review

Cancer immunotherapy with CAR T cells: well-trodden paths and journey along lesser-known routes

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Background. Chimeric antigen receptor (CAR) T cell therapy is a clinically approved cancer immunotherapy approach using genetically engineered T cells. The success of CAR T cells has been met with challenges regarding efficacy and safety. Although a broad spectrum of CAR T cell variants and applications is emerging, this review focuses on CAR T cells for the treatment of cancer. In the first part, the general principles of adoptive cell transfer, the architecture of the CAR molecule, and the effects of design on function are presented. The second part describes five conceptual challenges that hinder the success of CAR T cells; immunosuppressive tumour microenvironment, T cell intrinsic properties, tumour targeting, manufacturing cellular product, and immune-related adverse events. Throughout the review, selected current approaches to address these issues are presented.

Conclusions. Cancer immunotherapy with CAR T cells represents a paradigm shift in the treatment of certain blood cancers that do not respond to other available treatment options. Well-trodden paths taken by pioneers led to the first clinical approval, and now the journey continues down lesser-known paths to treat a variety of cancers and other serious diseases with CAR T cells.

Key words: chimeric antigen receptor; adoptive cell therapy; cancer; cellular immunotherapy; gene-engineered immune cells

Introduction

It took a series of ground-breaking ideas and clever experiments to establish the role of the immune system in controlling cancer (reviewed in[1]). Current understanding of cancer immunosurveillence also considers the notion that the immune system not only controls tumour formation and growth, but also influences the immunogenicity of the tumour and potential outgrowth. This hypothesis is referred to as cancer immunoediting, in which the three phases of elimination, equilibrium, and escape can be distinguished (reviewed in[2]). These foundations are important for understanding the concepts of cancer immunotherapy, which aims to enhance the immune system's responses to tumour cells.

In the landmark study in 1988[3], *ex vivo* expanded autologous tumour-infiltrating lymphocytes (TILs) in combination with human interleukin-2 (rhIL-2) were developed and demonstrated objective responses in patients with metastatic malignant melanoma. In addition, this work provided the unequivocal evidence of tumour-specific T cell mediated immunity leading to cancer recognition and elimination in humans.[3] The next milestone was the development of a T cell-based cancer immunotherapy using genetically engineered T cells,

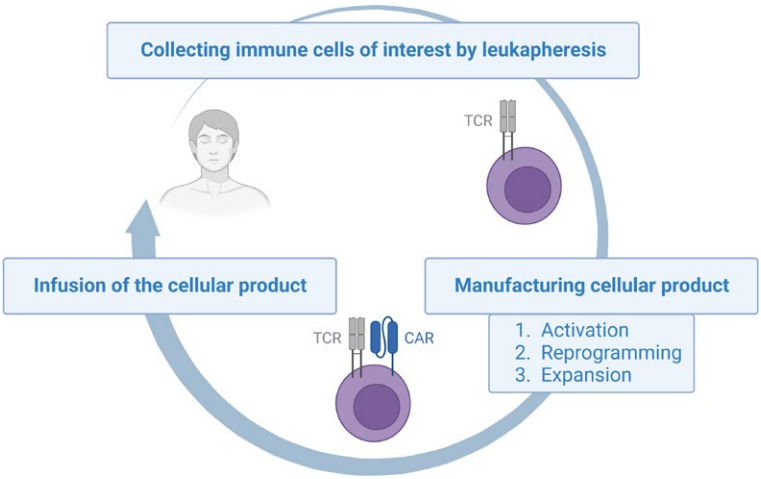

FIGURE 1. The principle of adoptive cellular immunotherapy.

CAR = chimeric antigen receptor; TCR = T-cell receptor

made possible by a better understanding of basic T cell biology and genetic engineering approaches.[4]

Currently, the two most widely used immune receptors that confer tumour specificity and functionality to genetically engineered T cells are a tumour-reactive synthetic chimeric antigen receptor (CAR) and an identified (e.g., from TILs) or further engineered T-cell receptor (TCR). To date, CD19-targeting CAR T cells emerged as the most successful cellular immunotherapy approach. Clinical trials in relapsed or refractory paediatric acute lymphoblastic leukaemia (ALL)[5–7] and high-grade B-cell lymphoma in adults[8–16] have demonstrated that CAR T cell immunotherapy can produce effective, long-lasting, and overall unprecedented clinical responses. CD19-targeting CAR T cells received the U.S. Food and Drug Administration and European Medicines Agency approval in 2017 and 2018, respectively. To date, genetically engineered T cell immunotherapies have mediated unprecedented clinical responses in hematologic malignancies[5–16] but the efficacy of these therapies is limited in solid tumours and also in certain blood cancers due to several factors, some of which are discussed in this review. In addition, adoptive cellular immunotherapies can cause potentially life-threatening complications such as cytokine release syndrome (CRS) and neurological toxicities.[17,18]

Nowadays, cellular immunotherapies include exciting research and clinical successes with TILs and T cells genetically modified with TCRs and CARs. In addition, alternative immune cells are being engineered with CARs[19,20] and CAR T cells are now being used outside of cancer treatment.[21–27] This review article focuses on CAR T cells to treat cancer. First, the concepts of adoptive cellular immunotherapy with CAR T cells are introduced. Then, the architecture of the CAR molecule is described and how design affects function. Current challenges and limitations regarding efficacy and safety are then presented, focusing on the immunosuppressive tumour microenvironment (TME), T cell intrinsic properties, tumour targeting, cellular product manufacturing and immune-related adverse events. Throughout, this paper presents selected recent next-generation approaches to the development of CAR T cells that have the potential to overcome some of these challenges.

Principles of cellular immunotherapy

Adoptive cell transfer

In its broadest sense, adoptive T cell transfer (ACT) involves the isolation of T lymphocytes from blood and their reinfusion into patients for the treatment of disease. Advances in the understanding of basic mechanisms in T cell biology, including target recognition, T cell activation, signal transduction, role of soluble factors, and co-stimulation signals, have led to a better understanding of T cell function, expansion, and persistence.[28] This knowledge has been critical for establishing optimized protocols for *ex vivo* culturing conditions, activation, and expansion. To redirect the specificity of T cells, genetic engineering approaches had to be developed to introduce the genetic cassette encoding TCR or CAR into primary T cells.[4] These significant advances enabled the development of sophisticated T cell-based therapies such as CAR T cells that transformed oncology.

Current clinical adoptive transfer of CAR T cells involves three steps (Figure 1). (1) Collection of T cells: The patient's own T cells (in the autologous ACT setting), which are the body's primary component for fighting infection and cancer, are first isolated from the blood in a procedure called leukapheresis. These cells express endogenous TCR. (2) *Ex vivo* reprogramming and manufacturing of the cellular product: Primary T cells are first activated using activation beads and then a genetic cassette encoding the CAR molecule is introduced into the primary T cells by viral transduction, which transforms donor T cells into CAR T cells. Introduction of these molecules reprograms T

cells to specifically recognize, target and eliminate cancer cells, while *ex vivo* expansion allows manufacturing of sufficient numbers of CAR T cells. (3) Infusion: Patients are treated with a preparatory chemotherapy and then reinfused with the modified T cells. After *ex-vivo* expansion, the reprogrammed cells are infused back to the patient where they find and eliminate the disease.[29]

Design of a CAR molecule

The Chimera is a creature of Greek mythology that consists of parts of various animals. Based on this analogy, CAR is a molecule that combines the properties of a monoclonal antibody that enables antigen recognition with the components of the TCR that drive T cell signalling and activation. CAR is a molecule composed of different domains, each of which contributes to a specific functionality, and together they effectively redirect T cells to the target of interest and elicit T cell responses (Figure 2).

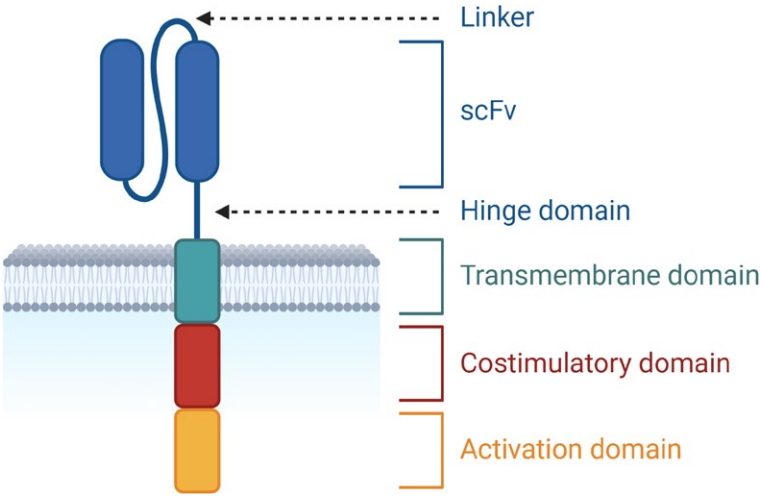

FIGURE 2. Schematics of the basic CAR architecture.

CAR = chimeric antigen receptor; scFv = single-chain variable fragment

Design of CAR molecule continues to evolve as we gain more knowledge from basic immunology and clinical trials. First-generation CARs consisted of an extracellular antigen-binding domain, usually in the form of an antibody-derived single-chain variable fragment (scFv) linked to intracellular signalling domains, most often derived from the components of the TCR complex, for example the CD3 zeta chain (CD3ζ).[30,31] This molecule was capable of recognizing antigens independent of HLA (human leukocyte antigens) presentation. First-generation CARs provided proof of principle but did not enable long-term T cell persistence and effector responses due to their limited signalling capacity.[32] This section describes CAR molecule architecture and its individual domains.

Antigen recognition domain

The specificity of the CAR molecule is defined by the antigen-targeting ectodomain. In most current designs, this is scFv, which is a fusion between variable heavy and variable light chains of an antibody connected by a flexible linker. The affinity of CAR has been shown to have important effects on the functions of CAR T cells. In a clinical study, enhanced CAR T cell expansion and prolonged persistence were observed with a low affinity CD19 CAR compared to CAR T cells with FMC63, a scFv in clinically approved CD19 targeting CAR T cells.[33] Interestingly, in a different study, linker length has also been shown to influence CAR clustering, antigen-independent signalling and function of CAR T cells.[34] ScFv have now been designed to target several cell surface molecules associated with cancer, most often proteins, but also glycans such as the aberrant cancer-associated Tn glycoform of MUC1, which is expressed in a variety of cancers.[35] Although the mechanism by which binding of CAR to its cognate antigen leads to T cell activation shares key similarities, it also differs substantially from the mechanism by which TCR binding leads to T cell activation. While CARs generally exhibit higher affinity that can also be tuned, the sensitivity is higher in TCRs.[36] Currently, CAR T cells that target CD19 (tisagenlecleucel, axicabtagene ciloleucel, lisocabtagene maraleucel and brexucabtagene autoleucel) and B-cell maturation antigen (BCMA also known as TNFRSF17) (idecabtagene vicleucel, ciltacabtagene autoleucel) are being FDA-approved and marketed[37,38] while several others are in clinical trials, including CD20, CD22, CD33, CD5, and CD7 (reviewed in[39]). Some of widely explored targets in solid tumours include alpha folate receptor (FOLR1), human epidermal growth factor receptor 2 (HER2), carcinoembryonic antigen (CEA), ganglioside G2 (GD2), mesothelin, epidermal growth factor receptor variant III (EGFRvIII), mucin1 (MUC1), interleukin-13 receptor subunit alpha-2 (IL13Ra2), prostate specific membrane antigen (PSMA), B7 homolog 3 (B7-H3), epidermal growth factor receptor (EGFR), and fibroblast activation protein (FAP) (reviewed in[39,40]).

Hinge and transmembrane domain

The scFv domain is connected via a hinge region to the transmembrane (TM) domain. The TM domain is often derived from CD8 or CD28 molecules and functions to anchor CAR in the membrane and facilitate signal transduction. The choice or engineering of TM domain may affect the interactions between CAR molecules themselves[41], or with other endogenous molecules such as CD28.[42] Innovative designs in hinge and TM domains may provide opportunities to tune CAR signalling.

Co-stimulatory domain

In the clinically approved CARs, the membrane proximal intracellular domain is the co-stimulatory domain. The need for costimulatory domain arose when limited clinical efficacy of the first generation CAR T cells was observed.[43] The authors concluded that genetically engineered tumour-reactive T cells are safe but do not persist and that strategies to prolong T cell persistence are needed. The first domain included in the CAR design was the CD28 costimulatory domain, initially alone[44] and then in combination with CD3ζ.[45,46] The CD28 domain provides robust response with an effector phenotype and high levels of secreted IL-2 and tumour lysis activity.[47] The other widely used co-stimulatory domain introduced into CAR design is CD137 (4-1BB). Compared to CD28, 4-1BB provides improved persistence, shift towards central memory phenotype differentiation, a lower propensity to exhaustion and reduced toxicity.[15,47,48] A recent comparison between the two marketed products, axicabtagene ciloleucel and tisagenlecleucel examined the differences between CD28 and 41BB in relapsed or refractory diffuse large B cell lymphoma and concluded that axicabtagene ciloleucel provides higher efficacy and also a higher toxicity.[48] Other co-stimulatory domains are also being studied including CD27[49], ICOS[50], and OX-40[51], each of which has certain favourable properties. Finally, third generation CARs comprise a combination of two costimulatory domains and some of these have already been tested in clinical trials.[52] However, excessive stimulation can lead to dysfunctional CAR T cells.[53]

The design of the second-generation CARs, which includes additional co-stimulatory domains that enhance the expansion, persistence, and effector functions of CAR T cells, has been key to the success of clinical trials. A recent study revealed that CAR T cells persisted for more than ten years after infusion, with sustained remission in a patient treated with CD19 targeting 4-1BB CAR T in 2010.[54] Selection of the co-stimulatory domain influences important parameters of CAR T cell therapy including effector function, response kinetics, expansion, differentiation, metabolism and toxicity.[47] Innovative studies are attempting to address the complexities and unknowns by characterizing multiple intracellular signalling domains in a high throughput manner to identify to the CAR designs that have improved functions compared to clinically used CAR T cells.[55]

Activation domain

The distal intracellular domain is CD3ζ, a signal transduction component of the TCR complex that has been repurposed to drive CAR signalling after recognition of its cognate target. Immunoreceptor tyrosine-based activation motifs (ITAMs) are key motifs in the CD3ζ domain. When the TCR recognises its target, ITAMs are phosphorylated through a series of molecular interactions mediated by Lck kinase (lymphocyte-specific protein tyrosine kinase). This leads to the recruitment and activation of ZAP-70 (Zeta-chain-associated protein kinase 70), which orchestrates a series of downstream phosphorylation events that result in the complex and highly regulated signal transduction required for T cell activation and effector functions.[56] CAR signalling resamples key features of TCR signalling but also differs in important ways. Analogous to the "two-step" T cell activation model, CD3ζ provides signal 1 whereas the co-stimulatory domain provides signal 2. CAR signalling is active area of research in basic T cell biology and has direct importance for the therapeutic implementations. As an alternative to the CD3ζ, other domains are investigated for CAR T cell therapy including the CD3ε.[57] An example of rational tuning and calibration of CAR activation and signalling demonstrated that combinatorial mutation of ITAM motifs directs differentiation towards memory T cell states, which translated in improved persistence and therapeutic potency in preclinical mouse models.[58] Moreover, using the genome editing approach, the TRAC locus was modified in primary human T cells to target cell-surface molecules via their TCR complex, which was reconfigured to use the same targeting component as a corresponding CAR. These HLA-independent TCRs, referred to by the authors as HIT receptors, have been shown to be particularly sensitive compared to CD28-based CARs.[59]

Challenges and opportunities of cellular immunotherapy

The success of CAR T cells is countered by challenges in efficacy in solid tumours[40] and immune-related adverse events. Underlying causes of limited efficacy include immunosuppressive TME and T cell and tumour intrinsic properties. In addition, the manufacturing of the cellular product and lack of tumour specific targets represent a major challenge. Here, some of these aspects are outlined and selected recent publications are presented that attempt to meet these challenges (Figure 3).

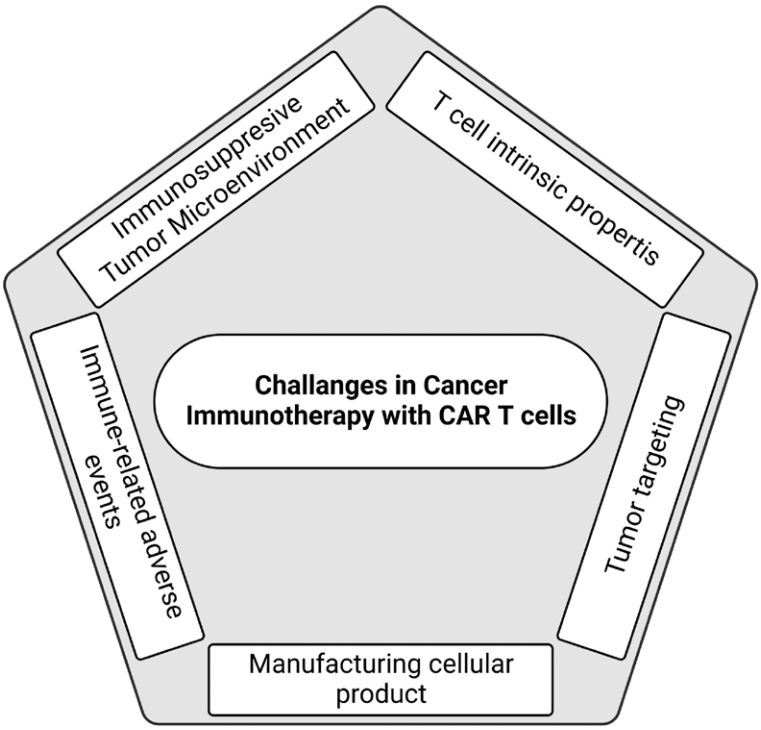

FIGURE 3. Challenges of cellular immunotherapy with chimeric antigen receptor (CAR) T cells.

Immunosuppressive tumour microenvironment

Immunosuppressive TME limits the efficacy of CAR T cells by interfering with their function. Various approaches have been developed to address these challenges, including upgrading engineered T cells with the expression of accessory molecules. Pioneering work has been done with tumour infiltrating-lymphocytes (TILs) engineered with inducible expression of the potent immune-enhancing molecule IL-12.[60] This approach was tested in human clinical trials and clinical activity but also toxicity were observed. Similarly, CAR T cells have been equipped with accessory molecules to counteract various aspects of the hostile immunosuppressive TME. These molecules include IL-18[61–64], PD-1[65], CTLA-4, or TIM3[66] blocking scFvs and minibodies, CD40L[67], dominant-negative Fas[68] or Fas-41BB switch[69] receptors, pro-inflammatory neutrophil-activating protein (NAP) from *Helicobacter pylori*[70] and dominant-negative TGFβ Receptor.[71] Recently, a pooled knock-in platform has been developed to screen for genetic constructs that can improve T cell functions for effective cell therapies when constitutively overexpressed.[72] Additional genetic approach coupling expression of effector molecule with specific antigen recognition was developed using a synNotch platform.[73] These approaches improve the efficacy of T cell therapy and highlight the need to develop robust and efficient gene expression systems suitable for clinical translation.

Depleting of cells that limit the efficacy of CAR T cells is a viable approach to increase CAR T cell activity in TME. One approach is the depletion of immunosuppressive M2 tumour-associated macrophages (TAMs) by CAR-mediated targeting of a folate receptor β (FRβ) positive subset of TAMs that exhibit an immunosuppressive M2-like profile. CAR T cells eliminated these FRβ+ TAMs, resulting in recruitment of endogenous tumour-specific CD8+ T cells, improved tumour control, and prolonged survival.[74]

Therefore, overcoming immunosuppressive TME with innovative approaches is an important pillar in improving the activity of CAR T cells.

T cell intrinsic properties

It is becoming increasingly clear that intrinsic T cell dysfunctions, such as T cell exhaustion limit the success of CAR T cells in solid tumours but also in hematologic malignancies that induce dysfunctional T cell states. A recent correlative study examined the determinants of response at genomic, phenotypic and functional levels and demonstrated that clinical efficacy in patients with chronic lymphocytic leukaemia (CLL) treated with CAR T cells is affected by complex intrinsic immune cell functions and dysfunctions.[75] Chronic stimulation of T cells with an antigen, as occurs also with CAR T cells targeting solid tumours, is an important reason for the dysfunction.[76] One approach to overcome this problem is a temporary resting period in which the functionality of the CAR T cells is restored.[77] Innovative approaches

have been developed to maintain functionality of CAR T cells, including overexpression of c-Jun[78] or a combination of BATF and IRF4.[79] Recent studies linked the heterogeneity of autologous CAR T cells in terms of cellular and molecular characteristics of the infusion products to differences in efficacy and toxicity following CD19 CAR T therapy.[80] In a distinct approach, CAR T cells were designed to express interleukin IL-7 and CCL19 to mimic a favourable milieu that forms and maintains T cell zones in lymphoid organs.[81] These upgraded CAR T cells demonstrated enhanced recruitment of T cells and dendritic cells into tumour and augmented therapeutic effects against solid tumours. Favourable effect on differentiation and persistence of CAR T cells has been demonstrated with the constitutive IL-7 receptor[82], IL-15[83], and synthetic receptors combining orthogonal extracellular IL-2 and intracellular IL-9 domains.[84] In a recent study, overexpression of more than 10,000 barcoded human open reading frames (ORFs) identified positive regulators of T cell function, with the aim of developing improved cellular immunotherapies including CAR T cells.[85]

The intrinsic properties of T cells in the context of CAR T cell therapy require careful study from the perspective of basic immunology. This knowledge is important to overcome the dysfunction that limits the activity of CAR T cells.

Tumour targeting

CD19 is an example of a target that is also expressed on normal cells (B cells), but humans can live with B cell aplasia and appropriate treatment, namely intravenous immunoglobulin (IVIG) treatment, which overcomes antibody deficiencies. However, a major challenge in the development of CAR T cells is to identify targets that are homogeneously expressed at sufficient levels on the surface of tumour cells and are not present on healthy tissues at levels that would cause damage. A tragic example is described in a case report where CAR T cells based on the humanized monoclonal antibody trastuzumab (Herceptin), which recognizes ERBB2, led to the patient's death.[86] The authors hypothesize that the large number of CAR T cells infiltrated in the lungs and triggered cytokine release after recognizing low levels of ERBB2 on lung epithelial cells.

Acute myeloid leukemia (AML) is a candidate disease for cellular immunotherapy. However, targeting the myeloid marker CD33 in (AML) leads to toxicity from destroying normal myeloid cells. The authors demonstrated the artificial generation of a leukaemia-specific antigen by deleting CD33 from normal hematopoietic stem and progenitor cells (HSPCs), generating a hematopoietic system resistant to CD33-targeted therapy and enabling specific targeting of AML with CAR T cells.[87] In this approach, the host was genetically engineered to avoid on-target and off-tumour toxicity.

Heterogeneity[88] and loss of antigen expression on cancer cells under selective pressure of targeted immunotherapy can lead to evasion strategies by cancer cells.[89] This has sparked the development of CARs with multiple specificities. Examples for hematologic malignancies include a dual CD19 and CD22 CAR T cells expressing two CAR receptors[90] or CAR T cells with a tandem scFv CAR molecule with dual targeting of CD19 and CD22.[91,92]

Another approach that allows on demand multiple antigen targeting to mitigate a potential antigen escape in CAR T cell therapy is adapter CAR platform. One example is the universal immune receptor based on SpyCatcher-SpyTag chemistry. The SpyCatcher immune receptor redirects primary human T cells upon adding SpyTag-labeled targeting ligands.[93] Another example is the so-called SUPRA CAR, a split-CAR design that allows the development of CAR T cells with multiple features and provides the ability to switch targets without re-engineering the T cells.[94]

TCRs have been shown to enable targeting of neoantigens[95–98] and recently CARs have also been developed that specifically target peptides derived from intracellular proteins presented by HLAs.[99] These results demonstrate that CAR T cells are not limited to recognizing molecules expressed on the surface, but can now be engineered to recognize intracellular targets presented by the HLAs, which mimics recognition by TCRs. This significantly increases the potential pool of CAR T targets.

Tumour targeting represents a challenge and an opportunity for innovative approaches and advances will be necessary to develop CAR T cell therapies for new disease indications, particularly in solid tumours.

Manufacturing cellular product

The manufacturing process, which involves the collection of autologous T cells and the generation of CAR T cells for each individual patient, is expensive and complex from an infrastructural and logistical perspective. In addition, unexpected challenges can emerge with some of the existing pipelines. One such example is the discovery that

the lentivirally delivered CAR gene was inadvertently introduced into a single leukemic B cell during T cell manufacturing. This anti-CD19 CAR molecule then bound the CD19 epitope on the surface of the same leukemic cells, which masked it from being recognized by the CD19-targeting CAR T cells, resulting in relapse.[100] Therefore, there is great interest in optimizing the manufacturing of the cellular product to make it safer, more effective and broadly available.

Recent study presented the shortened process of manufacturing of non-activated CAR T cells with improved functionality.[101] Another study investigated the approach where CAR T cells have been manufactured from the defined CD4+ and CD8+ T cell subsets and infused in a defined CD4+: CD8+ composition.[102] Recent study investigated the efficacy and safety of CAR T cells generated from preselected naïve/stem memory T cells, observing a superior safety and efficacy profile compared to unselected bulk T cells.[103] In addition, alternative sources of donor T cells are being explored, including allogeneic off-the-shelf approaches.[104,105] Recently, the first human clinical trials were reported with CRISPR/Cas9-engineered T cells that edited PD-1[106] or even demonstrated multiplex CRISPR/Cas9 editing of the endogenous T cell receptor and PD-1.[107]

Currently CAR T cells are produced via lentiviral or retroviral transduction, where integration of a gene encoding CAR is semi random and poses certain risks and challenges. Recent studies have demonstrated that genome editing technologies can be used for CRISPR/Cas9-mediated targeted integration of a CAR into an endogenous locus via homology-directed repair (HDR) and an adeno-associated virus (AAV) vector as a HDR donor template.[108,109] Further, a non-viral strategy using a double stranded DNA as a HDR donor template for CRISPR/Cas9-mediated targeted integration has been demonstrated.[110] CAR T cells generated with non-viral targeted integration have even been tested in a clinical trial.[111] Finally, approaches to generate CAR T cells *in vivo* are also being explored.[112]

Bringing the manufacture of cellular products to a level that enabled clinical approval required extensive efforts by pioneers and now continues to represent an area of opportunity to make CAR T cells safer, more effective, and broadly available.

Immune-related adverse events

Unfortunately, adoptive cancer immunotherapy carries safety risks such as cytokine release syndrome (CRS) and neurologic toxicities[113], that have led to life-threatening complications.[17] Current management strategies include systemic use of the antibody tocilizumab, which blocks IL-6 receptor.[114] CRS and neurotoxicity are the two main toxicities associated with clinically used CD19-targeting therapies. B-cell aplasia is on-target, off-tumour adverse effect of CARs that target B-cell differentiation antigens such as CD19[17] and can be effectively managed by IVIG, as mentioned earlier in the paper. Further, on-target off-tumour toxicity can have devastating effects[86] as described in previous sections.

A recent study illuminated a contributor to severe neurotoxicity observed in a subset of patients treated with CD19-targeting therapies. The authors show that brain mural cells, which surround the endothelium and are critical for the integrity of the blood-brain-barrier, express CD19, implying that on-target off-tumour toxicities may occur.[115]

Several approaches are being developed to mitigate toxicities, including platforms in which the activity of CAR T cells can be regulated by genetically encoded transient functions in a combination with the small molecules[116–118] or targeting ligands.[93,94] Suicide switches based on inducible caspase-9[119] or on expression of surface molecules, such as a truncated version of epidermal growth factor receptor (EGFRt) are being developed. In the latter case, EGFRt is expressed together with CAR on the surface of T cells, so that CAR T cells can be eliminated by addition of an antibody targeting EGFRt.[120]

SynNotch enabled AND-gate combinatorial targeting, in which the synNotch receptor first recognized one tumour antigen, which led to the release of a transcriptional activator domain to drive expression of a CAR targeting another tumour antigen.[121]

New insights into the biology of CAR T cells, experience from clinical trials, and advances in engineering approaches now provide the basis for making CAR T cells safer while maintaining their efficacy.

Conclusions

This review article focuses on CAR T cells for cancer immunotherapy. However, it is important to note that cellular immunotherapy using TILs[122,123] or T cells with engineered TCRs has achieved remarkable success in clinical studies in solid tumours and established approaches to target intra-

cellular antigens presented in the context of major histocompatibility complex (MHC) molecules including neoantigens.[95–97] The success of CAR T cells in treating cancer has led to their use outside of cancer treatment, including autoimmunity[21–23], infections[24,25], senescence-associated pathologies[26], and cardiac fibrosis.[27,112] Several cell types including Natural Killer (NK)[19] cells and macrophages[20] are being explored as alternatives to T cells that have certain advantages and provide new features. Cancer immunotherapy with CAR T cells represents a paradigm shift in the treatment of certain blood cancers that do not respond to other available treatment options. Well-trodden paths blazed by pioneers led to the first FDA and EMA approval, and the journey now continues on lesser-known paths to treat a variety of cancers and other serious diseases with CAR T cells.

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