated to alter their activity: For example, CAR T cells engineered to be heat sensitive can be stimulated with local temperature increases mediated by external cues applied to the tumor (*59*, *60*). It is also becoming increasingly clear that the mechanical properties of tumors (e.g., stiffness) can be a barrier to immune cell and therapeutic transport and function; thus, modulation of mechanosensing provides a means to alter immune cell activity in vivo (*61*).

Engineered systems to modulate immune cells

Biomaterial strategies provide another opportunity to enhance ICT efficacy, manipulate immune cells directly in the body, and/or synergize host immunity and adoptive cell therapy. Biomaterials can protect molecular and cellular cargoes in harsh environments, localize therapies to a desired anatomic location, and control the trafficking and activation of host cells. The poor solid tumor localization of transferred T cells and exhaustion of tumor-resident T cells have motivated the fabrication of biomaterial carriers that provide continuous stimulation (e.g., TCR stimulation, cytokines) to cargo T cells after transplantation, allowing their direct placement in the vicinity of tumors and maintaining desirable phenotypes (*62*). Antigen-presenting cells (APCs) have also been engineered, using biomaterial scaffolds designed to accumulate large numbers of APCs in situ, load them with sustainably released antigen, and stimulate their migration to draining lymph nodes by activation with codelivered adjuvants (*63*). A wide array of nanoparticles have also been developed to traffic antigen and adjuvant directly to lymph nodes, demonstrating robust, specific immune responses (*64*).

Direct gene modification in vivo

Engineering cells in culture before transfer is expensive, is complex, and requires considerable time, motivating approaches to genetically modify host cells in situ to bypass ex vivo manipulation (*65*). CAR-encoding retroviral particles have been incorporated into implantable scaffolds used to transfer human immune cells, leading to CAR T cell generation in vivo (*66*). Bypassing any ex vivo cell manipulation, lentiviruses and polymer nanoparticles are being developed to specifically target T cells, and single infusions have demonstrated the ability to transduce sufficient host cell numbers to generate effective CAR T cell responses in humanized mouse cancer models (*65*). In vivo transduction of T cells and other immune cells is now an intensive area of research and may be critical for the long-term success of cell therapy.

Conclusions and future outlook

The science and technology of engineering immune cells for therapeutic purposes has made great progress, but there remains an enormous space for exploration to find safer and more effective immune cell–based treatments. In addition, new ways of applying ICTs to treat disease are being discovered, such as the use of CAR T cells to block fibrosis during heart failure (*67*). Notably, human trials of immune cell therapies are providing important insights into the function of the immune system in health and disease, leading to discoveries such as the identification of genes that regulate lymphocyte survival and function (*68*).

Two critical challenges facing the long-term utility of immune cell therapies in medicine include their cost and complexity of manufacture. In some settings, successful development of injectable drug alternatives [e.g., use of bispecific T cell engager proteins for treatment of hematologic malignancies (*69*)] may eventually supplant the use of ICTs if shown to be equivalently effective. However, the ability of immune cells to be chemically and/or genetically engineered with multiple environment-responsive, controllable functions not present in native cells gives ICTs the potential to enact changes in the disease microenvironment that cannot be achieved by traditional therapeutics. There are ongoing clinical and regulatory efforts to explore decentralized, faster, and automated manufacturing to increase efficiency and reduce costs (*70*). In addition, intensive efforts focused on engineering ICTs directly in vivo or to use engineered "off the shelf" third-party cell sources may eliminate some or all of the practical issues surrounding cell therapy.

To enable engineered immune cell therapies to reach their full potential, a diverse scientific effort is needed. Exciting progress is currently being made through collaborative efforts cutting across immunology, oncology, synthetic biology, and molecular biology. Combination therapies employing the expertise of protein, chemical, materials, and biological engineers may open new ways to enhance immune cell therapies that cannot be achieved by genetic engineering alone. As data from human trials continues to expand, there is also scope for a substantial impact of computational modeling to make predictions of key parameters to be optimized in cell therapy and machine learning–based data analysis. Finally, the intersection of ICTs with the larger systems biology of the immune system must be accounted for—interactions of cell therapies with the endogenous immune system, the nervous system (*71*), and the role of the microbiome (*72*) in cell therapy outcomes are areas that may hold important new discoveries.

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