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Therapeutic T cell engineering

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Abstract

Genetically engineered T cells are powerful new medicines, offering hope for curative responses in patients with cancer. Chimaeric antigen receptors (CARs) are a class of synthetic receptors that reprogram lymphocyte specificity and function. CARs targeting CD19 have demonstrated remarkable potency in B cell malignancies. Engineered T cells are applicable in principle to many cancers, pending further progress to identify suitable target antigens, overcome immunosuppressive tumour microenvironments, reduce toxicities, and prevent antigen escape. Advances in the selection of optimal T cells, genetic engineering, and cell manufacturing are poised to broaden T-cell-based therapies and foster new applications in infectious diseases and autoimmunity.

T lymphocytes develop in the thymus[1], where they acquire their antigen receptor, known as the T cell receptor (TCR)[2]. T cells are an essential component of adaptive immunity, contributing to tumour rejection and pathogen clearance. The adoptive transfer of T cells was a pivotal experimental technique used more than half a century ago to establish that cellular components of the immune system could reject tumours[3,4]. Over time, adoptive transfer experiments in mice have contributed to the identification of tumour antigens and to elucidating the obstacles to establishing effective tumour immunity[5–7]. This laboratory procedure inspired clinical applications of adoptive cell transfer, including the use of autologous lymphokine-activated killer cells[8] and tumour-infiltrating lymphocytes (TIL)[9] to treat human solid tumours. Independently, borne out of the field of bone marrow transplantation, allogeneic donor T cells were shown to sometimes eradicate haematological malignancies via the graft-versus-leukaemia (GVL) effect[10]. Allogeneic T cells can also induce a devastating pathology known as graft-versus-host disease (GVHD). The dual-edged role played by T lymphocytes spurred a search to identify and separate beneficial and deleterious T cells[11,12], which spawned allogeneic therapies such as donor leukocyte infusion[13] and virus-specific T cell therapy[14–16]. Altogether, these early clinical

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investigations eventually pointed to the need to better control the composition of therapeutic T cell products by increasing their content of tumour-specific T cells and removing T cells with harmful potential[17].

A shared feature of late 20th century approaches is the focus on selecting and expanding naturally occurring T cells found in the patient or a healthy donor. The prospect of T cell engineering would singularly alter these original concepts. The emergence of replication-defective viral vectors[18] provided new possibilities for cell therapies, such as the potential to genetically modify T lymphocytes[19]. In this perspective, it would no longer have to be a cell harvested from the patient or a donor that would be adoptively transferred, but a cell product designed and repurposed through *ex vivo* genetic modification[20,21]. Starting from easily accessible cells collected from a patient's blood, genetic engineering provided a means to rapidly generate anti-tumour T cells for any cancer patient by introducing tumour-targeting receptors and other attributes intended to improve therapeutic efficacy and safety. Advancing therapeutic T cell engineering required progress on multiple fronts including target identification, antigen receptor isolation or design, T cell differentiation, genetic engineering, cell manufacturing sciences, and regulatory compliance. This immunotherapeutic modality thus draws not only on principles of immunology but genetics, synthetic biology, stem cell biology and a range of manufacturing technologies. The poster child for this new paradigm is CD19 CAR therapy.

Redirecting the specificity of T cells

The most natural approach to target a T cell to a chosen antigen is to express therein rearranged TCR α and β chains of defined antigen specificity, conforming to the physiological TCR–CD3 complex (Fig. 1a). This procedure was used in transgenic mice to demonstrate that the TCR was sufficient to direct antigen-specific T cell differentiation[22] and later applied to human T cell clones to redirect their cytotoxicity[23]. The transfer of TCR genes aims to phenocopy naturally occurring T cells, thereby supplying tumour- or virus-specific T cells to individuals whose endogenous immune response is insufficient to combat the disease. Current efforts to implement this approach focus on isolating TCRs with optimal specificity and affinity[24], and devising molecular strategies that eliminate potential TCR cross-reactivities[25,26] and minimize αβ chain mispairing[27–29]. TCR gene transfer however remains constrained by TCR competition for rate-limiting amounts of the signalling molecules of the CD3 complex[30] (Fig. 1a), and by human leukocyte antigen (HLA) restriction, which imposes that multiple TCRs be identified for any given antigen to ensure that patients with different HLA haplotypes be eligible for therapy. These biological limitations notwithstanding, TCR gene transfer is a compelling therapeutic concept that utilizes physiological antigen recognition and T cell activation to generate antigen-specific T cells. Indeed, TCR-engineered T cells have been shown to have significant anti-tumour activity in patients with melanoma and sarcoma in small clinical trials[31,32].

An alternative approach to retarget T cells rests on the design of artificial receptors for antigen[33]. In order to support a productive T cell response, receptors for antigen must encompass CD3 components that are sufficient to initiate T cell activation, such as the CD3 ζ chain[34–36]. Fusion molecules that linked the ζ chain to an extracellular ligand binding

domain, initially termed T bodies[37] or chimaeric TCRs[38], and later first-generation CARs[33], are in essence a simplified TCR–CD3 complex serving as a TCR surrogate. However, experimentation in primary T cells and in transgenic mice revealed that signalling through such receptors was insufficient to direct productive immune responses[39–41], indicating that a better adapted and less natural design was needed to engineer sustainable T cell responses. Based on the incorporation of co-stimulatory components into antigen-specific receptors[42], the design of receptors that simultaneously provided both activation and co-stimulatory signals opened a path for the generation of antigen-specific T cells capable of interleukin-2 secretion[43] and, most importantly, expansion upon repeated exposure to antigen[44]. This receptor design, dubbed second-generation CAR[33] (Fig. 1b), is the backbone of current CAR therapies.

Building up the potency of engineered T cells

The second-generation CAR design is amenable to a vast number of variations that include differences in antigen specificity, co-stimulatory signalling domain(s) and T-cell-activating components. The structural features afforded by the hinge and transmembrane regions (Fig. 1b) also affect antigen binding and signalling[45]. The term CAR intends to encompass this modular potential[33], and over 100 different CAR specificities, predominantly using antibody single chain variable fragments (scFv) to impart antigen recognition, with at least eight co-stimulatory components, have already been reported[46–48].

The best studied second-generation CARs are those using CD28 or 4-1BB co-stimulatory domains[48]. Both these designs have been tested in clinical trials for relapsed B cell malignancies where they have induced major clinical responses (see below). These two variant CAR designs impart different functional and metabolic profiles upon the engineered T cell. CD28-based CARs promote brisk T cell proliferation, glucose metabolism, and self-limited T cell persistence, while 4-1BB-based CARs induce a less potent effector T cell but stimulate lipid oxidation and support greater T cell persistence[48–50]. These differences illustrate the potential to differentially reprogram T cells using second-generation CARs.

The genetic optimization of T cells is not limited to the expression of a CAR or TCR to redirect specificity. The ease with which genes can be introduced into T cells enables the expression of multiple gene products to further shape the targeting and functional attributes of engineered T cells. The molecules used in conjunction with CARs for the purpose of increasing T cell potency or safety include a range of synthetic receptors, including chimaeric co-stimulatory receptors[42,51,52], Notch-based receptors[53], antigen-specific inhibitory receptors[54] and others (Fig. 1c), and additional gene products designed to act in the tumour microenvironment[55] or augment T cell safety[56,57] (see below). Thus, while second-generation CARs have already demonstrated and validated the enormous potential of synthetic T cell engineering, one may anticipate a number of further advances in T cell therapy based on multiplexed enhancements.

Clinical proof-of-concept: the CD19 paradigm

To successfully translate CAR therapy in the clinic, one not only needs a powerful CAR but also a suitable target, which ideally is expressed on the surface of all tumour cells and absent from all normal cells, or at least any vital cells. We identified CD19 as a promising target[58] based on its cell-surface expression in most leukaemia and lymphomas and its function[59,60]. The targeting of CD19 was expected to induce a B cell aplasia, as eventually documented in several mouse models[57,61–63], but deemed to be tolerable and clinically manageable if it were limited in time. We also reasoned that B cell elimination might prevent the emergence of anti-CAR antibodies, which were eventually observed in CAR T cell trials using other CARs encompassing murine components[64,65]. Our studies on CD19 CARs were the first to demonstrate complete eradication of established, systemic lymphoma following a single infusion of human CAR T cells in mice[58], thus providing the foundation and rationale for clinical studies. Three groups reported intriguing early results in three different B cell malignancies, diffuse large B cell lymphoma[66], chronic lymphocytic leukaemia (CLL)[67] and acute lymphoblastic leukaemia (ALL)[68]. These studies focused on relapsed, chemotherapy refractory patients, and made use of either CD28 or 4-1BB-based CARs[69]. Notably, despite differences in disease histology, scFv, vector utilization and manufacturing process[69], all groups reported remarkably high rates of complete response, especially in ALL[71–75] (Table 1).

CD19 CAR T cells must be administered following a cytotoxic conditioning regimen that depletes endogenous lymphocytes, without which no significant benefit is obtained[70,76]. If conditioning is too intensive, however, toxicities such as cytokine release syndrome and neurotoxicity can be exacerbated[71]. Whereas cytokine release is an expected outcome of T cell activation, the mechanism of neurotoxicity remains elusive. Toxicities from CAR T cells can usually be managed successfully by administering antibodies that block the interleukin-6 receptor and corticosteroids[72,77], and partly mitigated by adjusting conditioning intensity and T cell dosing. Further research is needed to improve treatment and prevention strategies for such toxicities.

It is noteworthy that complete response rates have been superior in ALL compared to CLL and lymphomas, using the same CARs (Table 1). Clinical observations point to the importance of disease location, with bone marrow disease in ALL and CLL being easier to eradicate than extra-medullary sites. Clinical data on T cell biodistribution are scarce, but suggest that CAR T cell efficacy is more likely to be limited by inhibitory molecules in the tumour microenvironment than by inadequate T cell trafficking, although it remains possible that accessibility to some tumour sites limits efficacy.

Where to apply CAR therapy

The success of engineered T cells expressing CD19 CARs is a milestone in cell therapeutics, and is one of two approaches currently revolutionizing the field of cancer immunotherapy[78]. The other approach uses antibodies that target immune checkpoint molecules such as CTLA-4 or PD1 to enhance priming or activity of endogenous T cell responses to tumour antigens. The efficacy of immune checkpoint blockade is often incomplete but is highest in

patients with tumours that carry a high burden of somatic mutations[79,80]. While patients with tumours with low mutation burden are typically less responsive to checkpoint blockade, it is noteworthy that it is in one such disease, ALL, that CAR therapy has proven its efficacy (Fig. 2). CAR therapy may thus be especially valuable in low mutation cancers and in those that fail checkpoint blockade therapy (Fig. 2).

The clinical benefits induced by, not a drug or an antibody, but an engineered cell, have generated enthusiasm amongst scientists, patients and industry[81]. Engineered T cells are a versatile platform that can be, in principle, applied to all cancers and be used in combination with other modalities. However, extending CAR therapy to more tumours requires advances in several realms, including the identification of suitable CAR targets and the generation of T cells equipped to overcome hostile tumour microenvironments.

Identification of new targets for engineered T cells

Given the success of CD19 CARs, one may anticipate similar outcomes with other leukaemia targets and haematological malignancies. Two other targets have been recently evaluated, CD22 in ALL and BCMA in myeloma. CD22 is a B cell inhibitory receptor expressed in B cell malignancies, including pre-B ALL[82]. Encouraging results have been obtained with CD22 CAR T cells in chemotherapy refractory paediatric patients who relapsed after CD19 CAR therapy or blinatumomab, although with a higher rate of relapse than after CD19 CAR therapy[83]. BCMA is a TNF superfamily receptor member expressed in myeloma cells, normal plasma cells and a subset of B cells[84]. A first trial of BCMA CAR-T cells in 12 subjects with chemotherapy refractory myeloma resulted in two significant responses[85].

T cells are also able to eliminate solid tumours, as exemplified in TIL therapy for melanoma, cholangiocarcinoma and colorectal cancer[31,32,86]. TCR-engineered T cells are likewise able to mediate tumour destruction, but the identification of suitable antigens requires further research to prevent or minimize off-tumour activity[25,26], and enhance efficacy. Ideal tumour antigens to target would be mutated or uniquely expressed molecules that are present in all tumour cells. Viral antigens, cancer/testis antigens and neoantigens have the advantage of being tumour-restricted, and TCRs can be identified for a number of them. However, TCR-directed therapies remain burdened by HLA-restriction and the low frequency of shared cancer neoantigens. This approach therefore requires amassing a vast collection of validated TCRs, or high-throughput efforts to rapidly derive TCRs for personalized therapy[87]. Notably, CARs specific for HLA–peptide complexes offer an alternative, antibody-based approach to target neoantigens[88–92].

Classic CAR targets are HLA-independent and more frequently shared in their expression on solid tumours of a particular histology, but they pose a different challenge in that they are more likely to be found in normal tissues. In contrast to CD19, CD22 or BCMA, which are expressed in normal but non-vital or temporarily dispensable tissues, other commonly cited targets such as ROR1, mesothelin or prostate-specific membrane antigen (PSMA), are detected in a subset of normal tissues[47,93,94]. The low expression of these antigens in normal tissues may avert significant toxicity, but the toxicities encountered in clinical trials targeting

carbonic anhydrase IX or carcinoembryonic antigen with T cells suggest caution for clinical efforts targeting such molecules[95,96]. EGFRvIII, a differentially spliced EGFR variant found in glioblastoma, offers stricter tumour specificity, but its heterogeneous expression limits its potential as a target for complete tumour eradication[97]. IL13Rα-2 has been successfully targeted in a single case of glioblastoma, in which local CAR T cell therapy induced a complete response lasting several months[98].

Alternative targeting concepts provide potential routes to bypass these limitations, based on the therapeutic window and combinatorial antigen recognition. The therapeutic window exploits differences in antigen expression such that malignant cells that express high antigen levels are eliminated while normal cells with low levels are spared. This may be implemented by tuning receptor expression or affinity to discriminate between the high and low antigen expression profiles[99–101]. Combinatorial antigen recognition strategies offer yet another alternative. Some are designed to avoid antigen escape[102–105] and others to reduce off-target effects by restricting T cell activity to tumour cells that co-express the targeted antigens[51]. Combinatorial targeting makes use of TCRs, CARs, split-signalling receptors[49,51,106,107], sequentially acting receptors[53] and inhibitory receptors[54] (Fig. 1c). The discovery of suitable CAR target antigens will require deeper analysis of antigen expression in both tumours and all normal tissues, and testing in relevant model systems to determine whether therapeutic efficacy and tumour selectivity are achieved.

Adapting CARs to overcome tumour microenvironments

The solid-tumour microenvironment is immunosuppressive and an obstacle for all immunotherapies[108]. It is comprised of multiple cell types including T cells (effector, regulatory and γδ), B cells, myeloid cells (macrophages, dendritic cells, granulocytes, monocytes), stromal cells and endothelial cells. The extracellular milieu is suboptimal for T cell function, owing to hypoxia, necrosis, acidification, nutrient shortage (glucose, glutamine, L-arginine) and an array of immunosuppressive molecules (PD-L1, IL-10, TGFβ, indolamine-2-3-dioxygenase).

The conditioning regimen usually administered before T cell infusion abates some of this resistance[109], but it is not sufficient to remove or disrupt all inhibitory compartments in most instances. Additional steps need to be taken to reinforce engineered T cells to overcome micro-environmental obstacles (Fig. 3). These may consist of therapeutic combinations or the design of more efficacious T cells armed to impart resistance to inhibition.

Small molecules that interfere with immunosuppressive cells and pathways, such as IDO inhibitors[110], lenalidomide[111] and adenosine antagonists[112], are likely to act synergistically with engineered T cells. The Btk inhibitor ibrutinib, which is active against CLL, has also been found to improve CAR T cell function in preclinical models[113]. Checkpoint blockade may sustain function and persistence of engineered T cells in some instances[114–116], albeit not universally[117]. Multiple approaches have been used to interfere with immune checkpoints, including the use of blocking antibodies[114,116], dominant-negative receptors[114] and targeted gene disruption[118].

Significantly, the latter two approaches rely on T cell engineering, thus averting the need for combining agents and the risk of cumulative toxicities. Several other approaches to adapt engineered T cells to overcome the tumour microenvironment have been recently reported (Fig. 3) and some will enter the clinic in the next year. One approach is to co-express the CAR with a cytokine, such as IL-12 or a γ_c cytokine[62,119–121]. The toxicities encountered in a first trial using IL-12 caution against excessive cytokine secretion. Other approaches make use of secreted molecules to block inhibitory ligands[55] or constitutive expression of co-stimulatory ligands to enhance T cell function through auto- and trans-co-stimulation[122]. In the latter case, the display of a ligand such as 4-1BBL serves the dual purpose of increasing intrinsic T cell persistence as well as providing targeted co-stimulation to neighbouring tumour-infiltrating T cells[49].

What T cells to engineer

The anti-tumour effects of engineered T cells are predicated on their ability to migrate to tumour sites, proliferate, and mediate effector functions that destroy numerically larger tumour burdens. In addition to T cell genetic modifications that confer specificity to tumour antigens and enhance safety and efficacy, the phenotype of T cells that are engineered can affect potency[123]. It is now evident that the quality, efficacy, longevity and location of T cell immunity results from the diversification of naive T cells (T_N) into various phenotypically distinct subsets with specific roles in protective immunity. These include memory stem (T_{SCM}), central memory (T_{CM}), effector memory (T_{EM}), tissue resident memory (T_{RM}), and highly differentiated effector (T_E) T cells[124]. Fate mapping supports a model of progressive differentiation in which antigen-specific T_N give rise to long-lived T_{SCM} and T_{CM} that self-renew and provide proliferating populations of shorter-lived T_{EM} and T_E cells[125–127] (Fig. 4a). This conceptual framework suggests that selecting less differentiated T_N, T_{SCM} or T_{CM} subsets for genetic modification might provide cells with greater therapeutic efficacy[128].

The early clinical successes of genetically engineered T cells were accomplished by obtaining T cells from patients without regard to the phenotypic and functional heterogeneity in individual patients. Preclinical models have however shown that engineering T cells selected from T_N and T_{CM} subsets (or intermediates), or expanding T_N *in vitro* in the presence of small molecules or cytokines that inhibit T cell differentiation, provide cell products with superior engraftment, proliferation and anti-tumour effects after adoptive transfer[123,129–131]. These observations suggest that the potency of engineered T cells may be improved if therapeutic products were prepared from purified subsets with superior activity in preclinical models and formulated uniformly for infusion to the patient.

The concept of defining the subset formulation of engineered T cells is beginning to be explored in the clinic. The first clinical trials in which CD19 CAR T cells were engineered from selected T cell subsets and formulated in a uniform CD4/CD8 ratio demonstrated feasibility in greater than 90% of patients, including those with severe lymphopenia[75,132]. These studies also showed reproducible CAR T cell dose-related *in vivo* expansion, marked anti-tumour activity at lower cell doses, and a relationship between cell dose and toxicity that has not been clearly evident in earlier trials. Improvements in clinical cell selection methodology and use of culture conditions that retain less-differentiated phenotypes or

dictate production of specific effector cytokines are areas of investigation that are likely to be implemented in future clinical applications[133–136].

Alternative (non-autologous) T cell sources

The successful TCR- and CAR-based therapies have to date made use of autologous T cells, which imposes individualized cell manufacturing and makes inter-patient variability unavoidable, even with selection of defined subsets. The overarching rationale for autologous approaches is to prevent a T cell attack of the recipient and rejection of the therapeutic T cells by the recipient. Immunosuppressive drugs may mitigate such complications, but are not an option because unimpeded anti-tumour function of the infused T cells is essential. The risk of exacerbating GVHD after introducing CARs in donor T cells is real, albeit variable[137–140], depending in part on the CAR design[140].

Three strategies have been proposed to prevent GVHD. One is to transplant engineered haematopoietic stem cells or T cell precursors instead of mature T cells, thus allowing for the establishment of host tolerance. This approach however requires thymic function for positive and negative selection and yields progressive T cell reconstitution[141,142]. Another approach is to identify allogeneic T cells with a defined TCR specificity lacking GVHD potential[143,144]. VSTs may demonstrate alloreactivity *in vitro* but have been administered to allogeneic recipients to treat viral infections without causing serious GVHD[145]. However, initial clinical investigations of CAR-modified VSTs revealed limited therapeutic efficacy[146], which may owe in part to the deleterious consequences of having two functioning receptors in the same T cell[140]. A third approach to improve safety of allogeneic T cells is to remove the TCR, which may be achieved by disruption of the TCR α or β chain[147–149]. None of these approaches address the issue of rejection of allogeneic cells by the recipient's immune system, and are therefore only being evaluated in heavily immunosuppressed recipients[150], limiting their relevance. The latter strategies are constrained by their reliance on primary cells and the need for labour-intensive *in vitro* manipulations that may exhaust the replicative and engraftment potential of mature T cells[123].

An alternative to manipulating mature T cells is to generate CAR T cells *in vitro* from pluripotent stem cells[151–154] (Fig. 4b). Stem cell reprogramming offers potential access to an unlimited source of therapeutic T lymphocytes and provides an excellent platform for performing additional engineering intended to enhance the therapeutic potential of induced T cells[155].

How to engineer and manufacture better T cells

The genetic engineering of primary T cells began with recombinant retroviral vectors[19], which remain the most used vectors in clinical studies of TCR- or CAR-modified T cells[71–75]. Like retroviral vectors, transposons integrate semi-randomly and are thus also susceptible to variegated expression[156]. In hundreds of patients infused with TCR- or CAR-modified T cells, no occurrence of an oncogenic transformation has yet been reported. Alternative approaches to express the CAR rely on messenger RNA transfection, resulting in

transient expression lasting only a few days[157,158], and gene editing using targeted nucleases. Expressing CARs from the TCR locus affords added benefits in T cell functionality[159] that warrants clinical evaluation. Targeted nucleases enabling gene ablation or addition in T cells are poised to advance therapeutic T cell engineering.

The genetic engineering, selection, expansion and differentiation of T cells have to be integrated within manufacturing platforms that meet biological and regulatory requirements (Fig. 3). T cell manufacturing is a complex science that must coalesce biological optima with efficacy, safety, reproducibility, traceability as well as regulatory and economic imperatives. Clinical T cell production is regulated under current good manufacturing practices (cGMP) and requires facilities and manufacturing processes that meet FDA guidelines. Streamlining the process to augment performance while reducing scale and cost is contingent upon understanding how to optimally purify or induce T cells that bestow efficient tumour eradication with limited side effects. Seemingly small process changes can significantly alter the potency and safety profile of expanded T cell populations. The manufacture of optimal cellular components will not only increase safety, efficacy and reproducibility, but also decrease the effective T cell dose and hence the scale of production[75,160]. The high cost and limited number of GMP antibodies available to sort specific subsets of T cells will hopefully be assuaged by new technologies such as acoustic waves that allow the capture, separation and concentration of desirable T cell fractions, microfluidics and new phase-change hydrogel substrates[161–164]. These new platforms can potentially be combined with *in vitro* enrichment of desired T cell subsets using small molecules such as GSK-3β inhibitors or cytokines such as IL-7, IL-15 or IL-21[123,165,166]. These same principles apply to the induction of therapeutic T cells from induced pluripotent stem cells[155]. In order to mitigate costly cGMP operations and limit error-prone manual procedures, closed automated systems integrating cell-selection devices, microchips and bioreactors combined with biosensors for *in situ* monitoring, are being investigated[167,168]. New cell therapy platforms such as nanofluidic-based chip systems allowing individual cells to be isolated, cultured and assayed are becoming available for multiple applications including the characterization and manufacturing of T cell products. The rise of T cell therapies has incentivized the development of innovative manufacturing platforms and the establishment of standards driven by the formation of consortia such as CCRM (http://ccrm.ca), NIIMBL (http://www.niimbl.us) and the involvement of industry[167,168]. These initiatives will in time benefit all cell-based medicines.

Engineered T cells to fight infection and autoimmunity

The breakthroughs of T cell engineering in oncology have been remarkable, but the potential of engineered T cells extends far beyond cancer. One area awaiting further investigation is that of severe infectious diseases. Although early applications targeting HIV were not successful[169], the potential of T cells to combat intractable infections is intriguing. The other realm is that of autoimmunity and transplantation tolerance[170]. CAR T cells can eliminate auto-reactive B cells as demonstrated in a mouse model of pemphigus vulgaris[171]. Engineered T regulatory cells may also be harnessed to dampen immune responses[172], which may be useful in the context of GVHD, autoimmunity[173] or transplantation

tolerance[174]. This aspect of therapeutic T cell engineering is nascent and also poised for rapid development.

Perspectives

Twenty-five years of research on T cell engineering have culminated in the demonstration that genetically enhanced T cells can mediate profound responses in patients with refractory cancers. This progress was predicated on advances in cell engineering and manufacturing sciences that enabled successful clinical translation, the most striking example of which is CD19 CAR therapy. This new therapeutic modality is not yet fully mastered nor optimized. While high rates of complete response can be obtained in patients with B cell malignancies who do not have alternative treatment options, relapse may occur in up to half of patients, often owing to antigen loss variants. Despite progress in managing treatment-related toxicities, the mechanisms of severe cytokine release syndromes and neurotoxicities are poorly understood, precluding their effective prevention. There is therefore a need to further improve CAR therapies for B cell malignancies and other cancers.

A range of novel target antigens and CAR designs will soon be tested in haematological and solid cancers. The success of CD19 CAR therapy in some leukaemias and lymphomas is a good omen for tackling other haematological malignancies. There is, however, a need to identify suitable targets in solid tumours, whether neoantigens, cancer-testis antigens or non-mutated tumour-associated antigens, define the optimal T cell types for adoptive transfer, and address the immunosuppressive tumour microenvironment. New devices and processes are needed to decrease the cost of cell manufacturing. Autologous T cells are the corner stone of present cell-based cancer immunotherapy, but allogeneic cell sources, possibly including stem cell-derived, ‘off-the shelf’ T cells, may play an important role in the future.

While the recent therapeutic breakthroughs have all come to fruition in the realm of oncology, one may anticipate that T cell therapies will garner momentum in other fields. The success of CD19 CAR therapy and the rising proficiency of therapeutic T cell engineering is empowering all cell therapies, from immunotherapies to regenerative medicines.

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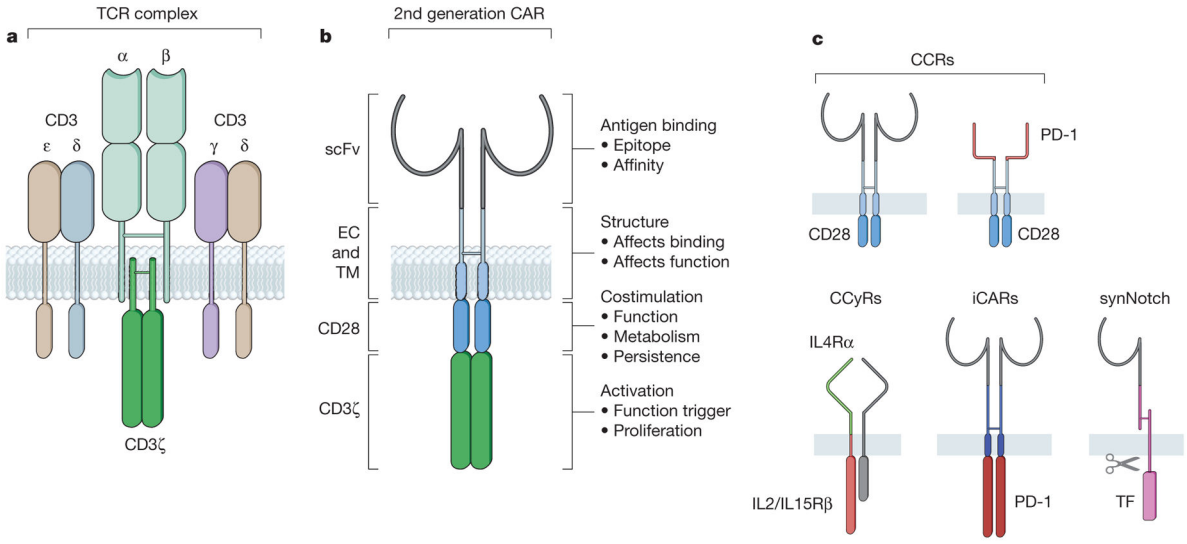

Figure 1. Receptors for antigen

a, T cell receptor (TCR). Physiological antigen recognition is mediated by the TCR–CD3 complex, which comprises six independent gene products: the TCR α and β chains, which together bind to HLA–peptide complexes, and the CD3γ, δ, ε and ζ chains, which initiate T cell activation. **b**, Chimaeric antigen receptor (CAR). A prototypic second-generation CAR comprises three canonical domains for binding to antigen (unrestricted by HLA), T cell activation (commonly via the CD3ζ cytoplasmic domain) and co-stimulation (via the CD28 cytoplasmic domain in this example). The hinge and transmembrane domain also contribute to overall CAR function. **c**, Synthetic receptors in the extended CAR family: chimaeric co-stimulatory receptors (CCRs) provide co-stimulation in response to antigen or alternate ligands; chimaeric cytokine receptors (CyCRs) bind to one cytokine but transduce the signal of another; antigen-specific inhibitory receptors (iCARs) inhibit T cell activation in response to an antigen; synthetic Notch (synNotch) receptors induce CAR expression after antigen recognition by a chimaeric Notch receptor. The scissors indicate a cleavage site that releases a transcription factor (TF).

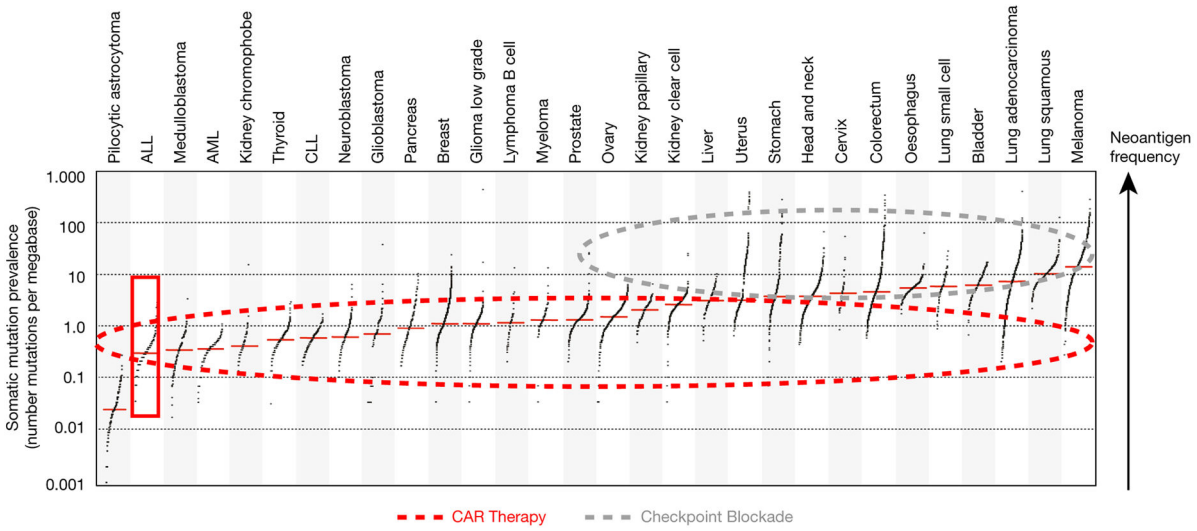

Figure 2. Where to apply CAR therapy

CARs are applicable in principle to any cancer for which suitable cell-surface target antigens are identified. CARs may be especially effective in cancers with low mutation burden that elude checkpoint blockade. Adapted from ref. 175 (Nature Publishing Group), previously adapted from ref. 176 (Nature Publishing Group).

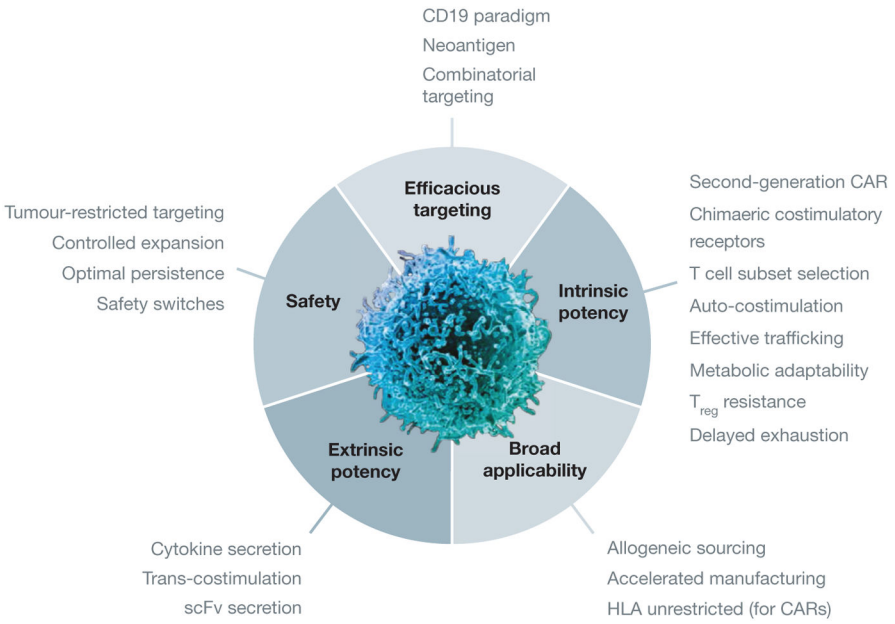

Figure 3. Therapeutic T cell design: goals and strategies

The major goals of T cell engineering address tumour targeting, T cell potency (intrinsic, that is, functionality and persistence; extrinsic: action on the tumour microenvironment), safety and cell manufacturing. Research strategies are exemplified for each one of these goals.

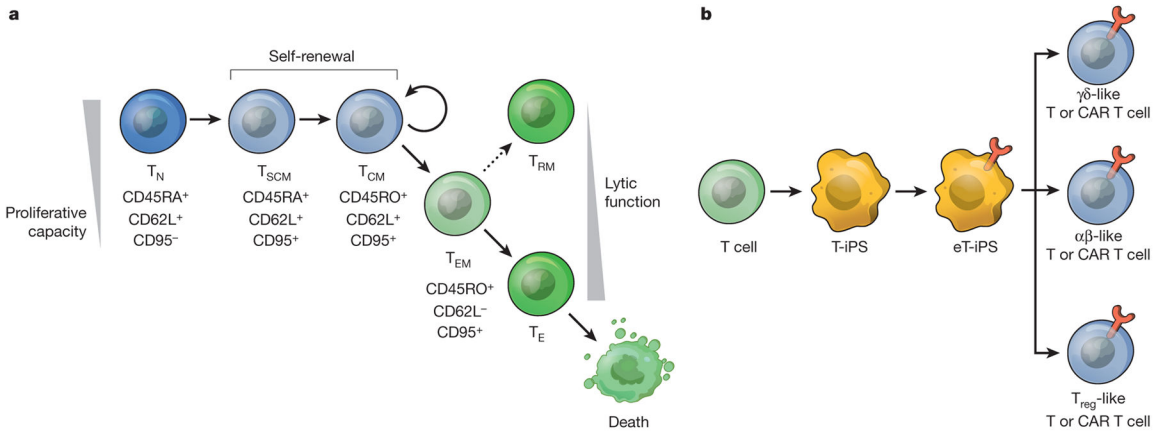

Figure 4. Cell sources for T cell engineering

a, Post-thymic differentiation of memory and effector T cell subsets. T memory stem cells (T_{SCM}) and central memory T cells (T_{CM}) are derived following antigen activation of naive T cells (T_N), and reside in low frequency in blood and higher frequency in lymphoid organs. T_{CM} have been shown in clonogenic assays to be capable of both self-renewal and differentiation to more distal effector memory (T_{EM}) and effector (T_E) cells. T_{EM} and T_E are incapable of self-renewal, and T_E, which can be present at high frequency at the peak of an immune response, are short-lived. A resident memory (T_{RM}) subset resides in peripheral tissues but recirculates very poorly, if at all. Acquisition of effector functions increases as T cells progressively differentiate. The individual memory T cell subsets can be distinguished by the cell surface markers, which can be used for purification for genetic modification. **b**, Pluripotent stem-cell-derived T cells. Human T lymphocytes may be derived in culture from pluripotent stem cells. Several functionalities (cytotoxic, helper, regulatory) could be obtained in principle. αβ-like T cells normally proceed through a double-positive ($CD4^+CD8^+$) intermediate stage, which is not required for the genesis of γδ-like T cells. The stem cell platform is attractive for its ease of genetic engineering and function as a permanent T cell reservoir. T-iPS, T cell-derived induced pluripotent stem cell; eT-iPS, engineered T-iPS (here expressing a CAR).

Table 1

Clinical responses to CD19 CAR therapy

Study	Disease	CAR	V	N	Cond.	T cells	CR rate
Davila, 2014 (ref. 72)	ALL (Ad.)	CD28	γRV	16	CY	Auto	88%
Maude, 2014 (ref. 73)	ALL (Paed.)	4-1BB	LV	25	CF	Auto	90%
Lee, 2015 (ref. 74)	ALL (Paed.)	CD28	γRV	21	CY	Auto	68%
Turtle, 2016 (ref. 75)	ALL (Ad.)	4-1BB	LV	29	CY/CF	1:14/8	93%
Qasim, 2017 (ref. 150)	ALL (Paed.)	4-1BB	LV	2	IC	Allo	100%
Kochenderfer, 2015 (ref. 71)	NHL/CLL	CD28	γRV	15	CF	Auto	53%
Kochenderfer, 2016 (ref. 137)	B-mix	CD28	γRV	20	IC	Allo	30%
Turtle, 2016 (ref. 132)	NHL	4-1BB	LV	32	CY/CF	1:14/8	79%

Clinical responses to CD19 CAR therapy in patients with relapsed/chemorefractory B cell malignancies. These trials make use of different CAR signalling elements (CD28 or 4-1BB), different vector types (gamma-retroviral vector (γRV) or lentiviral vector (LV)) and different conditioning regimens.

Ad., adult; Paed., paediatric; V, vector type; N, number of patients; Cond., conditioning regimen; CY, cyclophosphamide; CF, cyclophosphamide – fludarabine; IC, intense conditioning; Auto T cells, autologous T cells; Allo, allogeneic T cells; 1:1 4/8, autologous T cells with defined (1:1) $CD4^+$ to $CD8^+$ T cell ratio; CR rate, complete remission rate.