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Genome Engineering for Next-Generation Cellular Immunotherapies

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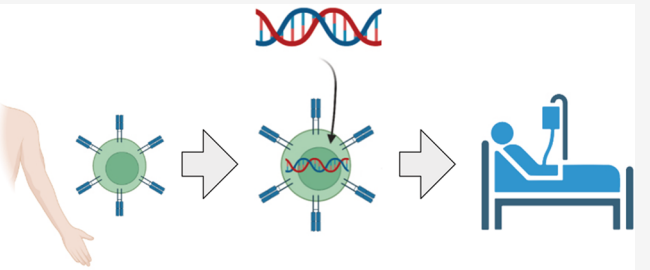
ABSTRACT: Over the past decade, cellular immunotherapies such as CAR-T, TCR-T, and NK cell therapies have achieved tremendous success in cancer treatment. However, various challenges and obstacles remain, including antigen escape, immunosuppression in the tumor microenvironment, toxicities, and on-target off-tumor effects. Recent strategies for overcoming these roadblocks have included the use of genome engineering. Multiplexed CRISPR-Cas and synthetic biology approaches facilitate the development of cell therapies with higher potency and sophisticated modular control; they also offer a toolkit for allogeneic therapy development. Engineering approaches have targeted genetic modifications to enhance long-term persistence through cytokine modulation, knockout of genes mediating immunosuppressive signals, and genes such as the endogenous TCR and MHC-I that elicit adverse host−graft interactions in an allogeneic context. Genome engineering approaches for other immune cell types are also being explored, such as CAR macrophages and CAR-NK cells. Future therapeutic development of cellular immunotherapies may also be guided by novel target discovery through unbiased CRISPR genetic screening approaches.



Cell therapies have expanded new frontiers in oncology. By harnessing our immune system to eliminate cancer, adoptive cell therapies (ACTs) effectively function as "living drugs" with unique pharmacological characteristics, including the ability to migrate, localize, and proliferate in the target tissue.[1] In recent years, cell therapies have seen an explosion of interest in both clinical translation and scientific research, heralded by the U.S. Food and Drug Administration (FDA) approval of tisagenlecleucel and axicabtagene ciloleucel for treatment of acute lymphocytic leukemia and diffuse large B-cell lymphoma, respectively.[2] Advances in genome-editing technologies such as clustered regularly interspaced short palindromic repeats (CRISPR) have also helped facilitate much of the rapid development in ACT.[3]

There are several approaches to immune cell therapies, including isolation and expansion of tumor-infiltrating lymphocytes (TILs), engineering T cell receptor transgenics (TCR-Ts), and engineering chimeric antigen receptor T cells (CAR-Ts). Natural killer (NK) cells, macrophages, and other innate immune cells are also currently being explored for their therapeutic potential (Figure 1). CAR-T cells, which have been a particular focus for ACT development, are T cells genetically modified to express a chimeric antigen receptor (CAR) comprising antigen binding, hinge, transmembrane, and intracellular signaling domains. The extracellular domain confers antigen specificity, allowing the CAR-T cell to target specific tumor cells, and is typically a single-chain variable fragment (scFv) derived from the heavy and light chains of a monoclonal antibody. The functions of the intracellular signaling domains, which have received much consideration in recent engineering strategies, include activation and co-stimulation of the CAR-T cell, which are essential for a durable response. However, various challenges and obstacles remain in CAR-T cell therapeutic development with respect to safety, efficacy, and cost.[4] Subsequent sections of this Perspective will discuss how novel engineering approaches help address these issues, for example, through improvements to the design of CAR constructs as well as gene knockouts to confer improved tumor infiltration, tumor killing, and reduced exhaustion.

GENOME-EDITING TOOLS FOR CELLULAR THERAPIES

By enabling engineering modifications to circumvent these limitations, next-generation genome-editing technologies are helping realize the promise of immune cell therapies (Figure 2). These tools enable direct, specific modifications of DNA sequences and include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and, most recently, CRISPR.[3] The capability of CRISPR to introduce facile genetic modifications into primary T cells, coupled with its advantage over ZFNs and TALENs, which require protein engineering, naturally led to cell therapy applications, and

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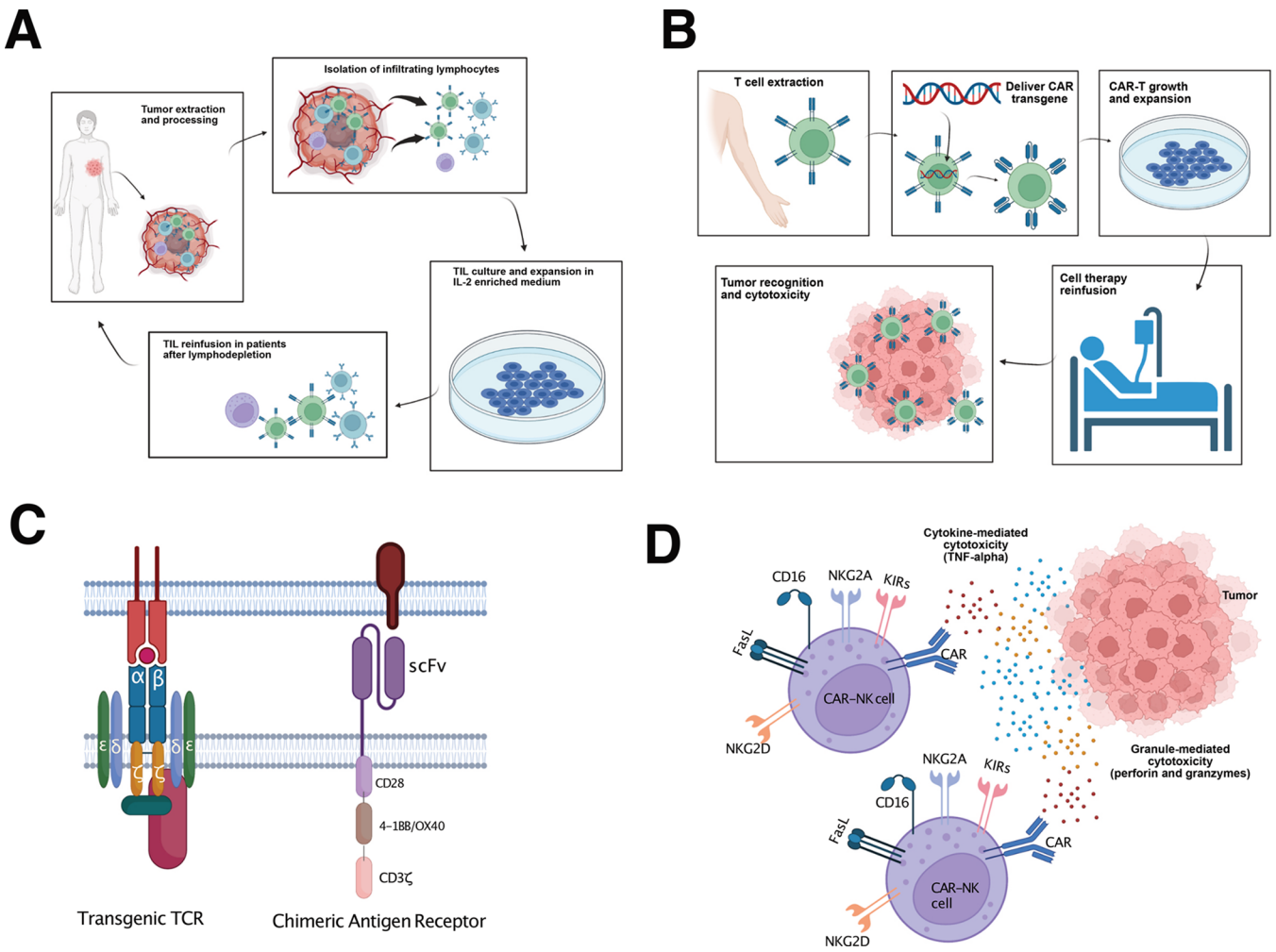

Figure 1. Overview of cellular immunotherapies. (A) TIL therapy is based on harvesting antigen-specific T cells that have already infiltrated tumors, activating and expanding them *ex vivo*, and then re-infusing them into patients. (B) Autologous CAR-T cell therapy typically involves extraction of T cells from peripheral blood, followed by CAR gene transfer and re-infusion. (C) TCR-Ts typically involve delivering transgene-containing, clonally selected, cancer antigen-specific TCRs to T cells that have been isolated from peripheral blood. Unlike CAR-T cells, TCR-T cells can act in an MHC-dependent manner, which allows them to target intracellular antigens but makes them susceptible to poorer recognition in the context of downregulation of antigen processing and presentation in cancer cells. (D) CAR-NK cell therapies are emerging as a complementary therapeutic modality to CAR-T cells, as CARs engineered into NK cells for allogeneic therapeutic development can circumvent issues in CAR-T cells such as GVHD.

studies soon demonstrated its potential for engineering with enhanced precision, efficiency, and efficacy. One landmark study demonstrated that CRISPR-Cas9 could be used to direct a CD19-specific CAR to the native T cell receptor α-constant (TRAC) locus, which was found not only to knock out the TCR but also to enhance the potency and stability of the CAR-Ts.[5] Another study showed that short single-stranded oligodeoxynucleotide homology-directed repair templates could be used to replace endogenous TCR loci with tumor-specific TCRs for nonviral CRISPR-Cas9 genome targeting.[6] Non-Cas9 CRISPR proteins such as the tracrRNA-independent Cpf1, which can mediate DNA cleavage at multiple targets using a single customized CRISPR array, have also been harnessed; Cpf1 and adeno-associated virus (AAV) enabled a knock-in of two CARs in the same T cell in one step, illustrating how CRISPR can be used for modular engineering.[7] These and other studies have contributed to the growing body of preclinical evidence that precise genome editing with CRISPR can be used for therapeutic engineering of primary human immune cells.

However, CRISPR-Cas9 genome editing has limitations, including off-target editing and large-scale genomic rearrangements.[8] Because CRISPR proteins such as Cas9 induce double-stranded DNA breaks (DSB), which often result in unintended insertions and deletions and chromosomal rearrangements, advances in genome engineering methods to circumvent these issues have been explored in the context of immune cell therapy development.[9] Base-editing technologies in particular have shown promise as the next frontier for highly specific gene knockouts in T cells. Base editing allows the introduction of point mutations into the DNA without double-stranded breaks or the need for a template of exogenous DNA. Cytidine base editors (CBEs) allow C → T conversions, and adenine base editors (ABEs) allow A → G conversions through a deaminase enzyme that carries out the desired chemical modification of the target DNA base.[10] In one noteworthy study, base editing was used to generate fratricide-resistant T cells by removing TCR/CD3 and CD7 ahead of lentiviral-mediated expression of CARs.[11] While chromosomal trans-

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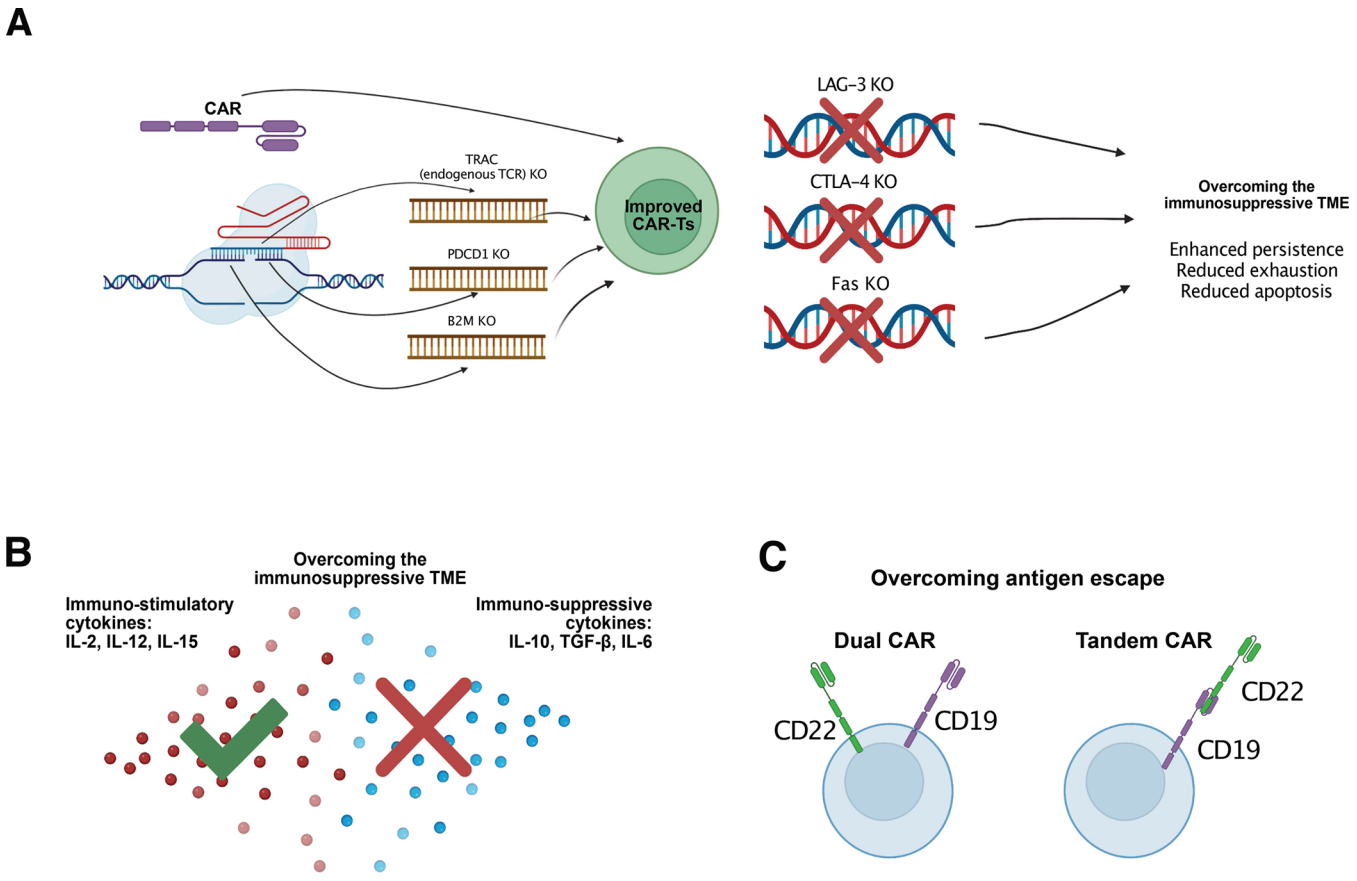

Figure 2. Engineering strategies for next-generation CAR-T cells. (A) Multiplex CRISPR-Cas9 editing to enable the introduction of increasingly sophisticated phenotypes during CAR-T cell generation, including for enhanced effector function, weakened off-target effects, resistance to exhaustion, and allogeneic therapy designs. (B) Cytokine modulation in CAR-T cell engineering as a strategy for overcoming immunosuppressive TME. (C) Dual and tandem CARs as mechanisms for overcoming antigen escape.

locations are commonly detected in Cas9-treated cells, base-edited T cells remained free of these adverse effects.

IMPROVING SAFETY BY INCREASING THE TARGET SPECIFICITY AND MITIGATING TOXICITIES

To improve the safety of CAR-T cell therapies, the ability of CAR T cells to selectively exert cytotoxic effects on cancer cells without harming healthy cells is of great interest. Targeted antigens are often found in both tumor and healthy cells, and as a result, CAR-T cells targeted to these surface molecules may kill indiscriminately both cancerous and healthy tissue, a phenomenon known as "on-target off-tumor" effects. One strategy for preventing the on-target off-tumor effects is using a masked CAR construct, whereby a masking peptide, connected to the CAR by a protease-sensitive linker, blocks the CAR from targeting normal tissue outside of the tumor microenvironment (TME).[12] Once the CAR-T is in the TME, proteases—which are abundant in the TME as they mediate cancer metastasis—will cleave the masking peptide and unleash the cytolytic power of CAR-T cells.

The use of "Boolean logic gates" to enhance the target specificity of CAR-T cells is another approach for constructing a design that can help distinguish tumor cells from healthy cells. One example of a CAR with a Boolean "AND" gate is a dual CAR design consisting of two extracellular domains with specificities for different antigens, each coupled to separate components of the intracellular stimulatory apparatus.[13] This design is characterized as an "AND" gate because a combination of antigens are required for activation of the CAR-T cell.[14] Other examples of Boolean AND gates are synNotch-CAR circuits, which operate via a two-step mechanism. First, the synNotch receptor binds a tissue- or tumor-specfic antigen to locally prime expression of a CAR. Once the CAR is expressed, the CAR-T cells can begin killing tumor cells in the setting of both cognate antigens.[15] These studies highlight how multiantigen targeting and synthetic biology approaches can improve the efficacy and safety of cell therapies.

Moreover, crucial to enhancing the safety of CAR-T cell therapies is mitigating toxic side effects. One of the severe side effects of CAR-T therapies is cytokine release syndrome (CRS), a highly prevalent side effect among patients who receive CAR-T cell therapy. Among B-NHL patients receiving CAR-T cell treatment, anywhere from 42% to 93% experienced CRS and 2−22% experienced grade >3 CRS.[16] The cytokines released into the bloodstream by infused CAR-T cells can cause high fever and hypotension, which is potentially life-threatening for patients. Although steroids and IL-6 blockers such as tocilizumab can be used to control CRS, the need to constantly administer these suppressive molecules is less desirable than having control mechanisms at the CAR level itself. Given that CAR-related toxicities often arise acutely, genetic engineering approaches are being explored to mitigate these adverse events in CAR-T cell therapy.

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Human studies have shown that hinge and transmembrane domains of the CAR are strong determinants of cytokine release.[17] Replacing the CD28 hinge and transmembrane domain with the hinge and transmembrane domain of CD8a significantly decreased cytokine levels while maintaining a similar level of antitumor response.[17,18] Furthermore, this redesigned CAR exhibited a lower level of activation-induced cell death, which greatly enhanced CAR-T persistence *in vivo*. The results of preliminary clinical trials for these redesigned CAR-T cells have shown improved safety for patients.

Another common side effect of CAR-T therapies is neurotoxicity, resulting in severe phenotypes such as impaired speech and seizures. Although the exact cause of immune effector cell-associated neurotoxicity syndrome is still unclear, it is suspected to be related to on-target off-tumor effects.[19] Solid tumor antigens targeted by CAR-T therapies are often expressed in normal tissue at some level, leading to collateral damage and toxicity by CAR-Ts.

The ability to regulate CAR-T cell activity by inducible inactivation or elimination has become important as further research has detailed the toxic and adverse side effects of CAR-T cells. Inducible inactivation of CAR-T cells may be promising for controlling and mitigating adverse events such as CRS and neurotoxicity. Especially in the context of allogeneic CAR-T cell development, which carries the risk of graft versus host disease (GVHD), the ability to rapidly "turn off" or remove CAR-T cells upon the onset of GVHD is critical.

Classic "off switch" strategies typically involve the use of a suicide gene for selective and specific elimination, such as with inducible caspase 9 (iCasp9),[20−22] herpes simplex virus tyrosine kinase (HSV-TK),[23,24] or human thymidylate kinase (TMPK).[25] The incorporation of the iCasp9 suicide gene into a CAR construct has been clinically validated in patients receiving haploidentical stem cell transplants, where the iCasp9 system was used to rapidly remove the CAR-T cells in patients with GVHD onset.[26] Alternatively, co-expression of cell-surface elimination markers in the CAR construct can allow for antibody-mediated degradation of CAR-T cells. One study showed how CD4CAR-T cells can be systematically depleted using alemtuzumab as a natural safety switch to prevent toxicities from CD4 T cell aplasia.[27] The development of an "off switch" that is directly embedded within a CAR construct has been proposed to address cellular autoactivation and T cell fratricide.[28] Via incorporation of a self-cleaving degradation moiety controlled by a protease/inhibitor pair, the CAR acquires a tight, reversible control system for its cell-surface expression and subsequent CAR-induced signaling and cytolytic functions. Control via lenalidomide-induced degradation with an on/off switch has also been explored. Upon activation of the on switch, CAR-T cells demonstrated lenalidomide-dependent antitumor activity, while off switch degradable CARs were successfully depleted.[29] These and other studies demonstrate how engineering genetic circuits and the CAR design itself can lead to a higher degree of control over ACT for improved safety and weakened side effects.

IMPROVING EFFICACY BY OVERCOMING CELLULAR EXHAUSTION, IMMUNOSUPPRESSIVE TME, AND ANTIGEN ESCAPE

In addition to addressing safety concerns of CAR-T cell therapy, improving its efficacy by mitigating cellular exhaustion and antigen escape are critical research objectives. T cell exhaustion is a state characterized by poor effector function, sustained expression of inhibitory receptors, and a transcriptional profile that differs from those of effector or memory T cells.[30] This state of cellular dysfunction has proven to be a major barrier to the clinical efficacy of CAR-T cell therapy. As interest in bioengineering T cells to express CARs has grown, an increasing need has emerged for CAR constructs that achieve sustained T cell activation via reduced exhaustion and potent cytotoxicity against tumor cells. CAR-T cells engineered to overexpress c-Jun, a canonical AP-1 transcription factor, demonstrated resistance to exhaustion.[31] Reducing T cell exhaustion is also facilitated by removing T cell inhibitory receptors or signaling molecules, such as CTLA-4, PD-1, LAG-3, and TIM-3, which act as "off signals" to limit T cell activity. Disruptingrelated genes in CAR-T cells has yielded robust antitumor activity with reduced exhaustion. For example, CRISPR-Cas9-mediated *LAG3* knockout in CAR-T cells exhibited robust tumor killing *in vitro* and *in vivo*, illustrating the potential for harnessing knockout of immune checkpoint molecules to reduce CAR-T cell exhaustion and thus potentiate tumor killing with a durable response.[32]

Immune checkpoint-based approaches can also be harnessed directly in the delivered transgenes. Though checkpoint blockade is traditionally associated with monoclonal antibody-targeted approaches, engineering checkpoint-based CAR constructs is promising for offering CAR-T cells as a monotherapy that combines multiple mechanisms of antitumor immunity. Modifying CAR-T cells to secrete PD-1 blocking single-chain variable fragments (scFv) has been demonstrated to boost CAR-T cell function in an immunosuppressive tumor microenvironment.[33] Administering CAR-T cells engineered to secrete PD-1 blocking scFvs showed efficacy in mouse models comparable to or better than that achieved by combination therapy with CAR-T cells and a checkpoint inhibitor. The study also highlighted the potential of this novel construct to improve safety, as the secreted checkpoint blocking scFvs remain localized to the tumor, avoiding the toxicities of systemic checkpoint inhibition.

Another factor that has limited the success of CAR-T cells is tonic signaling, which induces T cell exhaustion due to noncoordinated and sustained activation. To address tonic signaling, one study combined the specificity of a CAR and the internal signaling machinery of an endogenous TCR through a double-chain chimeric receptor termed synthetic T cell receptor and antigen receptor (STAR).[34] This engineered construct incorporates an antigen-recognition domain of an antibody and constant regions of TCR that engage endogenous CD3 signaling machinery, which can be leveraged to decrease the level of tonic signaling. STAR-T cells were also shown to proliferate better than traditional 28zCAR-T cells, exhibit higher antigen sensitivity, and elicit potent antitumor function. Higher antigen sensitivities displayed by STAR-T cells have important implications for the treatment of antigen-low tumors.

The efficacy of CAR-T cells can also be augmented through receptor and cytokine modulation. Multiplex CRISPR-Cas9 editing has been used to directly target the inhibitory genes PDCD1, PD-1, and death receptors CD95/Fas to circumvent the inhibitory signaling in the immunosuppressive tumor microenvironment (TME) that contributes to low CAR-T persistence.[35−37] Prolonging the persistence of CAR-T cells can also be achieved by engineering populations with less differentiated subsets, such as central memory T cells and stem

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cell memory T cells. Because IL-15 is fundamental to T cell memory, one study engineered a membrane-bound chimeric IL-15 cytokine-fusion molecule for co-expression with CAR.[38] These mbIL15-CAR T cells demonstrated memory-like phenotypes and improved T cell persistence and achieved potent cytotoxic activity against CD19+ leukemia cells. CAR-T cells have also been engineered to induce IL-12 signaling within the target tumor, maximizing NK cell cytotoxicity to amplify antitumor immunity.[39] In this study, IL-12 was inserted into the extracellular moiety of a CD28-ζ CAR and tumor recognition and killing were augmented not only in cancer cells with the CAR cognate antigen but also in antigen-negative cancer cells, thus achieving an NK cell-like phenotype of nonspecific, broad antitumor activity.

Examples of signal modulation for improving the function of CAR-T cells also include the generation of CAR-T cells that express IL-7 and CCL19 to enhance tumor infiltration and CAR-T cell survival. These 7 × 19 CAR-T cells demonstrated complete regression of solid tumors and prolonged survival in mouse models.[40] CAR-T cells overexpressing CXCR1 or CXCR2 have also been found to enhance the migration and persistence of CAR-T in solid tumors, improve antitumor efficacy, and establish long-lasting immunologic memory.[41] CARs have also been designed to be resistant to immunosuppressive factors such as TGF-β.[42] One study employed CAR-T cells engineered to produce the RNA agonist RN7SL1 to activate RIG-I signaling, which promoted the expansion and effector memory differentiation of CAR-T cells. These cells also suppressed differentiation of immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs) and activated myeloid cells and dendritic cells, which altogether help to lessen the immunosuppressive nature of the TME.[43] These and other studies demonstrate that engineering novel CAR-T cell constructs can improve the function of cellular therapies through modulation of a variety of signaling mechanisms.

Finally, overcoming antigen escape is crucial for enhancing the efficacy of CAR-T cell therapy, especially in the solid tumor context. Antigen escape is characterized by a gradually diminishing level of expression of the target antigen in tumor cells, which results in diminished efficacy of tumor killing. The heterogeneity and mutability of cancer cells have enabled them to evolve under the selective pressure from CAR-T cell therapy. According to a follow-up study on CAR-T therapy for B-cell acute lymphoblastic leukemia, 24% of the patients who had a complete response to the treatment encountered relapse with cancer that completely lost CD19 expression.[44]

One strategy for addressing antigen loss is to target multiple antigens at the same time, through either dual CAR constructs or tandem CARs that contain two scFvs in a single CAR construct.[45,46] In preliminary clinical trial results, dual CAR-T cells targeting CD19 and CD22 demonstrated great efficacy in treating acute lymphoblastic leukemia, illustrating the promise of multiple targeting as a strategy for antigen escape.[47] Tandem CARs have also been used to target both EphA2 and IL13Ra2, demonstrating superior efficacy in a glioblastoma patient-derived xenograft (PDX) mouse model.[15] Another approach to addressing variations in tumor antigen representation in heterogeneous solid tumor tissue was to engineer a bicistronic construct to drive the expression of a CAR specific for EGFRvIII, a glioblastoma-specific tumor antigen, and a bispecific T cell engager (BiTE) against EGFR. The CAR-Ts secreted EGFR-specific BiTEs, which helped redirect CAR-T cells and recruit other bystander T cells to induce tumor killing. These CART.BiTE cells were robust in eliminating heterogeneous tumors in mouse models of glioblastoma.[48] Collectively, these studies illustrate that coordinated or multiantigen targeting strategies may help prevent antigen escape for CAR-T cells.

ADDRESSING COST THROUGH UNIVERSAL, OFF-THE-SHELF CAR-T CELLS

Even after issues of safety and efficacy are addressed, the major bottleneck to clinical translation remains: the exorbitant cost of manufacturing, which is largely due to the autologous production process. To date, six CAR-T cell therapies have been approved by the U.S. FDA to treat B-cell malignancies, all of which are autologous and therefore expensive. Four of these therapies are targeted toward CD19 (tisagenlecleucel, axicabtagene ciloleucel, brexucabtagene autoleucel, and lisocabtagene maraleucel), and two are directed against B-cell maturation agent BCMA (idecabtagene vicleucel and ciltacabtagene autoleucel). All of these approved therapies have cumbersome production processes. T cells must be isolated from patient peripheral blood mononuclear cells (PBMCs), engineered and expanded in the lab, and then infused back into patients. This process takes approximately one month, and the long development timelines, manufacturing bottlenecks, and high costs associated with autologous manufacturing limit the delivery of CAR-T therapies.[49]

To reduce the costs associated with this process, off-the-shelf CAR-T cell therapies derived from healthy donors have been explored. In addition to reducing costs, allogeneic CAR-T cells decrease the production time. They can be made available immediately to patients via cryopreserved batches and thus circumvent the lengthy process of tailoring CAR-Ts on an individual basis. Therefore, off-the-shelf immunotherapies could provide widely applicable therapies for many patients. However, there are challenges to be overcome regarding the compatibility of the host immune system with allogeneic cell therapy. Notably, multiple genetic modifications are necessary to avoid graft-versus-host disease to use allogeneic CAR-T safely for cancer treatment. For example, the endogenous TCR loci should be eliminated to avoid off-target effects, and major histocompatibility complex (MHC) class I on the CAR-T should be disrupted to prevent elimination by host T cells.[50]

Off-the-shelf innovations are still a work in progress. Several allogeneic CAR-T therapies have reached phase 1/2 clinical trials (ALLO-501A from Allogene, PBCAR0191 from Precision BioSciences, and WU-CART-007 from Wugen). However, the ALLO-501A clinical trial was put on hold by the U.S. FDA in October 7, 2021, due to the detection of a chromosomal abnormality thought to be potentially due to excessive on-target cutting.[51] The clinical hold has since been lifted because the abnormality was found to be unrelated to manufacturing and was not clinically significant. However, the effects of large-scale genetic perturbations on both on safety and efficacy will continue to be a concern for adoptive cell therapies.

GENETIC SCREENING FOR NOVEL TARGET DISCOVERY

Engineering CAR-T cells to overcome various hurdles is an ongoing process that continues to be advanced through the identification of new mechanisms of immunomodulation. One

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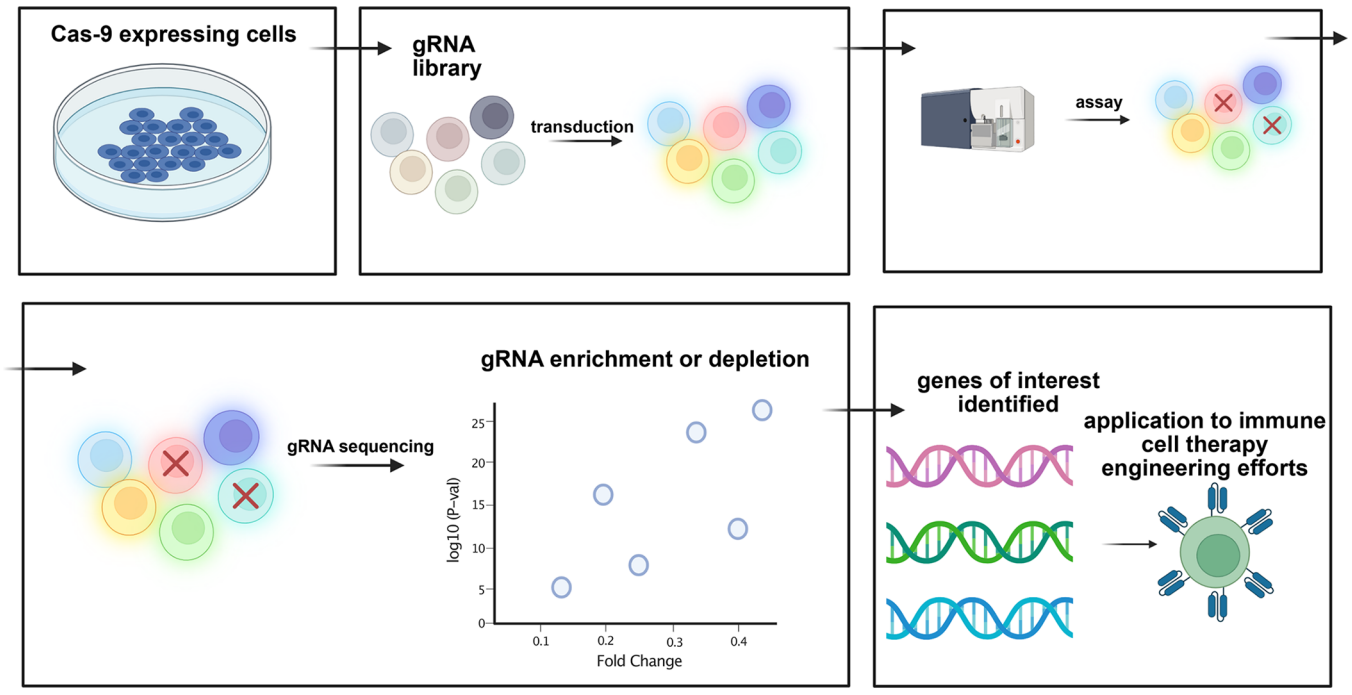

Figure 3. CRISPR screens for immunotherapy target discovery. Through unbiased target discovery via high-throughput genetic screens, novel regulators of T cell functions can be identified. Gene targets with therapeutic potential can be directly integrated into functional CAR-T cell engineering approaches.

way to discover novel targets is through unbiased, high-throughput genetic screens (Figure 3). In CRISPR screens, genetic perturbations are introduced into pools of cells to create a population of mutagenized cells. Using an optimized assay, a phenotype of interest can be selected for within this population of mutagenized cells. The perturbations that result in the desired phenotype can be retrieved using massively parallel sequencing, and further validation experiments can confirm the phenotypic effects. Through this approach, CRISPR screening can be used to identify modulators of T cell receptor activation and cytotoxic ability, which can inform CAR-T cell engineering strategies.

Genetic screens can identify intracellular signaling pathways and other biomolecular mechanisms that may be relevant for perturbations to potentiate CAR-T cell activity. For example, a recent paper conducted a CRISPR screen and identified *PRODH2*, a proline metabolism gene, as a potent modulator of antitumor efficiency; overexpressing *PRODH2* was found to enhance CAR-T cell effector function.[52] CRISPR screening has also been performed directly in CAR-T cells, identifying genes *TLE4* and *IKZF2* as molecular determinants of tumor killing in the specific context of glioblastoma stem cells.[53] Another CRISPR screen-based study identified death receptor signaling through FADD and TRAIL-R2 as key mediators of CAR-T cell cytotoxicity, suggesting death receptor signaling as an important mediator of tumor killing ability for CAR T cell therapies.[54] These and other studies demonstrate how screens in primary T cells can identify novel factors for improving adoptive cell therapies.[55−57]

Genetic screening also has several limitations, including methodological complexity, cost, and stochastic results. To mitigate the stochastic nature of genetic screening results, screens should be conducted with biological replicates[58] and unbiased analyses should be used. To evaluate gene function in a more clinically relevant setting, *in vivo* screens can be performed instead of *in vitro* screens; however, a key concern of *in vivo* screens is whether sufficient cells of interest can be retrieved, which affects the library coverage.[59] Coverage is dependent on the cells retrieved and library size and affects the stochasticity of guide/gene performance. One way to increase coverage is to reduce library size, by decreasing the number of genes targeted and/or the number of guides per gene. A successful CRISPR screen depends on the use of a suitable biological model, efficient perturbations of the target genes, and accurate readout of the perturbations.[60]

TCR-T AND INNATE IMMUNE CELL THERAPIES

Unlike CAR-Ts, which recognize tumor antigens in an MHC-independent fashion through the scFv, T cell receptor transgenics (TCR-Ts) express tumor antigen-specific T cell receptors with α and β chains that are produced from antigen-specific T cell clones. By engaging antigens in an MHC-dependent manner, TCR-Ts have an advantage over CAR-Ts as they are able to target both intracellular and cell-surface tumor antigens. However, they require matching HLA alleles and are susceptible to evasion through downregulation of antigen processing and presentation machinery.[61] CRISPR-Cas9 has been shown to combine TCR-targeted integration into the TCR-α constant locus with a TCR-β constant knockout, which avoids chain mispairing and maximizes TCR expression and function.[62] Thus, genetic engineering can also fine-tune the development of effective TCR-T cells against a novel cancer-associated antigen.

A recent case report of a TCR-T therapy demonstrated high efficacy in the solid tumor context. This study engineered autologous T cells to clonally express two allogeneic HLA-C*08:02-restricted T cell receptors targeting mutant KRAS G12D expressed in pancreatic tumor tissue. The engineered T cells demonstrated long-term persistence, comprising >2% of all of the circulating peripheral blood T cells six months after

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the cell transfer, and achieved regression of visceral metastases (72% overall partial response) in a metastatic pancreatic cancer in the patient who received this therapy.[63]

Immune cell therapies are not restricted to T cells. Innate immune cells with tumor killing ability such as NK cells[64] and macrophages[65] can also be harnessed to target tumors, and some studies have also explored applications of induced pluripotent stem cells (iPSC) to derive antitumor functions.[66] NK cells, which are of lymphocyte lineage but are part of the innate immune response, are particularly promising for allogeneic therapeutic contexts as GVHD is less of a concern; some preclinical studies suggest that NK cells may even protect against GVHD.[67,68] When NK cells are engineered to express a CAR, NK cells can elicit tumor-specific killing through mechanisms including direct tumor cytotoxicity and generation of cytokines.[69] Additionally, NK cells do not secrete high levels of IL-6 and are likely to have reduced toxicities compared to those of CAR-T cells, mitigating the risk of cytokine release syndrome and neurotoxicity.[70] The use of CAR-engineered macrophages is also promising for modifying the immunosuppressive tumor microenvironment. As macrophages are potent antigen-presenting cells that are capable of infiltrating solid tumor tissue, CAR-engineered macrophages may modulate efficacious antitumor responses in a solid tumor context as has been demonstrated in preclinical models.[65]

CONCLUSIONS AND FUTURE DIRECTIONS

An improved understanding of how the immune system controls cancer has contributed to the development of immune cell therapies. Over the past decade, cellular immunotherapies such as CAR-T, TCR-T, and NK cell therapies have achieved tremendous success in cancer treatments. Several clinical trials are in progress to bring the technology from the bench to the bedside. Although promising, there are challenges with expanding the potential of these cellular immunotherapies. T cell exhaustion has limited cancer killing efficacy and persistence. Side effects such as cytokine release syndrome, neurotoxicity, and graft-versus-host disease have raised safety concerns. Insufficient tumor infiltration and the immunosuppressive microenvironment have hindered the application of cellular immunotherapies in treating solid tumors.

We have discussed in this Perspective genome engineering strategies for improving the safety, efficacy, and cost of cellular immunotherapies. Inducible CAR-T activation and other such strategies hold promise for mitigating safety concerns such as on-target off-tumor effects, neurotoxicity, and GVHD. By engineering constructs such as STAR-Ts and tandem CARs, researchers have found ways to remedy efficacy limitations such as exhaustion, poor persistence, and antigen escape. Finally, efforts are in progress to advance allogeneic therapies and NK-CAR treatments, both of which hold promise for reducing the high manufacturing costs of ACT. Innovations in genome engineering approaches will continue to expand the frontiers of cell therapies by circumventing key hurdles in their application and treatment. Recent developments in synthetic biology to exploit combinatorial targeting and signaling circuitry are highly promising for increasing clinical efficacy and safety. Through varied, complementary approaches ranging from improved synthetic circuits to inducible CARs that mitigate T cell exhaustion, future therapies are likely to achieve greater clinical success.

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