Cellular Cancer Immunotherapy Development and Manufacturing in the Clinic

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ABSTRACT

The transfusion of naturally derived or modified cellular therapies, referred to as adoptive cell therapy (ACT), has demonstrated clinical efficacy in the treatment of hematologic malignancies and metastatic melanoma. In addition, cellular vaccination, such as dendritic cell–based cancer vaccines, continues to be actively explored. The manufacturing of these therapies presents a considerable challenge to expanding the use of ACT as a viable treatment modality, particularly at academic production facilities. Furthermore, the expanding commercial interest in ACT presents new opportunities as well as strategic challenges for the future vision of cellular manufacturing in academic centers. Current trends in the production of ACT at tertiary care centers and prospects for improved manufacturing practices that will foster further clinical benefit are reviewed herein.

Introduction

The application of *ex vivo* culturing and cellular engineering in the development of novel adoptive cellular therapies (ACT) has produced durable clinical responses in a variety of different relapsed and treatment-refractory cancer histologies. Tumor infiltrating lymphocytes (TIL) and chimeric antigen receptor (CAR) T cells have consistently generated robust immune responses leading to objective tumor regression in patients with melanoma and B-cell malignancies, respectively (1, 2). Given this success, these modalities, along with T-cell receptor (TCR) transduced T cells and dendritic cell (DC)-based cancer vaccines, are now being developed for the treatment of more aggressive cancers with lower mutational burden.

Given the highly personalized nature of ACT, many of the challenges associated the implementation of these cellular therapies arise from their lack of scalability, intensive resource demands, and cost. This is particularly pertinent to tertiary care medical centers, where much of the pioneering immunotherapy research and clinical trials that has driven the development of ACT are conducted. With these manufacturing hurdles in mind, a growing body of knowledge offers feasible means of improving cellular therapy production at these academic immunotherapy facilities. This review on the development and production of ACT in the clinic will highlight innovations that could refine academic workflows and ultimately improve the efficacy of future cellular therapies, with an emphasis on TIL, engineered T cells, and DC vaccines.

TIL

ACT using TIL has established itself as a proven treatment modality for refractory melanoma, mediating objective responses and durable complete responses in a number of clinical trials (3). When administered with a lymphodepleting chemotherapy regimen, TIL therapies produced objective complete responses in 22% of refractory patients with melanoma as judged by RECIST, and 95% of the subset exhibiting ongoing complete regression beyond 3 years (4). Responders were also found to have a significantly larger number of infused cells remaining in circulation 1-month posttransfer when compared with nonresponders (4). Notably, this TIL manufacturing scheme is reproducible, with multiple independent clinical trials being conducted at a number of research hospitals reporting similar patient populations and success rates (5–7).

The adoptive transfer of TIL used in these trials is considerably resource and time-intensive, as evidenced by manufacturing protocols that require rigorous sterility safeguards and personnel training. This is necessitated by significant good manufacturing practice (GMP) regulations—a bottleneck for all ACT, be it TIL, genetically engineered T cells, or DC vaccines—that outline minimum requirements for the workflows, cleanroom facilities, and controls used in the production of cellular cancer immunotherapies in both academia and industry. In order for academic programs to establish themselves as decentralized, point-of-care manufacturing centers, considerable financial, logistical, and regulatory hurdles must be cleared to build or convert facilities, validate protocols, train and certify technicians, and acquire and maintain the necessary reagents and instruments in accordance with GMP standards.

TIL growth and expansion

TIL are generated through the isolation and serial expansion of lymphocytes following surgical resection or biopsy of a lesion greater than 2 cm in diameter (8). TIL are typically cultured in complete media (CM), an Roswell Park Memorial Institute (RPMI) based medium containing human serum and IL2 and expanded directly from freshly dissected tumor fragments or tumor digest samples that have been enzymatically digested and mechanically dissociated into a single cell suspension (8). Culture conditions are optimized for lymphocytes only, leading to the death of plated tumor cells over the course of 2 to 3 weeks, until a pure culture of T cells remain (**Fig. 1**). TIL are then tested for tumor reactivity and neoantigen specificity through co-culture assays, with cultures exhibiting the highest IFNγ secretion

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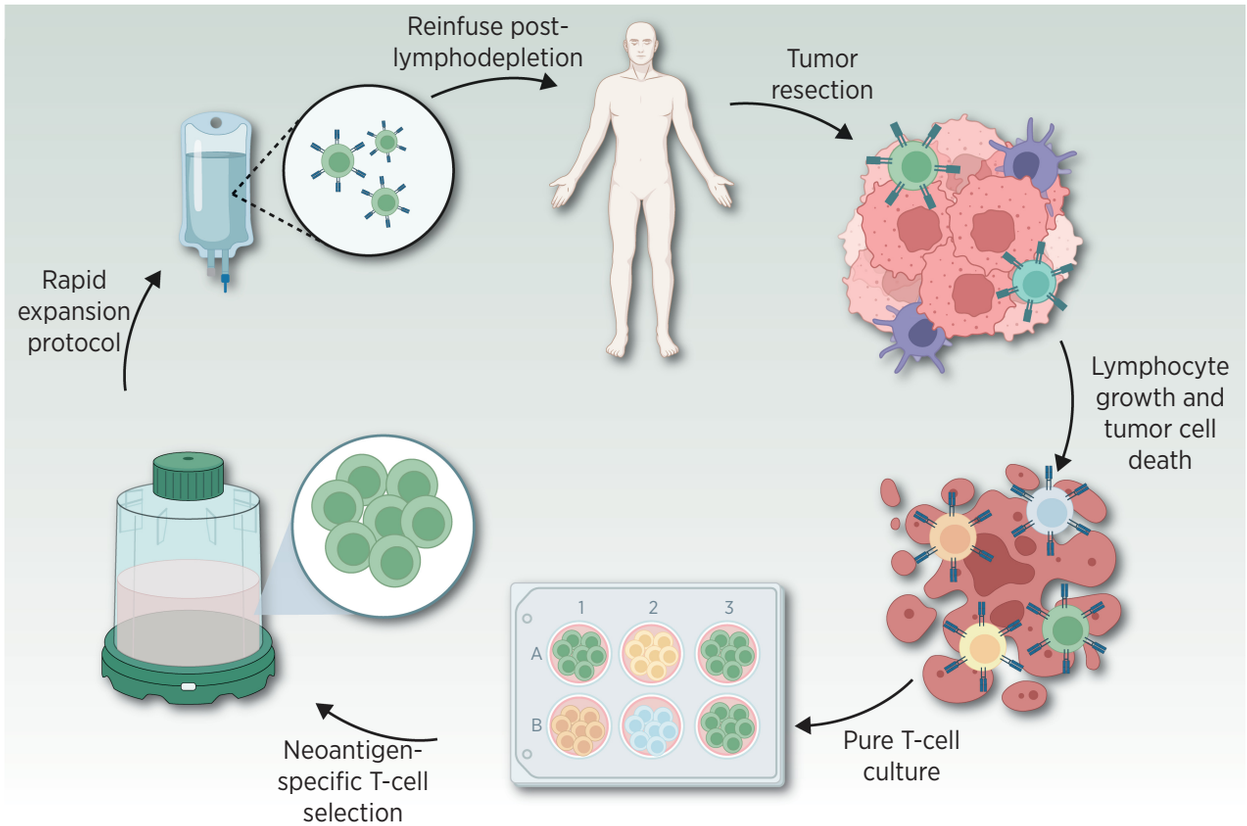

Figure 1.

Overview of the culturing and selection of autologous TIL for use in ACT. A resected tumor sample is enzymatically digested and plated into a single cell suspension. Cell culture conditions are suitable only for lymphocyte growth, yielding a pure T-cell culture as tumor cells die out. TIL are selected on the basis of tumor reactivity and neoantigen specificity as demonstrated by co-culture and ELISA assays. Selected TIL are then rapidly expanded in culture supplemented with IL2, OKT3, and PBMC "feeder" cells, after which the cells are harvested and prepared for infusion back into the patient. (Adapted from an image created with BioRender.com.)

being selected for further expansion (9). This rapid expansion protocol (REP) often involves the serial expansion of selected TIL cultured in a mixture of complete media and AIM-V media supplemented with IL2, OKT3, an anti-CD3 mAb, and irradiated allogeneic peripheral blood mononuclear cells (PBMC) that serve as "feeder" cells (10). The REP can be completed in 2 weeks with lymphocyte harvest and infusion taking place on day 14 of culture (9). In total, TIL growth and harvest takes between 5 and 6 weeks to complete with up to 1×10^{11} lymphocytes being generated for infusion (3). Technical issues associated with previous clinical trials do raise concern, however, as a retrospective analysis of previous clinical trials found that roughly 5% of patients accepted into trials did not successfully produce TIL for use in treatment and were, thus, dropped from study (10).

TIL selection and differentiation

Inherent in the immunotherapeutic potential and successful manufacturing of TIL is the generation of tumor reactive $CD8^+$ T cells, which effectively proliferate and persist postinfusion. As such, T-cell selection and differentiation may well offer means of circumventing technical issues associated with TIL moving forward. Numerous studies have demonstrated that the infusion and tumor infiltration of a high percentage of $CD8^+$ T-cell was associated with improved objective responses (7, 11–13). Of note, the TIL clinical trial overseen by Ithzaki and colleagues reported a 48% objective response rate in patients with metastatic melanoma, with the TIL cultures of responding patients containing 20% more cytotoxic T cells than those of nonresponders (13). Analysis performed by Radvanyi and colleagues found similar results as the proportion of $CD8^+$ T cells infused was found to be significantly higher for the 48.4% of patients with metastatic melanoma who responded to treatment (7). An analysis of the phenotypic traits associated with neoantigen-specific $CD8^+$ lymphocytes found that reactive cells expressed significantly higher levels of programmed cell death 1 (PD-1), lymphocyte-activation gene 3 (LAG-3), and T-cell immunoglobulin and mucin domain 3 (TIM-3), while simultaneously expressing a lower level of the costimulatory receptor 4–1BB (CD137; ref. 14). Growth in IL2 altered the expression levels of these markers, indicating the enhancement of tumor-reactive lymphocyte populations is not feasible following expansion in culture (14). PD-1 expression prior to expansion does, however, offer a means of selecting for tumor-reactive $CD8^+$ cell populations and generating enriched populations of neoantigen-specific lymphocytes (15). $CD8^+$ lymphocyte populations can also be enriched prior to resection. An Anti-OX40 (CD134) mAb neoadjuvant treatment prior to surgical resection was shown to increase $CD8^+$ T-cell proliferation, tumor-reactive activation, and TCRβ clonality, indicating a potential means of improving successful TIL growth and infusion products prior to culturing (16).

A growing body of research indicates that $CD8^+$ T cells can exist in a stem cell-like state, occupying an intratumoral niche and possessing the ability to both effectively kill tumor cells and clonally expand (3). Although *in vitro* antitumor reactivity is associated with terminally differentiated $CD8^+$ T cells, these cell populations were found to have significant impairments in functionality, proliferative capacity, and persistence when adoptively transferred into murine models (17). $CD8^+$ T-cell populations are, in fact, largely heterogeneous, with the differentiation state of cells and their observed rate of expansion and persistence to be inversely proportional (18). A memory T-cell subset was later discovered in humanized mouse models that exhibited both stem cell-like self-proliferative and multipotent character with simultaneous memory phenotype expression and activity (19). Retrospective analysis of ACT products similarly found that patients who responded to treatment possessed a population of neoantigen specific, stem-like memory-progenitor T cells ($CD39^-$, $CD69^-$) with improved TIL persistence when compared with tumor reactive cells in the terminally-differentiated $CD39^+$,69^+ state (20).

TIL for solid malignancies and tumors with low mutational burden

Despite the success of ACT with TIL in the treatment of melanomas with high levels of somatic mutation, expanded use will rely on the ability of the immunotherapy to effectively treat common epithelial cancers with lower mutational burden as well as tumors with intracranial metastases. Analysis of advanced melanoma with associated intracranial metastases proved that TIL were capable of mediating immune responses in the central nervous system (CNS) that were safe and elicited objective clinical responses, however systemic impact of circulating TIL had less effect on survival or the progression of disease at sites that had not received localized therapy prior to treatment (21). This immune response underscores the potential for TIL in the treatment of CNS tumors, as glioblastoma and other brain tumors have historically had minimal response to therapeutic intervention, are often characterized by a severe immunosuppressive environment, and are difficult to surgically resect, making TIL culture more logistically challenging. A population of circulating $CD4^+$ memory T cells with specificity for a cancer neoantigen was identified in 1 patient from a small cohort of 5 patients with glioblastoma, reinforcing how challenging the development of TIL for low-mutational burden CNS tumors will likely be while suggesting that targeted immunotherapy is nonetheless feasible in this group of cancers (22). Glioma-derived TIL were successfully expanded using a combination of IL2, IL15, and IL21 with a 100% success rate (23). Notably these results could not be replicated with a standard IL2-based expansion protocol. This unique combination of cytokines required for effective TIL growth from CNS tumors underscores the fact that manufacturing workflows may need to be tailored to each solid tumor histology to fully optimize TIL culture and production. The transcriptomic signatures of TIL within glioblastoma also indicate that resident $CD8^+$ T cells can potentiate robust antitumor responses, however this response is likely suppressed by the presence of T_H17 lineage $CD4^+$ T cells capable of inducing a terminal state of exhaustion in cytotoxic T cells (24). The presence of these subpopulations suggests that neoadjuvant therapy specifically targeting this T_H17 lineage could potentially improve the antitumor response of tumor-resident $CD8^+$ T cells and increase efficiency of TIL harvest for CNS tumors.

With limited clinical trial data, the safety profile of CNS-targeted TIL is largely unknown. A pilot study using locally infused TIL induced transient and asymptomatic cerebral swelling in all 6 patients treated but did not generate significant Grade 3 or 4 complications (25). Off-target effects and severe CNS complications must nonetheless be considered when infusing a substantial number of tumor-reactive TIL.

Outside of CNS tumors, multiple clinical trials have demonstrated limited but significant clinical responses in other cancers characterized by lower levels of mutation, with associated manufacturing strategies posing a potential paradigm shift in how TIL are selected. TIL screened for reactivity and selectively expanded on the basis of specificity for both the KRAS G12V mutation and human leukocyte antigen (HLA)-C*08:02 initiated a 9-month complete response in a patient with metastatic colon cancer, which progressed only after the genetic loss of the HLA-C*08:02 allele (26). Similarly, TIL selectively reactive for four mutant proteins identified using whole exome sequencing and RNA sequencing induced an ongoing complete response in a patient with chemorefractory hormone receptor-positive breast cancer (27). It should be stressed that these results are from two individual patients enrolled in an ongoing phase II clinical trial that has yet to be completed (NCT01174121). Although the objective tumor regressions associated with targeting specific mutations using HLA-restricted TIL suggests that this highly personalized therapy can be a valuable new approach for the treatment of solid tumors moving forward, this strategy has yet to demonstrate effectiveness to the extent that it can be expanded to a more generalized patient population.

The future of TIL at academic centers

The most significant challenge impeding the widespread adoption of TIL therapies is the sheer volume of resources required to produce a single therapy—be it the reagents used, the requisite labor and training needed to produce these therapies, or the production time needed to screen antigens and generate sufficient T-cell yield. Considerable overhauling of open-system manufacturing protocols must be made to ensure that TIL manufacturing is efficient, reduces reagent demand, and is financially feasible in an academic setting. Many academic institutions, for example, continue to rely on open systems consisting of T175 flasks or 3-L culture bags to expand cell products. Given the quantity of single-use flasks required for each individual TIL therapy, this quickly becomes a significant cost burden. Gas-permeable bioreactor platforms, such as gas permeable rapid expansion (G-Rex) flasks, offer the benefits of regular sterility testing and high-density cell growth necessary to manufacture all forms of ACT, however their price point largely precludes their use in academic centers. Donor PBMCs can also be replaced by artificial antigen-presenting cells (aAPC), a lentivirus transduced cell line constitutively expressing selected costimulatory molecules and cytokines optimized for the propagation of a desired T-cell phenotype. The generation of GMP grade aAPCs is complex, however, and does require additional resources to manufacture.

Although effective at manufacturing a small number of cell products simultaneously, the limitations associated with the open system platforms currently employed—the need for trained and experienced manufacturing technicians, operating cleanroom environments that must meet stringent GMP requirements, and the increased risk of microbial contamination of cell products—make them unsustainable for the large majority of academic immunotherapy programs to effectively scale up TIL production. Automated, closed-loop manufacturing platforms that can be more feasibly scaled to treat an expanding patient population would thus play the most pivotal role in reshaping academic manufacturing programs. The Xuri cell expansion system, a wave-mixed bioreactor that enables automated mixing and oxygen transfer within the reactor, and the LoVo cell processing system, which utilizes a spinning filtration membrane instead of centrifugation, are two devices that have demonstrated the ability to

rapidly expand and wash TIL as part of a closed and semi-automated system, albeit one that also uses G-Rex flasks for expansion (28). Used primarily for CAR T-cell manufacturing, the CliniMACS prodigy has also demonstrated 3,000 to 15,000 fold expansion of TIL when a REP is performed using the instrument, however the 4×10^9 cells harvested after one REP would not meet cell yield requirements for TIL products (29). Emphasis should not only be placed on the automation of expansion and purification, but of T-cell selection, allowing for the enrichment of TIL through bead-based $CD8^+$ selection or $CD39^+$ T-cell depletion for example, to effectively limit T-cell differentiation and exhaustion and thus improve TIL therapy efficacy. Given the current production capacity of closed system devices, subsequent or sequential production runs to produce cell numbers optimized for TIL therapies will be necessary until cell yield improves. Alternatively, TIL therapy can be supplemented by the addition of another ACT requiring reduced cell yield such as a pre-expanded TCR product or DC vaccine. Regardless of the modalities used, these automated systems require significant upfront expenditure but could effectively drive down year-over-year costs by requiring less infrastructure and staffing.

Beyond manufacturing, the accurate identification of antitumor T-cell receptors also poses a significant challenge to improving the efficiency and efficacy of TIL therapy development. Antigen screening and validation takes a considerable amount of time, delaying product manufacturing. Combined single-cell RNA sequencing (scRNA-seq) and T-cell receptor sequencing (TCR-seq) can potentially identify the transcriptomic state of TCR clonotypes that target neoantigens as well as nonmutated viral and tumor-associated antigens, circumventing the need for functional screening of candidate neoantigens (30). Refining the sensitivity of antigen identification will be invaluable to the development of TIL therapies for cancers with low-mutational burden, as the landscape of tumor antigens is proving to be far broader than that of somatic mutations alone (31, 32). Orphan tumor-reactive T-cell receptors, the term designated for receptors of unknown antigen specificity, have been found to be reactive to autologous tumor material in a number of solid tumors (33). Improved bioinformatic analysis, incorporating whole genome sequencing and T-cell functional analysis, will thus create the most opportunities to fully exploit the limited antigenic targets available within tumors possessing low mutational burden while simultaneously expediting the antigen identification timeline.

CAR

Recent FDA approval of CD19 B-lymphocyte–specific CAR T cells for the treatment of diffuse large B-cell lymphoma and pre-B-cell acute lymphoblastic leukemia underscores the potential CAR T cells exhibit in the treatment of both pediatric and adult hematologic malignancies. CARs have demonstrated clinical efficacy in the treatment of several other relapsed or refractory leukemias and lymphomas as well (2, 34–38). The duration of objective responses to these therapies is highly variable, with relapse continuing to be a barrier to more expanded use.

CAR T cells have had particularly little success in the treatment of solid tumors. CAR T-cell development for the treatment of high-grade gliomas may well serve as a model for developing new CAR for the treatment of solid neoplasms, however. As of 2019, the largest number of CAR T-cell clinical trials for solid malignancies was for brain tumors. Three of 4 patients with diffuse midline glioma exhibited clinical and radiographic improvement following infusion of CAR directed at GD2, a highly expressed disialoganglioside expressed on H3K27M-mutated glioma cells (39). Notably, on-target, off-tumor toxicity was not observed and resulting cytokine release syndrome, T-cell-mediated inflammation, and immune effector cell-associated neurotoxicity was reversible. A CAR T-cell product targeting IL13Rα2 induced regression of all intracranial and spinal glioblastoma tumors and elicited a clinical response lasting 7.5 months in a single patient with recurrent disease. HER2-specific CAR generated a partial response lasting more than 9 months in a single patient with progressive glioblastoma (40). CAR targeting EGFRvIII have also demonstrated clinical benefit in some glioblastoma patients while also suggesting that antigen loss is a likely pathway of therapeutic escape within these malignancies (41). As CAR T-cell technology continues to advance rapidly, these results underscore how more standardized and efficient manufacturing strategies are necessary to both meet growing demand and adapt to a rapidly evolving tumor microenvironment and antigen landscape following initial treatment.

T-cell collection

Current manufacturing techniques for CAR are significantly modified from those used in the production of TIL, however the cell yield required and, likewise, the timeline for production, is significantly reduced. Furthermore, CAR production is significantly more standardized than TIL production, as evidenced by the seven FDA approved products available across multiple indications. T lymphocytes are collected via leukapheresis and patients often receive anywhere from 1×10^6 to 1×10^7 cells/kg of patient bodyweight at infusion following conditioning chemotherapy (42). Commercial CAR T-cell products can be administered at a maximum dose of 2×10^8 cells, a significantly lower absolute cell number than the cell count necessary for the manufacturing of TIL therapies. This does not completely circumvent the issue of cell yield and availability for manufacturing purposes, as patients are often profoundly lymphopenic following previous chemotherapy regimens, limiting the yield of viable T cells for expansion and transduction (43). Once an apheresis product is collected, density gradient centrifugation can be used to wash and fractionate T lymphocytes, however this process is laborious. A number of automated devices are now available that can remove gross platelet and red blood cell contamination, such as the Cytomate cell washer and COBE2991, or provide size-based cell fractionation to isolate lymphocytes and deplete monocytes (44).

T-cell selection

Following isolation, T-cell subpopulation selection or depletion can then be performed. T-cell cultures have historically been enriched with a $CD3^+$ population of cells, with the number of PBMC used to initiate culture determined by the $CD3^+$ cell percentage as identified by flow cytometry (42, 45). T-cell subsets can be further enriched or depleted using instruments such as the CiniMACS Prodigy and associated antibody-conjugated magnetic beads. $CD4^+$, $CD8^+$, and $CD62L^+$ T-cell selection and enrichment has yielded CAR T-cell products with improved persistence and antitumor activity (46, 47). Clinical-scale GMP protocols have already been developed to isolate, transduce, and expand specific T-cell populations (48). Selection is not a prerequisite to CAR T-cell production, however. Tisagenlecleucel (tisa-cel) and Axicabtagene ciloleucel (axi-cel) therapies, for example, are generated from bulk T cells whereas lisocabtagene maraleucel (liso-cel) therapy requires the sequential infusion of selected $CD4^+$ and $CD8^+$ cells at equal target doses; all three therapies are FDA approved and have shown promising efficacy and safety profiles (49).

The selection of T cells can serve as a point of focus for academic manufacturing programs to optimize product efficacy and proliferative

capacity using established workflows and instrumentation. CD4/CD8 T-cell selection of starting apheresis product was found to improve manufacturing feasibility via increased cell expansion, transduction efficiency, and $CD3^+$ cell recovery as well as the ability to salvage previously unusable apheresis products as confirmed by CD3/CD28 enrichment product analysis (50). Similar to TIL manufacturing, highly proliferative naïve and stem-like CAR T cells were found to exhibit more robust clinical responses in murine models independent of their transduced costimulatory domain (43). A growing body of evidence suggests that subsets of $CD62L^+$ T cells, in particular naïve ($CD62L^+$, $CD45RA^+$), stem cell memory ($CD45RA^+$, $CD62L^+$, $CD95^+$), and central memory T cells ($CD62L^+$, $CD45RA^-$) demonstrate superior antitumor response and proliferative potential (19, 51, 52). These stem cell memory T cells occupy a T-cell compartment characteristic of naïve T cells, yet possess functional attributes of memory cells and are associated with increased *in vivo* expansion of CAR T cells following infusion (53). Most notably, these $CD62L^+$ T cells are less prone to inducing cytokine release syndrome and thus demonstrate a wider therapeutic index compared with unselected T cells (54). It should also be noted that cell selection is not exclusive to T cells, as NK cells harvested from cord blood and transduced with a CD19 construct generated objective clinical responses in patients with recurrent or refractory $CD19^+$ chronic lymphocytic leukemia and non-Hodgkin's lymphoma with no cytokine release syndrome or neurotoxicity (55).

Alternatively, selective depletion of particular PBMC subpopulations has also yielded more efficacious CAR T-cell therapies. $CD14^+$ monocytes have been noted to adhere to cell culture bags and phagocytose magnetic beads used for activation. By depleting these cells from apheresis products via rapid plastic surface adhesion in T175 flasks, the $CD14^+$ monocyte content of T cells cultures was significantly depleted while the $CD3^+$ content significantly increased (56). A threshold of at least 40% $CD14^+$ monocytes within an apheresis product was determined to warrant such depletion and resulted in successful CAR T-cell product manufacturing in 42 of 43 patients (56). Depletion of $CD25^+$ Tregs with the CD25-blocking mAb daclizumab resulted in Treg depletion and reprogramming as well as improved CD8 and CD4 T-cell priming (57).

T-cell activation

Prior to gene construct insertion, T cells must first be activated. T-cell activation can be performed using OKT3 and IL2, as with TIL activation, however bead-based activation has become standard, particularly in closed GMP cell culture systems. Dynabeads, for example, are superparamagnetic beads coupled to CD3 and CD28 antibodies. T-cell/Dynabead aggregates are agitated following stimulation and subsequently passed through a strong magnetic field to remove the beads, as the particles are hazard if infused into patients (58). Alternatively, ExpAct Treg beads employ the same magnetic particles while targeting CD3-biotin, CD-28, and anti-biotin mAbs, allowing for selective activation of regulatory T cells as well as conventional lineage T cells (44). TransAct, a colloidal polymeric nanomatrix conjugated to humanized anti-CD3 and anti-CD28 antibodies, eliminates the need for filtration as the nanomatrix is biodegradable and can be removed from cell suspension by centrifugation, however T-cell purification prior to activation is necessary (44). Manufacturing protocols utilizing bead-based activation or TransAct have shown comparable activation and both modalities demonstrate the ability to generate CAR T-cell products (53).

Gene transfer

Following T-cell activation, CAR expression can be induced by through construct delivery by viral and nonviral gene transfer systems. γ-Retroviral and lentiviral vector constructs are the two most common viral gene delivery systems used in CAR T-cell therapies given their high gene transfer efficiency and stable CAR expression. Viral vector production is particularly expensive, however, and must undergo strict FDA-regulated safety testing to assess for potential replicative capacity as well as annual testing for replication-competent virus in patient blood draws. Although insertional mutagenesis has occurred in early gene therapy trials for X-linked SCID, retrospective analysis of PBMCs from patient follow-ups indicate that these events were rare and, furthermore, that transduced cells have the ability to persist and function within patient circulation well after disease remission (59). More importantly, no clinical events induced by insertional mutagenesis have been noted in conventionally manufactured CAR T-cell products utilizing γ-retroviral and lentiviral transduction (60).

Alternatively, the Sleeping Beauty (SB) transposon/transposase system and clustered regularly interspaced short palindromic repeats (CRISPR) coupled with CRISPR-associated protein 9 (Cas9) endonuclease can also genetically engineer T cells without the need for viral transduction. These modalities circumvent the aforementioned safety testing associated with viral vector GMP requirements and can generate immunotherapeutic products at a fraction of the cost (61). DNA plasmids encoding the SB transposase and a CAR construct can be effectively electroporated into activated T cells inducing yield high enough for clinical use in a majority of patients, however the electroporation efficiency is low compared with viral transduction (62). SB electroporated T cells can be selected by aAPCs constitutively expressing selected costimulatory molecules and cytokines optimized for the propagation of a desired T-cell phenotype in culture supplemented with IL2 and IL21, allowing for the targeted expansion of T cells with integrated CAR capable of sustained propagation to clinically viable yields in an average of 28 days (62). In a phase I/II trial of B-ALL patients with relapsed disease, SB electroporated CAR T cells generated complete responses in a number of patients and CAR transgenes were measured up to 10 months following infusion (63).

Similarly, CRISPR edited cells are electroporated with Cas9 ribonucleoprotein complexes and guide RNA following T-cell stimulation with comparable levels of knock-in efficiency as the SB transposase (61). Although a recent clinical trial for refractory melanoma did not yield significant clinical responses, CRISPR electroporated T cells engineered to express TCRs for NY-ESO-1 or LAGE-1 elicited on-target tumor specificity and cellular persistence over 9 months, indicating that Cas9-based immune rejection is not a limiting factor with this methodology (64). As the technology associated with CRISPR and SB continue to rapidly progress, these early trials offer promise for the development of more efficient and cost-effective nonviral cell engineering.

Genomic integration can be circumvented through the use of a messenger RNA (mRNA) gene transfer system. *In vitro* transcribed mRNA can be introduced into T cells via electroporation, allowing for the highly efficient transfection of CAR transgenes that is sufficient in triggering antitumor responses (65). Gene expression is transient, however, with surface expression detected for roughly 1 week (66). The mRNA is translated in the cytoplasm, circumventing genomic integration and effectively eliminating the risk of genotoxicity or the creation of replication-competent retroviruses. This modality has demonstrated proof of concept for the *in vivo* production of CAR T cells in murine models using mRNA loaded nanoparticles (67). Likewise, an adeno-associated virus (AAV) encoding a CAR transgene

has also demonstrated the ability to generate *in vivo* CAR T cells targeting human T-cell leukemia, however there are concerns associated with nonspecificity and potential random insertion as is the case with other viral vectors (68). Although an *in vivo* strategy is far from in-human trials, the concept could significantly simplify and standardize manufacturing practices by largely eliminating cell production requirements.

CAR constructs

CAR T cells genetically differ from other forms of ACT, with vector constructs composed of single-chain variable fragment (scFv) from the variable region of a mAb, a hinged or spacer region that provides added receptor stability, a transmembrane domain, a CD3ζ intracellular signaling domain, and an additional costimulatory domain that can be generated from a variety of receptors including CD28 and 4–1BB (69, 70). This design bypasses some of the immunologic limitations common in other ACT, specifically the need for T-cell costimulation and MHC expression (71). CD28 is a costimulatory receptor constitutively expressed on resting and activated T cells that, when activated, induces a multiprotein signaling cascade and resulting transcription factor activation that ultimately leads to NF-κB expression, IL2 production, and the promotion of T-cell proliferation and survival through anti-apoptotic protein expression (72). 4–1BB is a transmembrane protein that, while not expressed on resting $CD4^+$ and $CD8^+$ T cells, is upregulated upon T-cell activation and induces IL2 production and anti-apoptotic protein expression via tumor necrosis factor receptor factor recruitment that inhibits NF-κB kinase activity and activates NF-κB signaling (73). Preclinical experiments demonstrate that T cells expressing CD28-costimulated CARs had increased cytokine production whereas large-cohort clinical trials likewise indicated that the CD28 costimulatory domain was associated with higher rates of neurologic toxicity (74). Although both costimulatory domains have been highly efficacious in patients with relapsed hematologic malignancies, confounding variables in the manufacturing process and variations in CAR scFV, hinge, and transmembrane domains limit the functional distinctions that the CD28 and 4–1BB costimulatory domains impart on CAR T cells. As CAR design continues to develop and improve, each component of the construct has proven to play a critical role in CAR T-cell functionality, proliferative potential, differentiation, exhaustion, and toxicities (75). The hinge and transmembrane domains used in CAR design were found to directly influence the severity of cytokine release syndrome and associated neurologic toxicity based on a clinical trial of patients with B-cell lymphoma (42). Notably, although this structural change reduced the grade of neurologic toxicity in patients, CAR blood levels and the clinical antilymphoma activity of transduced T cell were not impaired (42). Modifications to the costimulatory domain of CAR T cells have resulted in similar improvements in functionality, with a single amino acid change in a CD28-based CAR producing increased persistence, reduced exhaustion, as well as increased skewing towards a more stem-like Th17 fate (76). Constructs inducing the overexpression of the protein c-Jun, a T-cell-activating transcription factor found to have reduced expression in exhausted T cells, led to remarkably improved cell expansion both *in vitro* and *in vivo* as well as increased IL2 and IFNγ production, reduced terminal differentiation, and antitumor specificity (77).

T-cell expansion

Once transduced, CAR T cells can then be cultured and harvested in preparation for infusion. CAR T-cell culture and expansion has been commonly performed with IL2, however culture with exogenous IL7 and IL15 has been found to delay T-cell differentiation and preserve a greater proportion of stem cell memory T cells (78). This increase in stem cell memory T-cell population has yielded CAR T-cell products with greater antitumor activity and a shorter required culture time when compared with CAR T cells cultured in IL2 (79). *Ex vivo* expansion of CAR T cells does pose the risk for inducing T-cell exhaustion, however. Drug-regulation of CAR activity, where a small molecule is introduced into CAR T-cell culture, has demonstrated the ability limit T-cell exhaustion via receptor signaling cessation with the multikinase inhibitor dasatinib, redirecting cell fate towards a more memory-like phenotype with restored antitumor reactivity and transcriptional reprogramming (80).

The future of CAR at academic centers

As the seven FDA approved CAR T cell products continue to prove therapeutically and commercially viable, the development and scalability of CAR manufacturing platforms within academic cell production facilities will be critical to keeping pace with industry partners and ensuring academic workflows can be transferred to commercial production. CAR manufacturing must be further streamlined, standardized, and economized. To this end, closed and automated manufacturing systems offer a means of reducing labor costs, alleviating GMP manufacturing environmental requirements, and minimizing the risk associated with contamination or product variability. The CliniMACs Prodigy, a combination of a cell washer, magnetic cell separation system, and cell cultivation device is likely the most feasible means of achieving this goal, as it is one of the systems available that can enrich cell products within a closed environment (53). The Prodigy has already demonstrated the ability to select and expand T cells from preselected populations or whole apheresis, enabling the scalable production of CAR T cells in a controlled, GMP-compliant manner with no advanced manufacturing training necessary (81–83). Closed-system continuous perfusion bioreactors offer varying degrees of automatic T-cell selection, expansion, vector transfection or transduction, cell washing, concentration, harvesting, cell product formulation, and in-process control testing. A number of manufacturing studies conducted at academic institutions have demonstrated that closed-system bioreactors, including the Prodigy, limit microbial contamination and are capable of generating CAR T cells with tumor-specificity, functionality, and phenotypic expression similar to immunotherapies generated by other methods (28, 84–86). Although cell products did meet release criteria for expansion, cytotoxicity, and sterility, issues did arise with variability in cell growth, vector copy number, and myc overexpression (86). Transduction efficiency and cell yield are sufficient for clinical application in the manufacturing of CAR T-cell and DC therapies (85, 87, 88). Importantly, the use of an automated, closed-loop manufacturing system has already proven successful in the treatment of relapsed and refractory B-cell malignancies in trials conducted at academic medical centers (82, 89).

Refining transduction techniques beyond γ-retroviral and lentiviral vectors will also give academic manufacturing programs more flexibility in developing future CAR T-cell therapies. The use of SB, CRISPR Cas9, and mRNA gene transfer systems would circumvent the need for costly release testing and viral vector production. Concerns related to transduction efficacy, cell viability, duration of culture, and the duration of expression in the case of mRNA can only be dispelled through expanded clinical trials. Likewise, production variability in relation to treatment outcomes must be closely monitored before these modalities can be more widely adopted in academic manufacturing protocols.

The CAR transgene itself can also be updated through continued exploration of costimulatory molecules, suicide genes, and expanded CAR T-cell targets. CD20, CD22, CD30, CD33, CD138, CD171, CEA, EGFR, EFGRvIII, ErbB, FAP, GD2, Glypican 3, Her 2, Mesothelin, and NKG2D are all tumor associated proteins currently being targeted by academic programs designing CAR T cells (90). Targeting novel surface receptors is proving to be a key component of successful CAR T-cell products for the treatment of solid neoplasms. Tumor heterogeneity and antigen loss as a means of therapeutic escape reinforce that creating CAR T-cell therapies that target multiple surface markers through a pooled product may further improve clinical responses and prevent disease relapse.

Given the commercial success of multiple CAR T-cell products, collaboration with industry partners will further improve these manufacturing tools. When leveraged with the resources and finances available to the biopharma industry, the extensive experience academic programs have in the CAR T-cell arena can facilitate needed technological advancement, accelerate workflow development, and promote the expansion of CAR T-cell therapies to a greater patient population. The T-Charge platform, developed by Novartis, could be a particularly apt example of the benefit collaboration could pose for academic manufacturing centers; although only abstract data are currently available, the platform has demonstrated the ability to retain T-cell stemness and rapidly produce CAR T-cell products in less than 2 days.

TCR

Similar to CAR T cells, TCR transduced T cells offer a means of genetically engineered specificity via the transduction of viral vectors while simultaneously circumventing the need for naturally tumor-reactive T-cell availability. Although CAR T cells can target antigens independent of MHC expression, TCR-transduced T cells can recognize surface antigens as well as intracellular antigens presented by MHC proteins (**Fig. 2**). This added specificity necessitates more personalized therapies that are matched for a patient's unique neoantigen epitope and MHC complex, however TCR-transduced T cells do have a significantly larger number of viable immunogenic targets that can induce responses at much lower epitope densities (91). The

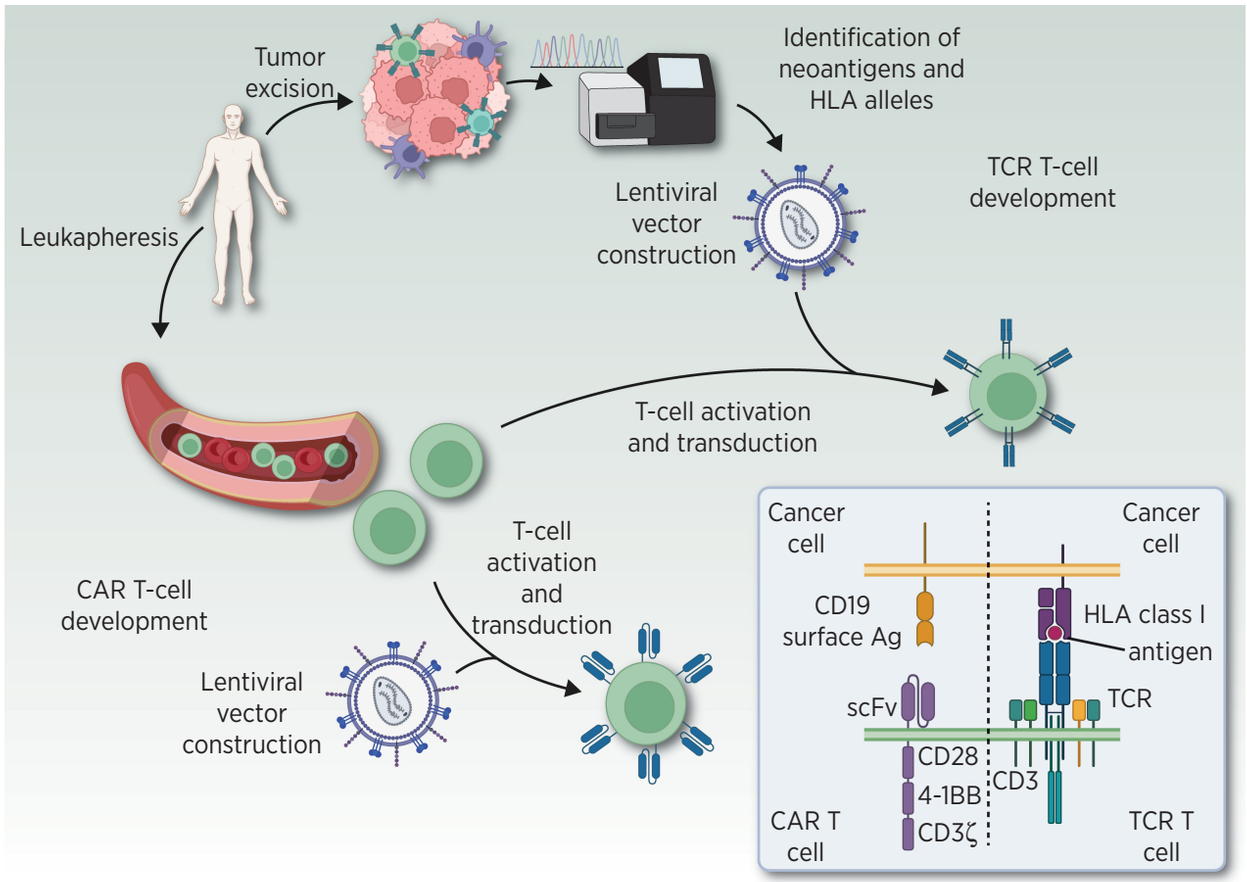

Figure 2.
Distinctions in the production of genetically engineered peripheral blood lymphocytes. Lentiviral or retroviral transduction of peripheral blood lymphocytes allows antitumor TCRs and CARs to be expressed in otherwise nonspecific T cells. T cells are harvested via leukapheresis and are activated prior to transduction. While transduction and expansion are similar, TCRs and CARs differ greatly in their structure, function, and selection. TCR selection requires tumor antigen screening and HLA matching to ensure proper recognition of an HLA-peptide complex presented to a TCR. Screening affinity-enhanced TCRs for off-target reactivity is also of great necessity, particularly when targeting TAAs, given past examples of severe toxicity after infusion. CAR genes are artificially designed and constructed out of a mAb-derived scFv, which binds directly to a tumor-associated surface antigen as well as intracellular signaling domains such as CD3ζ, CD28, and 4–1BB. (Adapted from an image created with BioRender.com.)

selection and generation of high-affinity TCRs for use in cell transduction requires significant development and testing, originally via the immunization of HLA transgenic mice with tumor-specific peptides and harvesting of splenocytes for further T-cell stimulation and TCR cloning (92). Phage display technology offers another means of TCR isolation without the need for activated T-cell clones, whereby a tumor-antigen peptide gene is incorporated into a bacteriophage virion and displayed on its surface with preserved immunogenicity, however TCRs generated using this technique have been found to improve affinity at the cost of decreased target specificity (93, 94). Manufacturing mirrors that of TIL, with IL2 and human serum being used to expand transduced cells to infusion counts of roughly 1×10^{11} cells. Clinically, TCR-transduced T cells have demonstrated *in vivo* engraftment, persistence, and prolonged TCR expression that has led to durable tumor regression of several solid tumors including synovial cell sarcoma, melanoma, and HPV-associated epithelial cancers (95–97). As a result of affinity enhancement and selection, TCR-transduced T cells have produced severe off-target toxicities when targeting cancer testes antigens expressed on melanocytes and neurons (92, 98). Off-target cardiotoxicity was later documented in 2 patients who received MAGE-A3 targeted TCR T cells and found to be due to the recognition of the striated muscle-specific protein titin (99). These events stress the importance of screening affinity-enhanced TCRs for off-target and organ-specific toxicity prior to product manufacturing and demonstrate that TAAs expressed at high levels in healthy tissues should be approached with caution when selecting target antigens. More recent trials have demonstrated reduced toxicities, however tumor resistance did develop as a result of immune editing associated with increased PD-1 expression by transduced lymphocytes as well as alterations in cytokine secretion and antigen presentation pathways (100).

The future of TCR at academic centers

Much like TIL, the future of TCR therapies will rely on improved antigen selection. Academic manufacturing centers must take advantage of new transcriptomic and high-throughput single-cell technology to isolate neoantigen-specific TCRs in a reliable manner, as has already been demonstrated (101). With the expansion of identified antigenic targets, the sensitivity of screening assays must also be improved to reduce the likelihood of off-target toxicities caused by affinity-enhanced TCRs. As with CAR T cells, nonviral gene transfer systems are an appealing alternative for use in academic production and must continue to be developed. Proof of principle in manufacturing lentiviral transduced TCR T cells with high yield, purity, viability, and transduction efficiency has already been demonstrated using the CliniMACS Prodigy in an academic medical center (102). Taken together, initial results and recent developments offer a compelling rationale for the further exploration of TCR T-cell therapy by academic immunotherapy programs.

Stem Cells

Hematopoietic stem cells

As described earlier, stem-like T cells have demonstrated improved expansion potential as well as a greater likelihood of objective clinical response compared with more terminally differentiated T-cell populations. The use of both hematopoietic stem and progenitor cells (HSC) and induced pluripotent stem cells (iPSC) in the development of novel genetically engineered cell therapies thus offers promise for increased persistence and therapeutic efficacy. Harvested from bone marrow or peripheral blood, HPSCs can recruit T cells through chemoattraction, differentiate into DC promoting intratumoral T-cell activation, and reliably engraft (103). This nonspecific, HSC-driven expansion is induced by cytokines IL7 and IL9 and can preferentially drive growth of adoptively transferred TIL following myeloablation (104). Administering HSCs following myeloablation also facilitated the migration and recruitment of tumor-specific T cells to intracranial tumors, causing improved efficacy of ACT in preclinical models of glioma (105). Likewise, lineage negative HSCs, harvested from bone marrow using magnetic bead isolation, which express C-C chemokine receptor type 2 (CCR2) confer enhanced immunologic tumor sensitization and rejection in PD-1-resistant preclinical models of medulloblastoma and glioblastoma (105). A clinical trial (NCT03334305) is currently recruiting patients to assess the role of immunomodulatory stem cell therapy in high-grade glioma. The point-of-care manufacturing of lentivirus gene-modified HSCs using the CliniMACS Prodigy has demonstrated proof-of-concept and is capable of generating $CD34^+$ cell products in a semi-automated, GMP compliant workflow (106).

Induced pluripotent stem cells

iPSCs, reprogrammed from an antigen-specific cytotoxic T cells, can generate functional T cells through the recapitulation of signal transduction events *ex vivo* (107). A total of 3×10^5 iPSCs are necessary to induce differentiation and can generate over 2×10^{10} iPSC-T cells, however the entire process, from T-cell clone induction to T-cell regeneration takes roughly 4 months (107).

More efficient production systems are also being developed for the manufacturing of iPSCs, avoiding the safety and scalability issues associated with stem cell proliferation on inactivated PBMC feeder cells traditionally used to generate T-cell lineage cells. A feeder-free system encompassing all stages of manufacturing from iPSC induction to T-cell expansion allows for the generation of banks of iPSC-T cells that can be used for future "off-the-shelf" therapies, however the timeline to induce iPSCs and regenerate T cells using this method is not currently feasible for regular use in clinical manufacturing (107). A closed-loop, automated, robotic cultivation system has also been developed that cultures and splits iPSCs with similar safety and efficacy as traditional culture methods (108).

The future of stem cell therapy at academic centers

Significant refinement of HSC and iPSC protocols and demonstrable evidence of efficacy in human trials is necessary for the wider adoption of stem cell-based therapies. The production timeline for iPSCs in particular makes these a largely unviable modality for current therapeutic use, however. Nonetheless, academic programs should continue to monitor the development of stem cell-based therapies moving forward, as they can provide valuable insight into the immunomodulation of tumor microenvironments, limit T-cell exhaustion and differentiation during expansion, and potentially supplement current ACT modalities through a combinatorial approach.

Cancer Vaccines

Cancer vaccines utilize DCs and other APCs, both *in vivo* and *ex vivo*, to induce innate inflammatory responses as well as prime T-cell-associated adaptive immune responses against tumor-specific antigens. A variety of tumor antigens, including somatically mutated epitopes, viral epitopes, carcinoembryonic antigens (CEA), and self-differentiated antigens, can be delivered to DCs for surface presentation to T cells via MHC molecules (109). The first adoptive cell therapy to be approved by the FDA, sipuleucel-T, was, in fact, a DC vaccine for

castration-resistant prostate cancer consisting of *ex vivo* activated DCs loaded with the fusion protein PA2024, a protein product consisting of the prostate cancer-specific antigen prostatic acid phosphatase and the cytokine GM-CSF (110). Even with the relative success of sipuleucel-T, the majority of trials involving cancer vaccine modalities have yielded limited objective clinical benefit (111). The administration of DC vaccines with immune checkpoint inhibitors has however, yielded objective responses in a number of patients with melanoma (112, 113). Although the lack of clinical responses is cause for concern, recent evidence of the immunogenicity of cancer vaccines, including the increased frequency of cancer-specific T cells in circulation, provides optimism for the future of the treatment methodology, particularly when considered in combination with other cancer immunotherapies.

Ex vivo DC stimulation

Ex vivo DC stimulation and expansion is one of a number of modalities available for the development of DC vaccines. Following fractionation of an apheresis product, immature DCs can be generated via monocyte enrichment in a culture of IL4 and GM-CSF (88). Sufficient monocyte recovery can be achieved in a majority of patients, which have been heavily pretreated with past regimens of myelosuppressive chemotherapy, however phenotypic and functional capacity of resulting DCs may be diminished in these cell populations (114). Mature DCs are usually generated 4 to 7 days after culturing begins via the addition of aAPCs, IFNγ, poly-ICLC, or other stimulatory cytokines (115, 116). Following stimulation, they can be pulsed with a variety of antigenic materials, including: tumor specific peptides, synthetic peptides, tumor RNA, and tumor lysates (**Fig. 3**). These stimulated and pulsed DCs can then be injected through multiple routes—intradermally, intracranially, intratumorally, subcutaneously, or intralymphatically—with subcutaneous and intradermal injection being the most common.

Peptide-loaded DC vaccines

One of the initial means of loading *ex vivo* DCs involved tumor-specific peptides containing immunogenic epitopes generated through isolation of peptides from whole tumor lysate or elution from the tumor cell surface, synthetic production of tumor associated antigens, or direct co-culture with the tumor lysate itself. Pulsing DCs with tumor-derived peptides relies on proper MHC class I and class II presentation as well as sufficient tumor availability to generate specific peptides. Because of these limitations, trials using any form of pulsed protein product are generally confined to patients with HLA A2 expression (109, 117). Clinical trials have proven, however, that this technique is capable of inducing neoantigen-specific immunity and T-cell-specific expansion in patients with advanced melanoma, glioblastoma, and ovarian cancer (115, 116, 118, 119). Issues with tumor availability for use in tumor lysate and peptide vaccines can be

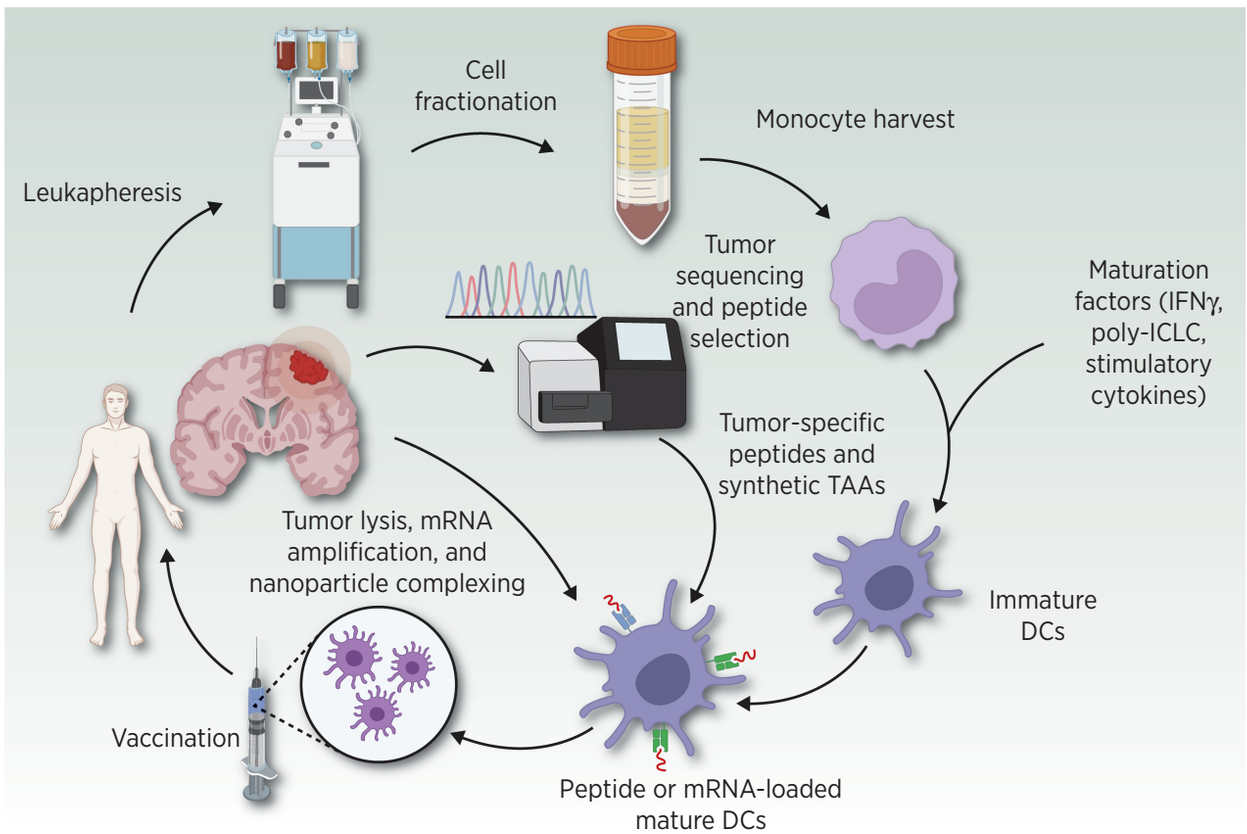

Figure 3.
General schema for the development and production of *ex vivo* stimulated DC vaccines. Patients undergo leukapheresis, after which the cell product is fractionated and monocytes are harvested. Monocytes are then cultured with stimulatory cytokines to generate immature DCs. Depending on the desired antigenic material, tumor harvested via biopsy or resection is used to generate tumor lysates, tumor mRNA, or antigen-specific peptides. Selected material is then pulsed into mature DCs, with the antigen-loaded DCs then being injected into the patient. (Adapted from an image created with BioRender.com.)

circumvented through the use of synthetic peptides and mRNA, modalities that provide greater flexibility for repeated vaccinations and serve as attractive strategies for more scalable, large-volume vaccine manufacturing. Vaccination can also be achieved through tumor neoantigen sequencing and the direct subcutaneous injection of synthetically manufactured long peptides containing antigen specific neoepitopes that are then purified and admixed with poly-ICLC (120). These long peptides are generated using prediction algorithms that model neoepitope binding affinity with patient-associated HLA molecules. Peptide-based DCs have been found to successfully generate both systemic and intratumoral neoantigen specific $CD4^+$ and $CD8^+$ T-cell responses against a majority of the neoantigens with a small number of clinical responses in patients with high-risk melanoma (120). It should be noted that, despite antigen selection based on predicted HLA class I binding, these peptide vaccines generate greater class II stimulation and a predominantly $CD4^+$ T-cell response (112). More detailed analysis of personalized neoantigen sequencing is beyond the scope of this review and has been discussed extensively in recent literature (121).

mRNA-loaded DC vaccines

Previously mentioned challenges associated with the MHC class restriction dictating peptide vaccine presentation can effectively be circumvented through the use of mRNA pulsing and vaccination, a modality that utilizes the intracellular translational and processing machinery of a DC cell to present tumor-associated neoepitopes specific to patient's HLA haplotype (109). Evidence of immunologic response, characterized by sustained T-cell antigen specificity and selective expansion of antigen-specific T cells, has been demonstrated against mRNA generated CEA, total tumor RNA, and tumor-associated self-antigens (111, 122, 123). Nonmutated tumor-associated antigens, such as the melanoma-associated antigens NY-ESO-1 and MAGE-A3, can also be targeted and have effectively produced expanded T-cell populations with specificity against a broad range of neoantigens (124). Further clinical trials showed that a robust immune response initiated by DC vaccination with nonmutated tumor-associated antigens can, in fact, override central T-cell tolerance and drive both $CD4^+$ and $CD8^+$ T-cell expansion (113). Uptake and expression of RNA encoded antigens can be further improved with the use of RNA-loaded cationic nanoliposomes (RNA-NP), which protect RNA from extracellular degradation while also triggering the innate immune response and secretion of inflammatory markers to further stimulate cellular activation of DCs and macrophages (125). The cationic lipid 1,2-dioleoyl-3-trimethylammonium-propane offers a promising safety profile and, when complexed to tumor derived RNA in murine models, increased MHC, B7, and APC maturation markers while simultaneously expanding antigen-specific T cells with greater efficacy than peptide vaccines (117). Loading RNA-NPs with iron oxide was also found to enhance DC migration to lymph nodes, stimulate the inhibition of tumor growth in mouse models, and allowing for the prediction of vaccine induced antitumor response via MRI (126).

Vaccine site preconditioning

Immunogenicity and DC migration to vaccine draining lymph nodes can be further improved through the preconditioning of the vaccine site with the recall antigen tetanus/diphtheria (Td) toxoid prior to injection (127). In murine studies, the increased migration of DCs in Td-treated mice was dependent on the expression of cytokine, CCL3 (74). Similar results were later inferred within human subjects in a series of clinical trials, demonstrating improved survival as well as increased DC migration to draining lymph nodes among patients preconditioned with Td, further confirming that CCL3 is an important mediator of DC homing and migration (75).

The future of DC vaccines at academic centers

Gene transfection and DC vaccination must also be optimized as manufacturing workflows shift toward automation. The electroporation of DCs with mRNAs has been performed in several DC vaccination clinical trials with results, demonstrating a substantial increase in immune stimulation as well as good safety and tolerability profiles (128, 129). Likewise, the electroporation of T cells with mRNA has demonstrated reduced cytotoxicity when compared with viral vectors, as mRNA is not capable of genomic integration, and does not have the capacity to generate replication competent retroviruses (130). mRNA electroporation also has high throughput capacity allowing for multiple rounds of electroporation when necessary, such as in the event of loss of transgene expression (131). Cytotoxicity associated with high voltage electroporation has been observed, however cell electroporation protocols at optimized voltages have yielded T-cell products with high viability and transfection efficiency (132, 133). Optimizing the concentration of genetic material electroporated, cell density, and length of T cell of stimulation were also found to improve transgene expression and cell viability (134).

A major limitation to the broader application of electroporation in ACT manufacturing continues to be the lack devices capable of producing cell product quickly and with high transfection efficiency. Most macroscale commercial electroporation machines are designed for research grade transfection, which is unsatisfactory for use in clinical grade manufacturing. Likewise, microfluidic electroporation devices are not capable of generating transfected cell products at a speed and volume necessary for the manufacturing of large-scale cell therapies. By integrating components of both systems, such as a larger sized, continuous flow tube and improved electrode arrays, new electroporation devices are being developed with the capability of processing cells at a rate of 20 million cells per minute (132, 135). These mechanical advancements, coupled with the ability to manufacture cell products with limited tumor material, make mRNA electroporation particularly amenable to scalable GMP production of DC vaccines moving forward.

Academic-Industry Partnership: A Catalyst for Future Innovation

These recent advancements in the scalability and optimization of academic manufacturing workflows underscore the need for collaboration between academia and industry to fully realize the therapeutic potential of ACT. The further development of closed and automated manufacturing systems is vital to creating more cost-effective, safe, and reproducible cell therapies that both meets the growing patient demand and offers feasible implementation within health care systems. With the success and FDA-approval of ACT platforms for hematologic malignances, industry interest and engagement in ACT development for a variety of malignant diseases has expanded rapidly. High-quality cellular manufacturing at clinical-scale has become recognized as an important and often critical bottleneck to the successful advancement and adoption of ACT platforms in clinical oncology. This exploding commercial interest presents both strategic opportunities and challenges for academic centers invested or considering investment in clinical-grade cellular manufacturing facilities and programs. The evolving regulatory landscape and expanded capabilities by the biopharma industry will threaten the niches enjoyed in this space by

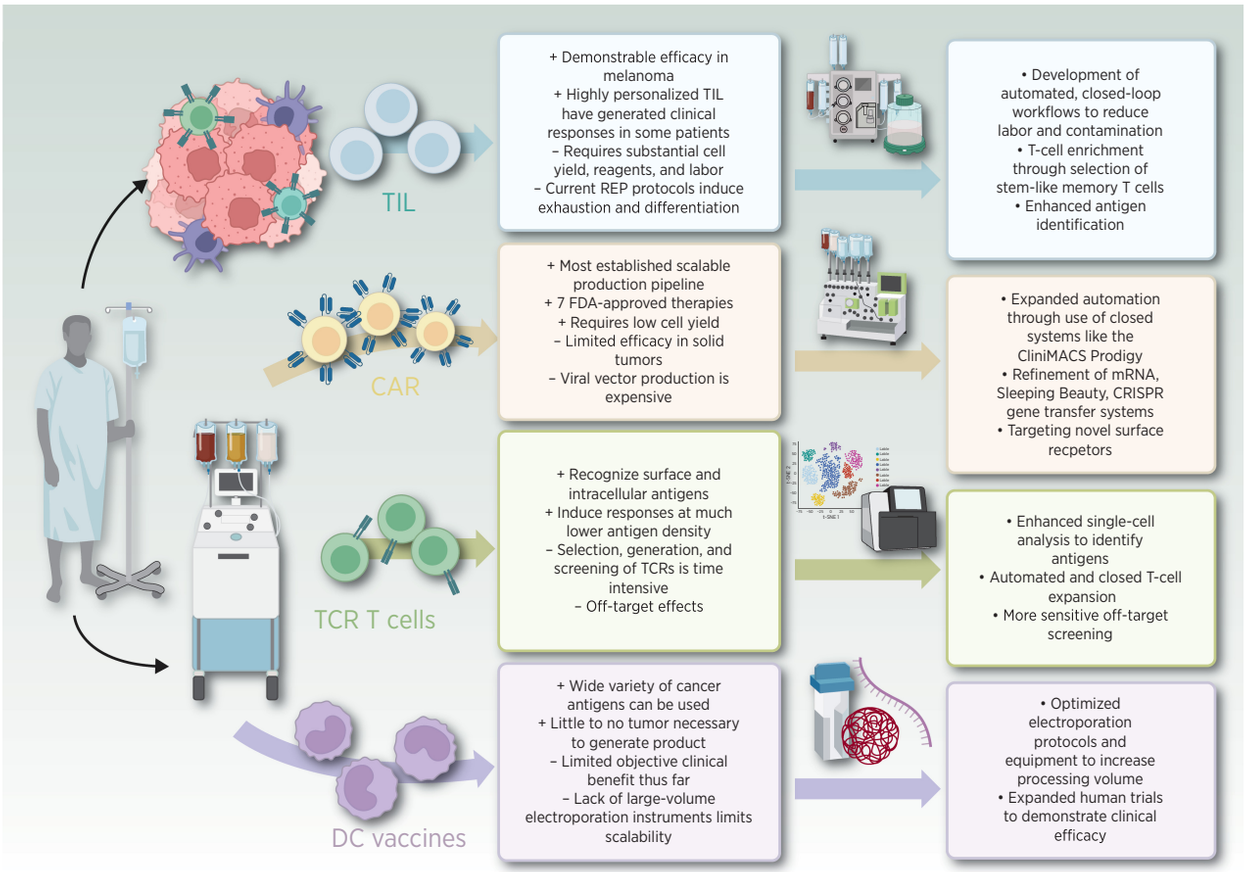

Figure 4.

Overview of adoptive cell therapies: their strengths, limitations, and promising new developments for academic production paradigms. The future of adoptive cell therapy at academic manufacturing centers will rely on the adoption and refinement of new technologies and production workflows. Academic centers must balance the unique strengths (indicated by "+" signs) and limitations (indicated by "−" signs) of each ACT modality with the logistical and regulatory pressures of GMP manufacturing at their respective institutions. The rapid development of closed-loop manufacturing systems, driven largely by industry and commercialization, and high-throughput cell sequencing platforms will enable the large-scale selection and expansion of T-cell products at academic centers. Associated protocols developed using these platforms will be crucial to future clinical trials, the development of academia-industry partnerships, and establishing ACT as a valuable component of cancer treatment for both hematologic and solid malignancies. (Adapted from an image created with BioRender.com.)

academic centers with clinical cellular manufacturing capabilities. However, this same competitive interest also opens the door to new collaborative possibilities and underscores the translational relevance of addressing scientific questions in the field of cellular therapy and manufacturing. Thus, academic centers have an expanded opportunity to develop a strategic vision for spearheading endeavors in ACT of cancer with relative assurance of numerous growth opportunities in the coming years.

Conclusion

As evidenced in this review, clinical trials of ACT, while faced with several key challenges in generating responses against a wide variety of solid cancers, has demonstrated feasibility, clinical activity, and an expanding foundation of knowledge regarding fundamental immunobiology. Utilizing this new-found knowledge, supplemented by innovations in molecular biology, genetic engineering, and cell processing, will be crucial to the development of more scalable, cost-effective, and, most importantly, efficacious cell therapies (**Fig. 4**). Initial demonstrations of improved clinical responses generated using ACT in combination with other therapies, be it additional forms of ACT or immune checkpoint inhibitors, suggests an intriguing path forward for future clinical trials. Ultimately, the unique position academic medical centers hold as clinical trial sites, cellular production facilities, and hubs of innovation, make them vital players in the development and manufacturing of more effective cancer immunotherapies.

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References

1. Goff SL, Dudley ME, Citrin DE, Somerville RP, Wunderlich JR, Danforth DN, et al. Randomized, prospective evaluation comparing intensity of lymphodepletion before adoptive transfer of tumor-infiltrating lymphocytes for patients with metastatic melanoma. J Clin Oncol 2016;34:2389–97.
2. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014;371:1507–17.
3. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science 2015;348:62–8.
4. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 2011;17:4550–7.
5. Besser MJ, Shapira-Frommer R, Itzhaki O, Treves AJ, Zippel DB, Levy D, et al. Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: intent-to-treat analysis and efficacy after failure to prior immunotherapies. Clin Cancer Res 2013;19:4792–800.
6. Pilon-Thomas S, Kuhn L, Ellwanger S, Janssen W, Royster E, Marzban S, et al. Efficacy of adoptive cell transfer of tumor-infiltrating lymphocytes after lymphopenia induction for metastatic melanoma. J Immunother 2012;35:615–20.
7. Radvanyi LG, Bernatchez C, Zhang M, Fox PS, Miller P, Chacon J, et al. Specific lymphocyte subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients. Clin Cancer Res 2012;18:6758–70.
8. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 2002;298:850–4.
9. Jin J, Sabatino M, Somerville R, Wilson JR, Dudley ME, Stroncek DF, et al. Simplified method of the growth of human tumor infiltrating lymphocytes in gas-permeable flasks to numbers needed for patient treatment. J Immunother 2012;35:283–92.
10. Dudley ME, Wunderlich JR, Shelton TE, Even J, Rosenberg SA. Generation of tumor-infiltrating lymphocyte cultures for use in adoptive transfer therapy for melanoma patients. J Immunother 2003;26:332–42.
11. Pagès F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med 2005;353:2654–66.
12. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A 2005;102:18538–43.
13. Itzhaki O, Hovav E, Ziporen Y, Levy D, Kubi A, Zikich D, et al. Establishment and large-scale expansion of minimally cultured "young" tumor infiltrating lymphocytes for adoptive transfer therapy. J Immunother 2011;34:212–20.
14. Gros A, Robbins PF, Yao X, Li YF, Turcotte S, Tran E, et al. PD-1 identifies the patient-specific $CD8^+$ tumor-reactive repertoire infiltrating human tumors. J Clin Invest 2014;124:2246–59.
15. Gros A, Tran E, Parkhurst MR, Ilyas S, Pasetto A, Groh EM, et al. Recognition of human gastrointestinal cancer neoantigens by circulating PD-1+ lymphocytes. J Clin Invest 2019;129:4992–5004.
16. Duhen R, Ballesteros-Merino C, Frye AK, Tran E, Rajamanickam V, Chang SC, et al. Neoadjuvant anti-OX40 (MEDI6469) therapy in patients with head and neck squamous cell carcinoma activates and expands antigen-specific tumor-infiltrating T cells. Nat Commun 2021;12:1047.
17. Gattinoni L, Klebanoff CA, Palmer DC, Wrzesinski C, Kerstann K, Yu Z, et al. Acquisition of full effector function in vitro paradoxically impairs the in vivo antitumor efficacy of adoptively transferred CD8+ T cells. J Clin Invest 2005;115:1616–26.
18. Buchholz VR, Flossdorf M, Hensel I, Kretschmer L, Weissbrich B, Gräf P, et al. Disparate individual fates compose robust CD8+ T cell immunity. Science 2013;340:630–5.
19. Gattinoni L, Lugli E, Ji Y, Pos Z, Paulos CM, Quigley MF, et al. A human memory T cell subset with stem cell-like properties. Nat Med 2011;17:1290–7.
20. Krishna S, Lowery FJ, Copeland AR, Bahadiroglu E, Mukherjee R, Jia L, et al. Stem-like CD8 T cells mediate response of adoptive cell immunotherapy against human cancer. Science 2020;370:1328–34.
21. Mehta GU, Malekzadeh P, Shelton T, White DE, Butman JA, Yang JC, et al. Outcomes of adoptive cell transfer with Tumor-infiltrating lymphocytes for metastatic melanoma patients with and without brain metastases. J Immunother 2018;41:241–7.
22. Leko V, Cafri G, Yossef R, Paria B, Hill V, Gurusamy D, et al. Identification of neoantigen-reactive T lymphocytes in the peripheral blood of a patient with glioblastoma. J Immunother Cancer 2021;9:e002882.
23. Liu Z, Meng Q, Bartek J, Poiret T, Persson O, Rane L, et al. Tumor-infiltrating lymphocytes (TILs) from patients with glioma. Oncoimmunology 2017;6:e1252894.
24. Mitsdoerffer M, Aly L, Barz M, Engleitner T, Sie C, Delbridge C, et al. The glioblastoma multiforme tumor site promotes the commitment of tumor-infiltrating lymphocytes to the T. Proc Natl Acad Sci U S A 2022;119:e2206208119.
25. Quattrocchi KB, Miller CH, Cush S, Bernard SA, Dull ST, Smith M, et al. Pilot study of local autologous tumor infiltrating lymphocytes for the treatment of recurrent malignant gliomas. J Neurooncol 1999;45:141–57.
26. Tran E, Robbins PF, Lu YC, Prickett TD, Gartner JJ, Jia L, et al. T-cell transfer therapy targeting mutant KRAS in cancer. N Engl J Med 2016;375:2255–62.
27. Zacharakis N, Chinnasamy H, Black M, Xu H, Lu YC, Zheng Z, et al. Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. Nat Med 2018;24:724–30.
28. Gannon PO, Harari A, Auger A, Murgues C, Zangiacomi V, Rubin O, et al. Development of an optimized closed and semi-automatic protocol for Good Manufacturing Practice manufacturing of tumor-infiltrating lymphocytes in a hospital environment. Cytotherapy 2020;22:780–91.
29. Heemskerk B, Maeder C, Criado-Moronati E, Boettcher L, Kaiser A, Assenmacher M, et al. Abstract B016: Automated ex vivo expansion of low numbers of tumor-reactive T-cells on the CliniMACS Prodigy®. Cancer Immunol Res 2019;7:2s (suppl. abstr. B016).
30. Lowery FJ, Krishna S, Yossef R, Parikh NB, Chatani PD, Zacharakis N, et al. Molecular signatures of antitumor neoantigen-reactive T cells from metastatic human cancers. Science 2022;375:877–84.
31. Yang W, Lee KW, Srivastava RM, Kuo F, Krishna C, Chowell D, et al. Immunogenic neoantigens derived from gene fusions stimulate T cell responses. Nat Med 2019;25:767–75.
32. Roudko V, Bozkus CC, Orfanelli T, McClain CB, Carr C, O'Donnell T, et al. Shared Immunogenic Poly-Epitope Frameshift Mutations in Microsatellite Unstable Tumors. Cell 2020;183:1634–49.
33. Gee MH, Han A, Lofgren SM, Beausang JF, Mendoza JL, Birnbaum ME, et al. Antigen identification for Orphan T cell receptors expressed on Tumor-infiltrating lymphocytes. Cell 2018;172:549–63.
34. Baird JH, Frank MJ, Craig J, Patel S, Spiegel JY, Sahaf B, et al. CD22-Directed CAR T-cell therapy induces complete remissions in CD19-Directed CAR-refractory Large B-cell lymphoma. Blood 2021;137:2321–5.
35. Cappell KM, Sherry RM, Yang JC, Goff SL, Vanasse DA, McIntyre L, et al. Long-term Follow-up of Anti-CD19 chimeric antigen receptor T-cell therapy. J Clin Oncol 2020;38:3805–15.
36. Kochenderfer JN, Dudley ME, Kassim SH, Somerville RP, Carpenter RO, Stetler-Stevenson M, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with

autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol 2015;33:540–9.
37. Chong EA, Ruella M, Schuster SJ., Lymphoma Program Investigators at the University of Pennsylvania. Five-year outcomes for refractory B-cell lymphomas with CAR T-cell therapy. N Engl J Med 2021;384:673–4.
38. Raje N, Berdeja J, Lin Y, Siegel D, Jagannath S, Madduri D, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. N Engl J Med 2019;380:1726–37.
39. Majzner RG, Ramakrishna S, Yeom KW, Patel S, Chinnasamy H, Schultz LM, et al. GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas. Nature 2022;603:934–41.
40. Ahmed N, Brawley V, Hegde M, Bielamowicz K, Kalra M, Landi D, et al. HER2-specific chimeric antigen Receptor-modified Virus-specific T cells for progressive glioblastoma: A Phase 1 Dose-escalation trial. JAMA Oncol 2017;3:1094–101.
41. Akhavan D, Alizadeh D, Wang D, Weist MR, Shepphird JK, Brown CE. CAR T cells for brain tumors: lessons learned and road ahead. Immunol Rev 2019;290:60–84.
42. Brudno JN, Lam N, Vanasse D, Shen YW, Rose JJ, Rossi J, et al. Safety and feasibility of anti-CD19 CAR T cells with fully human binding domains in patients with B-cell lymphoma. Nat Med 2020;26:270–80.
43. Barrett DM, Singh N, Liu X, Jiang S, June CH, Grupp SA, et al. Relation of clinical culture method to T-cell memory status and efficacy in xenograft models of adoptive immunotherapy. Cytotherapy 2014;16:619–30.
44. Wang X, Rivière I. Clinical manufacturing of CAR T cells: foundation of a promising therapy. Mol Ther Oncolytics 2016;3:16015.
45. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med 2013;368:1509–18.
46. Sommermeyer D, Hudecek M, Kosasih PL, Gogishvili T, Maloney DG, Turtle CJ, et al. Chimeric antigen receptor-modified T cells derived from defined CD8+ and CD4+ subsets confer superior antitumor reactivity in vivo. Leukemia 2016;30:492–500.
47. Terakura S, Yamamoto TN, Gardner RA, Turtle CJ, Jensen MC, Riddell SR. Generation of CD19-chimeric antigen receptor modified CD8+ T cells derived from virus-specific central memory T cells. Blood 2012;119:72–82.
48. Casati A, Varghaei-Nahvi A, Feldman SA, Assenmacher M, Rosenberg SA, Dudley ME, et al. Clinical-scale selection and viral transduction of human naïve and central memory CD8+ T cells for adoptive cell therapy of cancer patients. Cancer Immunol Immunother 2013;62:1563–73.
49. Meng J, Wu X, Sun Z, Xun R, Liu M, Hu R, et al. Efficacy and safety of CAR-T cell products axicabtagene ciloleucel, tisagenlecleucel, and lisocabtagene maraleucel for the treatment of hematologic malignancies: a systematic review and meta-analysis. Front Oncol 2021;11:698607.
50. Shah NN, Highfill SL, Shalabi H, Yates B, Jin J, Wolters PL, et al. CD4/CD8 T-cell selection affects chimeric antigen receptor (CAR) T-cell potency and toxicity: updated results from a phase I anti-CD22 CAR T-cell trial. J Clin Oncol 2020;38:1938–50.
51. Hinrichs CS, Borman ZA, Gattinoni L, Yu Z, Burns WR, Huang J, et al. Human effector CD8+ T cells derived from naive rather than memory subsets possess superior traits for adoptive immunotherapy. Blood 2011;117:808–14.
52. Berger C, Jensen MC, Lansdorp PM, Gough M, Elliott C, Riddell SR. Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates. J Clin Invest 2008;118:294–305.
53. Arcangeli S, Falcone L, Camisa B, De Girardi F, Biondi M, Giglio F, et al. Next-generation manufacturing protocols enriching T. Front Immunol 2020;11:1217.
54. Arcangeli S, Bove C, Mezzanotte C, Camisa B, Falcone L, Manfredi F, et al. CAR T cell manufacturing from naive/stem memory T lymphocytes enhances antitumor responses while curtailing cytokine release syndrome. J Clin Invest 2022;132:e150807.
55. Liu E, Marin D, Banerjee P, Macapinlac HA, Thompson P, Basar R, et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. N Engl J Med 2020;382:545–53.
56. Wang X, Borquez-Ojeda O, Stefanski J, Du F, Qu J, Chaudhari J, et al. Depletion of high-content CD14. Mol Ther Methods Clin Dev 2021;22:377–87.
57. Rech AJ, Mick R, Martin S, Recio A, Aqui NA, Powell DJ, et al. CD25 blockade depletes and selectively reprograms regulatory T cells in concert with immunotherapy in cancer patients. Sci Transl Med 2012;4:134ra62.
58. Abou-El-Enein M, Elsallab M, Feldman SA, Fesnak AD, Heslop HE, Marks P, et al. Scalable manufacturing of CAR T cells for cancer immunotherapy. Blood Cancer Discov 2021;2:408–22.
59. Scholler J, Brady TL, Binder-Scholl G, Hwang WT, Plesa G, Hege KM, et al. Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. Sci Transl Med 2012;4:132ra53.
60. Shah NN, Qin H, Yates B, Su L, Shalabi H, Raffeld M, et al. Clonal expansion of CAR T cells harboring lentivector integration in the CBL gene following anti-CD22 CAR T-cell therapy. Blood Adv 2019;3:2317–22.
61. Roth TL, Puig-Saus C, Yu R, Shifrut E, Carnevale J, Li PJ, et al. Reprogramming human T cell function and specificity with non-viral genome targeting. Nature 2018;559:405–9.
62. Kebriaei P, Singh H, Huls MH, Figliola MJ, Bassett R, Olivares S, et al. Phase I trials using sleeping beauty to generate CD19-specific CAR T cells. J Clin Invest 2016;126:3363–76.
63. Magnani CF, Gaipa G, Lussana F, Belotti D, Gritti G, Napolitano S, et al. Sleeping Beauty-engineered CAR T cells achieve antileukemic activity without severe toxicities. J Clin Invest 2020;130:6021–33.
64. Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E, et al. CRISPR-engineered T cells in patients with refractory cancer. Science 2020;367:eaba7365.
65. Parayath NN, Stephan SB, Koehne AL, Nelson PS, Stephan MT. In vitro-transcribed antigen receptor mRNA nanocarriers for transient expression in circulating T cells in vivo. Nat Commun 2020;11:6080.
66. Zhao Y, Zheng Z, Cohen CJ, Gattinoni L, Palmer DC, Restifo NP, et al. High-efficiency transfection of primary human and mouse T lymphocytes using RNA electroporation. Mol Ther 2006;13:151–9.
67. Xin T, Cheng L, Zhou C, Zhao Y, Hu Z, Wu X. Induced CAR-T cell for the potential breakthrough to overcome the barriers of current CAR-T cell therapy. Front Oncol 2022;12:809754.
68. Nawaz W, Huang B, Xu S, Li Y, Zhu L, Yiqiao H, et al. AAV-mediated in vivo CAR gene therapy for targeting human T-cell leukemia. Blood Cancer J 2021;11:119.
69. Larson RC, Maus MV. Recent advances and discoveries in the mechanisms and functions of CAR T cells. Nat Rev Cancer 2021;21:145–61.
70. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science 2018;359:1361–5.
71. June CH, Sadelain M. Chimeric antigen receptor therapy. N Engl J Med 2018;379:64–73.
72. Boomer JS, Green JM. An enigmatic tail of CD28 signaling. Cold Spring Harb Perspect Biol 2010;2:a002436.
73. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol 2013;13:227–42.
74. Cappell KM, Kochenderfer JN. A comparison of chimeric antigen receptors containing CD28 versus 4–1BB costimulatory domains. Nat Rev Clin Oncol 2021;18:715–27.
75. Stock S, Schmitt M, Sellner L. Optimizing manufacturing protocols of chimeric antigen receptor T cells for improved anticancer immunotherapy. Int J Mol Sci 2019;20:6223.
76. Guedan S, Madar A, Casado-Medrano V, Shaw C, Wing A, Liu F, et al. Single residue in CD28-costimulated CAR-T cells limits long-term persistence and antitumor durability. J Clin Invest 2020;130:3087–97.
77. Lynn RC, Weber EW, Sotillo E, Gennert D, Xu P, Good Z, et al. c-Jun overexpression in CAR T cells induces exhaustion resistance. Nature 2019;576:293–300.
78. Xu Y, Zhang M, Ramos CA, Durett A, Liu E, Dakhova O, et al. Closely related T-memory stem cells correlate with *in vivo* expansion of CAR.CD19-T cells and are preserved by IL-7 and IL-15. Blood 2014;123:3750–9.
79. Shah NN, Zurko JC, Schneider D, Yim S, Hamadani M, Fenske TS, et al. Phase 1/2 trial of IL7/IL15-expanded bispecific LV20.19 CAR T-cells for relapsed, refractory B-cell non-Hodgkin lymphoma. Blood 2021;138.
80. Weber EW, Parker KR, Sotillo E, Lynn RC, Anbunathan H, Lattin J, et al. Transient rest restores functionality in exhausted CAR-T cells through epigenetic remodeling. Science 2021;372:eaba1786.
81. Roddie C, O'Reilly M, Dias Alves Pinto J, Vispute K, Lowdell M. Manufacturing chimeric antigen receptor T cells: issues and challenges. Cytotherapy 2019;21:327–40.
82. Jackson Z, Roe A, Sharma AA, Lopes FBTP, Talla A, Kleinsorge-Block S, et al. Automated manufacture of autologous CD19 CAR-T cells for treatment of Non-hodgkin lymphoma. Front Immunol 2020;11:1941.

83. Castella M, Caballero-Baños M, Ortiz-Maldonado V, González-Navarro EA, Suñé G, Antoñana-Vidósola A, et al. Point-Of-Care CAR T-Cell Production (ARI-0001) using a closed Semi-automatic bioreactor: experience from an academic phase I clinical trial. Front Immunol 2020;11:482.
84. Straetemans T, Gründer C, Heijhuurs S, Hol S, Slaper-Cortenbach I, Bönig H, et al. Untouched GMP-ready purified engineered immune cells to treat cancer. Clin Cancer Res 2015;21:3957–68.
85. Klapper JA, Thomasian AA, Smith DM, Gorgas GC, Wunderlich JR, Smith FO, et al. Single-pass, closed-system rapid expansion of lymphocyte cultures for adoptive cell therapy. J Immunol Methods 2009;345:90–9.
86. Fernández L, Fernández A, Mirones I, Escudero A, Cardoso L, Vela M, et al. GMP-compliant manufacturing of NKG2D CAR memory T cells using CliniMACS prodigy. Front Immunol 2019;10:2361.
87. Mock U, Nickolay L, Philip B, Cheung GW, Zhan H, Johnston ICD, et al. Automated manufacturing of chimeric antigen receptor T cells for adoptive immunotherapy using CliniMACS prodigy. Cytotherapy 2016;18:1002–11.
88. Hopewell EL, Cox C. Manufacturing dendritic cells for immunotherapy: monocyte enrichment. Mol Ther Methods Clin Dev 2020;16:155–60.
89. Shah NN, Johnson BD, Schneider D, Zhu F, Szabo A, Keever-Taylor CA, et al. Bispecific anti-CD20, anti-CD19 CAR T cells for relapsed B cell malignancies: a phase 1 dose escalation and expansion trial. Nat Med 2020;26:1569–75.
90. Gill S, Maus MV, Porter DL. Chimeric antigen receptor T cell therapy: 25years in the making. Blood Rev 2016;30:157–67.
91. Chandran SS, Somerville RPT, Yang JC, Sherry RM, Klebanoff CA, Goff SL, et al. Treatment of metastatic uveal melanoma with adoptive transfer of tumour-infiltrating lymphocytes: a single-centre, two-stage, single-arm, phase 2 study. Lancet Oncol 2017;18:792–802.
92. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood 2009;114:535–46.
93. Smith GP. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science 1985;228:1315–7.
94. Zhao Y, Bennett AD, Zheng Z, Wang QJ, Robbins PF, Yu LY, et al. High-affinity TCRs generated by phage display provide CD4+ T cells with the ability to recognize and kill tumor cell lines. J Immunol 2007;179:5845–54.
95. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science 2006;314:126–9.
96. Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, Dudley ME, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol 2011;29:917–24.
97. Nagarsheth NB, Norberg SM, Sinkoe AL, Adhikary S, Meyer TJ, Lack JB, et al. TCR-engineered T cells targeting E7 for patients with metastatic HPV-associated epithelial cancers. Nat Med 2021;27:419–25.
98. Morgan RA, Chinnasamy N, Abate-Daga D, Gros A, Robbins PF, Zheng Z, et al. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. J Immunother 2013;36:133–51.
99. Linette GP, Stadtmauer EA, Maus MV, Rapoport AP, Levine BL, Emery L, et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. Blood 2013;122:863–71.
100. Doran SL, Stevanović S, Adhikary S, Gartner JJ, Jia L, Kwong MLM, et al. T-cell receptor gene therapy for human papillomavirus-associated epithelial cancers: a first-in-human, phase I/II study. J Clin Oncol 2019;37:2759–68.
101. Lu YC, Zheng Z, Lowery FJ, Gartner JJ, Prickett TD, Robbins PF, et al. Direct identification of neoantigen-specific TCRs from tumor specimens by high-throughput single-cell sequencing. J Immunother Cancer 2021;9:e002595.
102. Silva DN, Chrobok M, Rovesti G, Healy K, Wagner AK, Maravelia P, et al. Process development for adoptive cell therapy in academia: a pipeline for clinical-scale manufacturing of multiple TCR-T cell products. Front Immunol 2022;13:896242.
103. Wildes TJ, Flores CT, Mitchell DA. Concise Review: modulating cancer immunity with hematopoietic stem and progenitor cells. Stem Cells 2019;37:166–75.
104. Wrzesinski C, Paulos CM, Gattinoni L, Palmer DC, Kaiser A, Yu Z, et al. Hematopoietic stem cells promote the expansion and function of adoptively transferred antitumor CD8 T cells. J Clin Invest 2007;117:492–501.
105. Flores CT, Wildes TJ, Drake JA, Moore GL, Dean BD, Abraham RS, et al. $Lin^{-}CCR2^{+}$ hematopoietic stem and progenitor cells overcome resistance to PD-1 blockade. Nat Commun 2018;9:4313.
106. Adair JE, Waters T, Haworth KG, Kubek SP, Trobridge GD, Hocum JD, et al. Semi-automated closed system manufacturing of lentivirus gene-modified haematopoietic stem cells for gene therapy. Nat Commun 2016;7:13173.
107. Iriguchi S, Yasui Y, Kawai Y, Arima S, Kunitomo M, Sato T, et al. A clinically applicable and scalable method to regenerate T-cells from iPSCs for off-the-shelf T-cell immunotherapy. Nat Commun 2021;12:430.
108. Kami D, Watakabe K, Yamazaki-Inoue M, Minami K, Kitani T, Itakura Y, et al. Large-scale cell production of stem cells for clinical application using the automated cell processing machine. BMC Biotechnol 2013;13:102.
109. Sayour EJ, Mitchell DA. Manipulation of innate and adaptive immunity through cancer vaccines. J Immunol Res 2017;2017:3145742.
110. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010;363:411–22.
111. Cafri G, Gartner JJ, Zaks T, Hopson K, Levin N, Paria BC, et al. mRNA vaccine-induced neoantigen-specific T cell immunity in patients with gastrointestinal cancer. J Clin Invest 2020;130:5976–88.
112. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature 2017;547:217–21.
113. Sahin U, Oehm P, Derhovanessian E, Jabulowsky RA, Vormehr M, Gold M, et al. An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. Nature 2020;585:107–12.
114. Nair SK, Driscoll T, Boczkowski D, Schmittling R, Reynolds R, Johnson LA, et al. Ex vivo generation of dendritic cells from cryopreserved, post-induction chemotherapy, mobilized leukapheresis from pediatric patients with medulloblastoma. J Neurooncol 2015;125:65–74.
115. Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, et al. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science 2015;348:803–8.
116. Block MS, Dietz AB, Gustafson MP, Kalli KR, Erskine CL, Youssef B, et al. Th17-inducing autologous dendritic cell vaccination promotes antigen-specific cellular and humoral immunity in ovarian cancer patients. Nat Commun 2020;11:5173.
117. Sayour EJ, De Leon G, Pham C, Grippin A, Kemeny H, Chua J, et al. Systemic activation of antigen-presenting cells via RNA-loaded nanoparticles. Oncoimmunology 2017;6:e1256527.
118. Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med 2011;364:2119–27.
119. Wen PY, Reardon DA, Armstrong TS, Phuphanich S, Aiken RD, Landolfi JC, et al. A randomized double-blind placebo-controlled phase II trial of dendritic cell vaccine ICT-107 in newly diagnosed patients with glioblastoma. Clin Cancer Res 2019;25:5799–807.
120. Keskin DB, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, Li S, et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. Nature 2019;565:234–9.
121. Leko V, Rosenberg SA. Identifying and targeting human tumor antigens for T Cell-based immunotherapy of solid tumors. Cancer Cell 2020;38:454–72.
122. Morse MA, Nair SK, Mosca PJ, Hobeika AC, Clay TM, Deng Y, et al. Immunotherapy with autologous, human dendritic cells transfected with carcinoembryonic antigen mRNA. Cancer Invest 2003;21:341–9.
123. Nair SK, De Leon G, Boczkowski D, Schmittling R, Xie W, Staats J, et al. Recognition and killing of autologous, primary glioblastoma tumor cells by human cytomegalovirus pp65-specific cytotoxic T cells. Clin Cancer Res 2014;20:2684–94.
124. Sahin U, Derhovanessian E, Miller M, Kloke BP, Simon P, Löwer M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature 2017;547:222–6.
125. Kranz LM, Diken M, Haas H, Kreiter S, Loquai C, Reuter KC, et al. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. Nature 2016;534:396–401.
126. Grippin AJ, Wummer B, Wildes T, Dyson K, Trivedi V, Yang C, et al. Dendritic Cell-activating magnetic nanoparticles enable early prediction of antitumor response with magnetic resonance imaging. ACS Nano 2019;13:13884–98.
127. Mitchell DA, Batich KA, Gunn MD, Huang MN, Sanchez-Perez L, Nair SK, et al. Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. Nature 2015;519:366–9.
128. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov 2018;17:261–79.
129. Wilgenhof S, Corthals J, Heirman C, van Baren N, Lucas S, Kvistborg P, et al. Phase II study of autologous Monocyte-derived mRNA electroporated

dendritic cells (TriMixDC-MEL) Plus ipilimumab in patients with pretreated advanced melanoma. J Clin Oncol 2016;34:1330–8.
130. Wang Y, Zhang Z, Luo J, Han X, Wei Y, Wei X. mRNA vaccine: a potential therapeutic strategy. Mol Cancer 2021;20:33.
131. Choi Y, Yuen C, Maiti SN, Olivares S, Gibbons H, Huls H, et al. A high throughput microelectroporation device to introduce a chimeric antigen receptor to redirect the specificity of human T cells. Biomed Microdevices 2010;12:855–63.
132. Lissandrello CA, Santos JA, Hsi P, Welch M, Mott VL, Kim ES, et al. High-throughput continuous-flow microfluidic electroporation of mRNA into primary human T cells for applications in cellular therapy manufacturing. Sci Rep 2020;10:18045.
133. Alzubi J, Lock D, Rhiel M, Schmitz S, Wild S, Mussolino C, et al. Automated generation of gene-edited CAR T cells at clinical scale. Mol Ther Methods Clin Dev 2021;20:379–88.
134. Zhang Z, Qiu S, Zhang X, Chen W. Optimized DNA electroporation for primary human T cell engineering. BMC Biotechnol 2018;18:4.
135. Zhao D, Huang D, Li Y, Wu M, Zhong W, Cheng Q, et al. A Flow-through cell electroporation device for rapidly and efficiently transfecting massive amounts of cells in vitro and ex vivo. Sci Rep 2016;6:18469.