Engineered T cells: the promise and challenges of cancer immunotherapy

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Abstract | The immune system evolved to distinguish non-self from self to protect the organism. As cancer is derived from our own cells, immune responses to dysregulated cell growth present a unique challenge. This is compounded by mechanisms of immune evasion and immunosuppression that develop in the tumour microenvironment. The modern genetic toolbox enables the adoptive transfer of engineered T cells to create enhanced anticancer immune functions where natural cancer-specific immune responses have failed. Genetically engineered T cells, so-called 'living drugs', represent a new paradigm in anticancer therapy. Recent clinical trials using T cells engineered to express chimeric antigen receptors (CARs) or engineered T cell receptors (TCRs) have produced stunning results in patients with relapsed or refractory haematological malignancies. In this Review we describe some of the most recent and promising advances in engineered T cell therapy with a particular emphasis on what the next generation of T cell therapy is likely to entail.

T cell receptors (TCRs) provide a recognition signal for T cells complemented by a co-stimulatory signal that can provide an on/off signal to regulate the activation of T cells (FIG. 1). Naturally occurring immune responses against cancer have been described[1]; however, inherent to a persistent cancer is the ability to overcome such immune control. Since Medawar and colleagues[2] carried out their seminal work, it has long been recognized that adoptively transferred T cells have the potential to target and destroy cancer cells. In some cases, however, transferred T cells lacked sufficient specificity or numbers to completely reject a tumour[3–5]. T cells genetically engineered to express novel receptors have enhanced tumour specificity. In addition, advances in *ex vivo* expansion enable the production of clinically relevant doses of these therapeutic cells. Engineered T cells have produced unprecedented results in the clinic.

The earliest clinical trials of engineered T cells in cancer relied on the expression of cloned TCRs with targeted affinity for tumour antigens. A TCR may recognize either intracellular or extracellular antigen in the context of major histocompatibility complex (MHC) presentation. When designing a TCR to target tumour cells, having the option to target intracellular tumour antigen may be advantageous as this may expand the pool of potential targets. Conversely, many tumours downregulate MHC class I expression, potentially masking their presence from a TCR-engineered T cell[6]. More recently, artificial receptors, such as chimeric antigen receptors (CARs), have been used to enhance engineered T cell specificity (FIG. 2). Unlike TCRs, CARs enable highly specific targeting of antigen in an MHC-independent fashion. CARs are formed from a combination of antibody-derived or ligand-derived domains and TCR domains. A CAR is commonly composed of a specificity-conferring, B cell receptor (BCR)-derived, extracellular antibody single-chain variable fragment (scFv), a TCR-derived CD3ζ domain and one or more intracellular co-stimulatory domains. CAR design has evolved over the years to enhance efficacy and safety in particular immunological settings (FIG. 3). Until recently, however, CAR T cell targets were limited to extracellular tumour antigens.

Adoptive transfer of T cells expressing engineered receptors has shown enormous promise in humans. Adoptive transfer of CD19-directed CAR T (CART19) cells has generated complete and durable remissions in patients with refractory and relapsed B cell malignancies[7–10]. NY-ESO-1 (also known as CTAG1A)-specific TCR-engineered T cells have generated clinical responses in patients with advanced multiple myeloma and synovial cell sarcoma[11,12]. With the proof of concept established, engineered T cells have matured as a potential therapeutic option to treat malignancies. Building on this foundation, the field is broadening indications for current therapies, exploring new targets and using new techniques to create even safer and more effective therapies. In this Review, we describe some of the most recent and promising advances in engineered T cell therapy, with a particular emphasis on what the next generation of T cell therapy is likely to entail.

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doi:10.1038/nrc.2016.97
Published online 23 Aug 2016

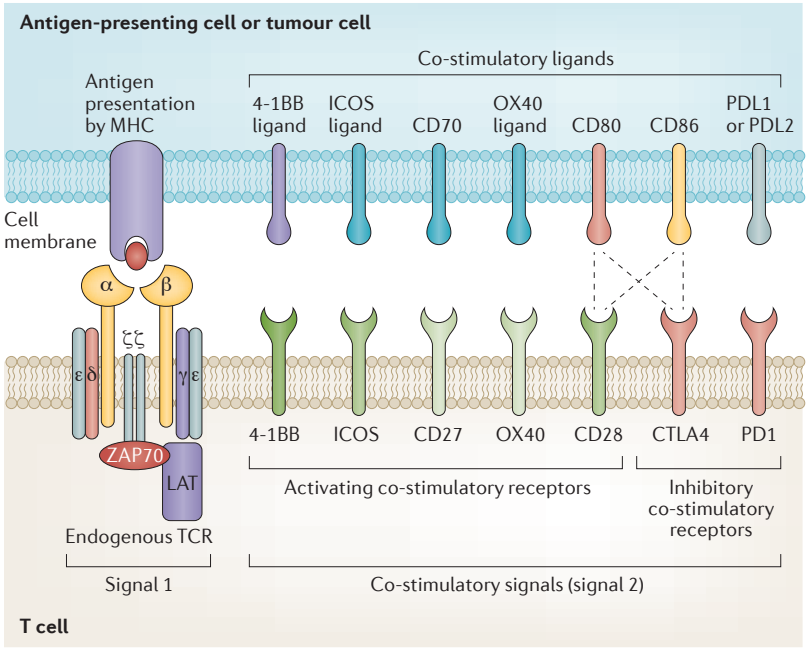

Figure 1 | **T cell receptor and co-stimulatory activation or inhibition of T cells.** Endogenous T cell receptors (TCRs) include paired α and β chains associated with δ, ε and γ chains, and signalling ζ chains. The antigen seen by the TCR is presented by either major histocompatibility complex (MHC) class I or MHC class II (class II shown). The specificity signal delivered through the TCR is commonly referred to as signal 1, as for complete activation leading to effector function, T cells require a co-stimulatory signal, referred to as signal 2. The most common activating co-stimulatory receptor domains that have been investigated in chimeric antigen receptor design are shown. If signal 1 can be thought of as the recognition signal, then signal 2 may be thought of as the on/off switch. The most prominent inhibitory co-stimulatory receptors are cytotoxic T lymphocyte-associated antigen 4 (CTLA4) and programmed cell death protein 1 (PD1). CD28 and CTLA4 both bind to ligands CD80 and CD86, with relative expression determining the course of activation and inhibition of T cells. Many tumours upregulate PD1 ligand (PDL1 or PDL2), among other inhibitory ligands not shown, to turn off T cells. Tumours may also downregulate MHC to evade an effective immune response. ICOS, inducible T cell co-stimulator; LAT, linker for activation of T cell family member 1; ZAP70, ζ-associated protein of 70 kDa.

Clinical trials in B cell malignancies

B cell malignancies are the most common tumour type to be targeted by engineered T cells. There are several reasons for this. B cell malignancies are relatively common and express several conserved cell surface markers. Acquired B cell aplasia is a treatable condition with mild to moderate long-term consequences. Circulating B cell tumours provide easy access for intravenously infused engineered T cells, reducing the requirement for therapeutic cells to traffic to the site of the tumour. Finally, the use of engineered T cells to treat B cell tumours, specifically B cell acute lymphoblastic leukaemia (B-ALL) has shown the greatest promise in the field to date.

The extracellular glycoprotein CD19 is the most common B cell target for engineered T cell therapies (TABLE 1). CD19 is expressed on both benign and most malignant B cells, with extremely limited non-B cell expression[13]. Several groups have reported response rates to CART19 cells in more than 80% of patients with relapsed and refractory B-ALL[7–10]. Moreover, several clinical trials have confirmed that CART19 cells are effective for treating refractory lymphoma with overall response rates of 50–80%[14,15]. Others have targeted rare CD19$^+$ multiple myeloma stem cells, demonstrating disease eradication at 12 months after adoptive transfer of CART19 cells[16]. Although further study is needed, engineered T cells have been shown to persist in at least one patient for more than a decade after transfer[17], suggesting that adoptively transferred T cells may be truly a living drug.

Although CD19 is frequently expressed initially, it may be downregulated[18] or mutated[19] in tumour cells, enabling these cells to acquire resistance to CD19-directed therapy. Relapse rates in B-ALL reported at the 2015 American Society of Hematology meeting ranged from 18% to 36%, with most of these (66–100%) due to CD19$^-$ relapses[20–24]. Alternative markers, such as CD20 and CD22, are also frequently expressed in non-Hodgkin lymphoma[25] and B-ALL[26]. Tolerability of CD20 monoclonal antibodies (rituximab) supports the safe use of anti-CD20 T cells. Although shown to be safe, autologous CD20-targeted CAR T (CART20) cells failed to persist *in vivo* in early trials, with loss of detectable modified cells occurring at between 1 and 9 weeks after transfer[27]. Inclusion of dual co-stimulatory domains (CD28 and 4-1BB (also known as TNFRSF9)) enhanced the persistence of CART20 cells in patients with indolent B cell lymphoma or mantle cell lymphoma[28]. These CART20 cells could be detected up to 1 year after transfer and two of the three patients treated had progression-free survival at 24-month follow-up. Preclinical data have demonstrated CD22-directed CAR T cell antitumour capacity[26] similar to that of CART20. Multiple phase I clinical trials using CART22 products are under way (TABLE 1). Relapse after single-target engineered T cell therapy suggests either the selection of a previously undetectable target-negative clone or acquired resistance by the tumour cells. Combination therapy, simultaneously targeting two tumour markers, may prevent such escape.

During B cell development, a given cell will express either immunoglobulin-κ (Igκ) or Igλ light chains. In humans, the ratio of Igκ$^+$ to Igλ$^+$ cells ranges from 4:1 to 0.5:1. When the ratio exceeds these limits, it is likely that a clonal, Ig light chain-restricted population has expanded. Ig light chain targeting by CAR T cells is a particularly attractive approach because, unlike CD19, Ig light chain-targeted CAR T cells have the potential to leave 20–80% of B cells and plasma cells untouched. In addition, Igκ light chain immunodeficiency does not confer increased risk of infection[29]. Igκ-targeted CAR T cells have been shown to generate specific cytotoxicity in response to Igκ$^+$ tumour cell lines[30]. These cells are now in use as part of a phase I clinical trial to investigate safety and efficacy in humans (TABLE 1).

Engineered T cells designed to target B cell malignancies serve as proof of concept that *ex vivo* modified T cells can eradicate tumour in humans (FIG. 4). These engineered T cells have shown the ability to serially kill malignant B cells, suggesting that transfer of very few cells may be sufficient to achieve remission[31,32]. Experience in manufacturing engineered T cells and

B cell aplasia
The complete *in vivo* absence of B cells.

Response rates
Determinants of whether cancer patients progress, stay the same or improve following therapy.

Autologous
From the same organism.

Plasma cells
B cell derivatives that produce immunoglobulin and are generally CD38$^+$CD138$^+$.

Cytokine release syndrome (CRS). A serious and in some cases potentially life-threatening toxicity that has been observed after administration of natural and bispecific antibodies and, more recently, following adoptive T cell therapies for cancer. CRS is associated with elevated circulating levels of several cytokines including interleukin-6 (IL-6) and interferon-γ (IFNγ).

clinically managing recipients of those cells has been instructive and challenging. Patient-to-patient differences in the T cells collected for *ex vivo* engineering inevitably led to variation between lots, despite refinements to standardize the manufacturing process. One strategy to reduce variability may be to initiate cell manufacturing with a purer starting T cell population, by enriching for central memory T cells, or to set the ratio of $CD4^+$ to $CD8^+$ T cells in the engineered product at 1:1 (REFS 33,34). Clinical management of recipients of engineered T cells with rapid tumour clearance following infusion of redirected T cells has been complicated by the associated immune activation and cytokine release syndrome (CRS). To control such responses, careful management of patients with high tumour burden, including the use of anti-cytokine therapies, and development approaches to adjust T cell activity *in vivo* are under active investigation. Efficacy in treating different lymphoma histologies and the different response rates compared with ALL suggest that specific disease factors may need to be considered to enhance potency. Ultimately, the successful eradication of B cell malignancies by engineered T cells has provided the foundation on which the field of adoptive T cell therapy is expanding.

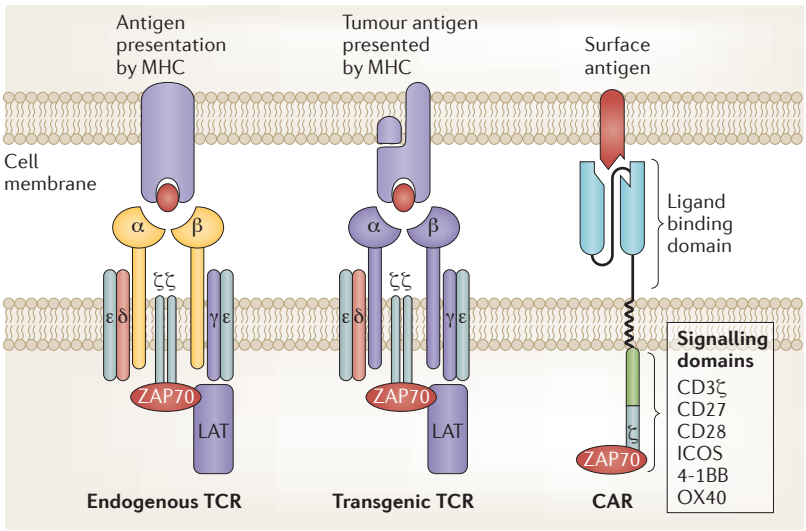

Figure 2 | **Comparing basic structure of engineered T cell receptors and chimeric antigen receptors.** Similar to the endogenous natural T cell receptor (TCR) shown on the left, most transgenic engineered TCRs also rely on recruitment of endogenous downstream signalling molecules such as linker for activation of T cell family member 1 (LAT) and ζ-associated protein of 70 kDa (ZAP70) to transduce the activation signal. Both endogenous and transgenic TCRs recognize intracellularly processed antigens that must be presented in the context of the major histocompatibility complex (MHC) and require co-stimulatory signals (see FIG. 1) for complete T cell activation. Chimeric antigen receptors (CARs), on the other hand, lack TCR α and β chains. The extracellular portion of a CAR consists of single-chain variable fragments derived from immunoglobulin heavy chain variable (V_H) and Ig light chain variable (V_L) domains. Typically these are then fused to a transmembrane domain, an intracellular co-stimulatory domain and an intracellular CD3ζ chain domain. Again, CARs must recruit endogenous downstream signalling molecules to transduce activating signal, but co-stimulation is provided in *cis* and in response to the same activating signal. CARs recognize cell surface antigens independently of the MHC and are therefore not tissue type restricted. ICOS, inducible T cell co-stimulator.

Moving beyond B cell malignancies

Novel target selection for non-B cell haematological malignancies. Several targets in non-B cell malignancies are under investigation (TABLE 1). Upon terminal differentiation, plasma cells downregulate many common engineered T cell targets such as CD19, CD20, CD22 and surface Ig light chains. Therefore, to effectively target malignant plasma cells in conditions such as multiple myeloma, new targets must be considered. One such target, B cell maturation antigen (BCMA; also known as TNFRSF17), is analogous to CD19 in that it is expressed in most cases of multiple myeloma and is not expressed on non-plasma cells[35,36]. Unlike CD19, however, BCMA signalling can induce plasma cell proliferation and survival[37–40]. Therefore, downregulation of BCMA by multiple myeloma cells to escape engineered T cell detection could limit tumour progression. BCMA-CAR T cells eradicate human multiple myeloma cell lines in xenograft models[41]. Two phase I trials are currently investigating the feasibility, safety and efficacy of BCMA-CAR T cells against multiple myeloma (TABLE 1). Cancer–testis antigens, such as NY-ESO-1, are also upregulated on plasma cell myeloma cells and can be highly immunogenic[42]. T cells engineered to express an affinity-enhanced, NY-ESO-1-specific TCR have been used to treat patients with advanced multiple myeloma. Clinical responses in 16 of 20 patients with advanced disease were observed, suggesting great promise in an otherwise incurable disease[11].

Treatment of myeloid malignancies has not progressed substantially over past decades; however, engineered T cell therapy may change this. Myeloid cell surface markers upregulated on malignant cells (for example, CD33, CD123 (also known as IL3Rα) and CD44v6) are under investigation as T cell therapy targets[43–45]. Importantly, CD33 and CD123 are expressed on normal haematopoietic stem cells. Therefore, targeting these markers risks ablation of the haematopoietic stem cell compartment, which would be an intolerable on-target, off-tumour effect. Although preclinical animal studies are equivocal on the question of *in vivo* myeloablation[44,46–48], some have proposed combining anti-myeloid T cell therapy with subsequent bone marrow transplant as salvage therapy[44]. A phase I clinical trial is investigating the use of CD123-targeted CAR T cells in treating myeloid malignancies (TABLE 1).

Interestingly, some potential haematological targets are not unique to haematological malignancy. For example, receptor tyrosine kinase-like orphan receptor (ROR1) is a transmembrane glycoprotein expressed on embryonal tissue and aberrantly on many adult malignant tissues[49,50]. Aberrant cell surface expression of ROR1 has been described in chronic lymphocytic leukaemia (CLL), mantle cell lymphoma, B-ALL and numerous types of solid tumour[49–52]. ROR1 expression seems to enhance cell survival and prevention of apoptosis, suggesting that downregulation of ROR1 may confer a proliferative disadvantage on tumour cells and may therefore be an unlikely mechanism of acquired resistance to ROR1-targeted engineered T cells[53,54]. ROR1-targeted T cells generate cytotoxicity against human $ROR1^+$

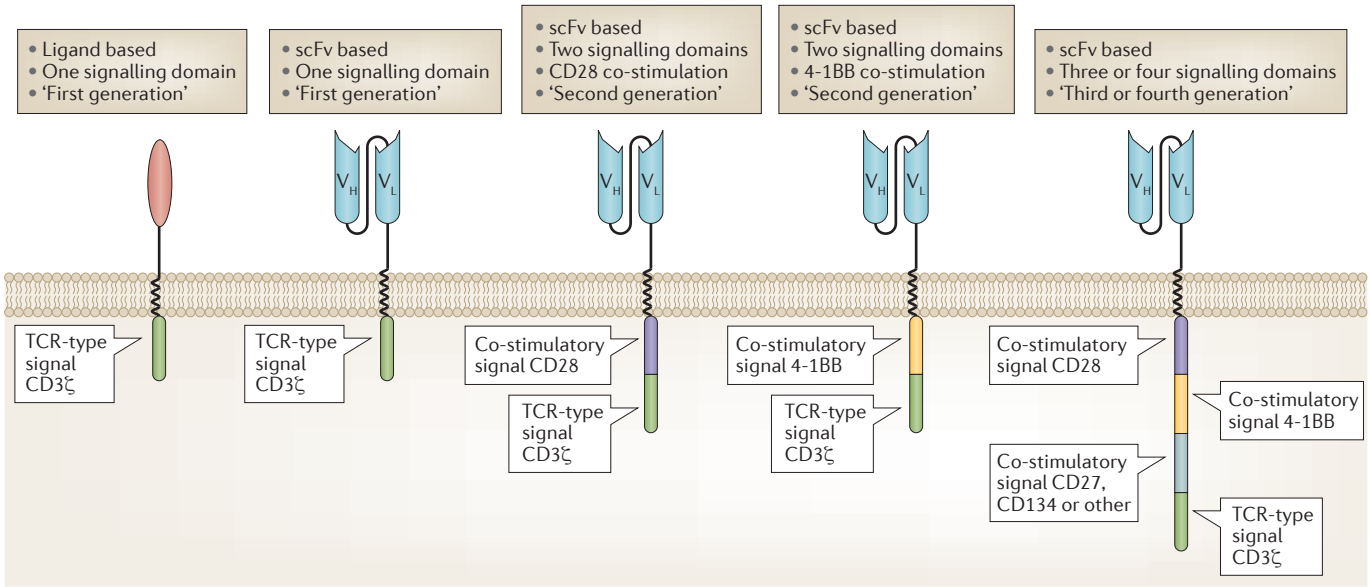

Figure 3 | **Chimeric antigen receptor design and evolution.** Chimeric antigen receptors (CARs) target surface antigens in a major histocompatibility complex (MHC)-independent manner and consist of an extracellular binding domain, hinge domain, transmembrane domain and intracellular signalling domains. The first clinical trials tested CARs that had a binding domain composed of native CD4 that bound to the envelope glycoprotein gp120 expressed by HIV-infected cells[188,189], with a single T cell receptor (TCR) signalling domain composed of the CD3ζ chain[190–192]. CARs with an extracellular domain composed of antibody single-chain variable fragments (scFvs) were first reported by Kuwana[193] and later by Eshhar and colleagues[194,195]. Second-generation CARs incorporating CD28 as a co-stimulatory domain were first developed by Roberts (US Patent 5,686,281) and reported by Finney[196], and those incorporating 4-1BB as a co-stimulatory domain were developed and reported by Finney[197,198], Imai[199] and then others[200,201]. CARs incorporating three or four signalling domains, so called 'third- and fourth-generation' CARs, have also been developed and are beginning to be tested in clinical trials[83,202,203]. V_H, immunoglobulin heavy chain variable domain; V_L, immunoglobulin light chain variable domain.

B cell malignancies and sarcoma in preclinical studies[52,55,56]. Importantly, despite low-level ROR1 expression in non-tumour tissue, transfer of ROR1-CAR T cells into non-human primates did not cause overt toxicity[57]. Autologous ROR1-directed CAR T cells are currently being investigated for safety and feasibility in a phase I trial to treat patients with CLL[58] (TABLE 1).

In search of specific solid tumour targets. Monoclonal antibodies directed against solid tumour antigens have shown promise in early clinical trials, although limited tissue penetration has restricted clinical responses[59]. Endogenous tumour-infiltrating lymphocytes (TILs) have long been known to generate antitumour immune responses and confer a positive prognosis; however, tumour immunosuppression prevents tumour clearance[60–62]. Given the ability of modified T cells to actively traffic to nearly every site in the body[63,64] and to overcome tumour immune evasion[65], engineered T cells possess unique potential to eliminate solid tumours. However, selecting appropriate solid tumour targets can be challenging. Most potential solid tumour targets are non-specific, being expressed on healthy tissue as well. At the same time, off-tumour effects may be less tolerable than the B cell aplasia associated with haematological CAR T cell therapies. Different levels of cell surface marker expression may enable engineered T cells to preferentially target malignant cells[56,66,67]; however, low-level expression on healthy tissue inherently increases the risk of on-target, off-tumour adverse effects. Those solid tumour targets that are highly specific for tumour tissue such as epidermal growth factor receptor variant III (EGFRvIII) may not be expressed throughout the tumour[68]. T cell therapy directed against a tumour target that is not present on all tumour cells runs the risk of selecting for target-negative tumour outgrowth. To date, most solid tumour targets of engineered T cell therapy rely on overexpression in tumour tissue and are relatively non-specific (for example, GD2, interleukin 13 receptor α2 subunit (IL13Rα2), mesothelin and human epidermal growth factor receptor 2 (HER2; also known as ERBB2)). Nonetheless, a wide variety of potential solid tumour targets are under consideration (TABLES 1,2).

Target selection for T cell treatment of glioblastoma illustrates the variety of approaches available. EGFRvIII is a mutant form of EGFR that generates a novel extracellular epitope. Unlike many other solid tumour markers, expression of EGFRvIII seems to be entirely limited to malignant tissue and is found in approximately 30% of cases of glioblastoma. Conversely, IL13Rα2 is also expressed in many cases of glioblastoma (44–100%, depending on methodology)[69,70]. Despite being present in more cases, IL13Rα2 is expressed on non-neoplastic tissues at either reduced[70,71] or comparable[69,72] levels. Engineered T cell therapy targeting either EGFRvIII or

Table 1 | **Examples of chimeric antigen receptor T cell clinical trials**

Target	Indication	Clinical trials and refs*
CD19- or CD20-directed trials		
CD19 or CD20	Leukaemia	NCT01860937, NCT02146924, NCT02228096, NCT02435849, NCT02028455, NCT02614066, NCT02625480, NCT01747486, NCT02030847, NCT02535364 and NCT01683279
		7,8,20,210–214
	Leukaemia or lymphoma	NCT02443831, NCT02529813, NCT02546739, NCT01430390, NCT01853631, NCT02050347, NCT02456350, NCT02081937, NCT02132624, NCT02349698, NCT01475058 and NCT02537977
		10,33,215–220
	Lymphoma	NCT02650999, NCT02431988, NCT02631044, NCT02445248, NCT02277522, NCT02624258, NCT01493453, NCT01840566, NCT02134262, NCT02247609, NCT02348216 and NCT02030834
		14,27,28,34,221–226
	Multiple myeloma	16,227
Additional targets for haematological CAR T cell trials		
CD22	B cell malignancy	NCT02588456 and NCT02315612
Igκ light chain	B cell malignancy	228
CD30	Lymphoma	NCT02259556 and NCT02274584
		229
CD138	Multiple myeloma	230
BCMA	Multiple myeloma	NCT02546167 and NCT02215967
CD33	Myeloid malignancies	231
CD123	Myeloid malignancies	NCT02623582 and NCT02159495
NKG2D ligands	Various haematological malignancies	NCT02203825
ROR1	Leukaemia	58
Solid tumour CAR T cell trials		
EGFR	$EGFR^+$ solid tumours	NCT02331693
		232
EGFRvIII	Glioblastoma	NCT02209376
		74,75,124,233
GD2	Neuroblastoma, Ewing's sarcoma, osteosarcoma and melanoma	NCT01822652 and NCT02107963
IL13Rα2	Glioma	NCT02208362
HER2	$HER2^+$ solid tumours	107,110
Mesothelin	Mesothelioma, pancreatic cancer and ovarian cancer	NCT02159716, NCT02414269, NCT01897415, NCT02580747 and NCT02465983
		99
PSMA	Prostate cancer	NCT01140373
		234
FAP	Malignant pleural mesothelioma	NCT01722149
GPC3	Hepatocellular carcinoma	NCT02395250
MET	Breast cancer	NCT01837602
MUC16	Ovarian cancer	NCT02498912
CEA	Various solid tumours	NCT02349724 and NCT01723306
Lewis-Y	Solid tumours and myeloid malignancies	NCT01716364
MUC1	Hepatocellular carcinoma, NSCLC, pancreatic carcinoma and triple-negative invasive breast carcinoma	NCT02617134 and NCT02587689

BCMA, B cell maturation antigen; CEA, carcinoembryonic antigen; EGFR, epidermal growth factor receptor; EGFRvIII, EGFR variant III; FAP, fibroblast activation protein; GPC3, glypican 3; HER2, human epidermal growth factor receptor 2; Ig, immunogloulin; IL13Rα2, interleukin 13 receptor α2 subunit; MUC, mucin; NSCLC, non-small cell lung carcinoma; ROR1, receptor tyrosine kinase-like orphan receptor. *Ongoing trials are indicated by NCT accession numbers and trials with published or presented results are denoted by references.

Minimal residual disease
Small amount of disease remaining, typically after treatment.

IL13Rα2 has shown promise. EGFRvIII-CAR T cells have been shown to control growth of EGFRvIII$^+$ human glioblastoma in preclinical models[63,73]. Phase I and phase I/II trials are now being conducted to determine the safety and efficacy of EGFRvIII-CAR T cells in treating malignant gliomas[74] (TABLE 2). EGFRvIII-CAR T cells administered intravenously have been reported to cross the blood–brain barrier, infiltrating tumour (examined following resection) and specific target loss was observed[75]. Despite low level non-neoplastic expression of the target, intracranial administration of IL13Rα2-CAR T cells has been shown to be safe and well tolerated in patients with glioblastoma[76]. IL13Rα2-CAR T cell treatment of IL13Rα2$^+$ brain tumours is under investigation in an active phase I clinical trial (TABLE 2).

Ganglioside GD2, a glycosphingolipid, is expressed on various tissues, both malignant and benign. GD2 is highly expressed on neuroectodermal tumours (for example, neuroblastoma, melanoma and glioma), sarcomas, brain cancer and small-cell lung cancer[77–79]. Low-level expression of GD2 is also found on non-malignant neurons, skin, melanocytes and peripheral nerves[80]. GD2 monoclonal antibodies have shown efficacy in the setting of minimal residual disease, suggesting that enhanced immune-mediated tumour clearance may be effective in treating GD2$^+$ tumours[81,82]. GD2 monoclonal antibodies have significant adverse effects, including neuropathic pain, potentially owing to targeting of GD2$^+$ peripheral nerves. GD2-CAR T cells can generate an antitumour immune response in preclinical models[83,84] and in phase I clinical trials treating patients with GD2$^+$ neuroblastoma[85,86]. These patients were treated with GD2-CAR T cells and some experienced durable remission regardless of disease status at the time of infusion. Importantly, despite low-level GD2 expression on benign tissue, GD2-CAR T cells were well tolerated with no dose-limiting toxicities observed[85,86]. These studies were carried out with first-generation CAR T cells, and whether toxicity will be acceptable with more potent CAR designs remains to be determined. A phase I clinical trial is investigating GD2-CAR T cells in patients with various GD2$^+$ malignancies (TABLE 1).

Mesothelin is a 40 kDa cell surface glycoprotein expressed on normal pleura, pericardium and peritoneum[87,88] and overexpressed on various solid tumours, including pancreatic cancer, mesothelioma and subsets of lung, oesophageal, ovarian and breast cancers[89–95]. The physiological function of mesothelin is unknown; however, some evidence suggests that in malignancy, the molecule is involved in metastasis, making this an attractive therapeutic target[96]. Intrathoracic and extrathoracic human mesothelioma lesions are eradicated by mesothelin-targeted CAR T cells in preclinical models[97,98]. These findings confirm that mesothelin-specific CAR T cells can traffic to appropriate body compartments and home to the tumour while retaining antitumour

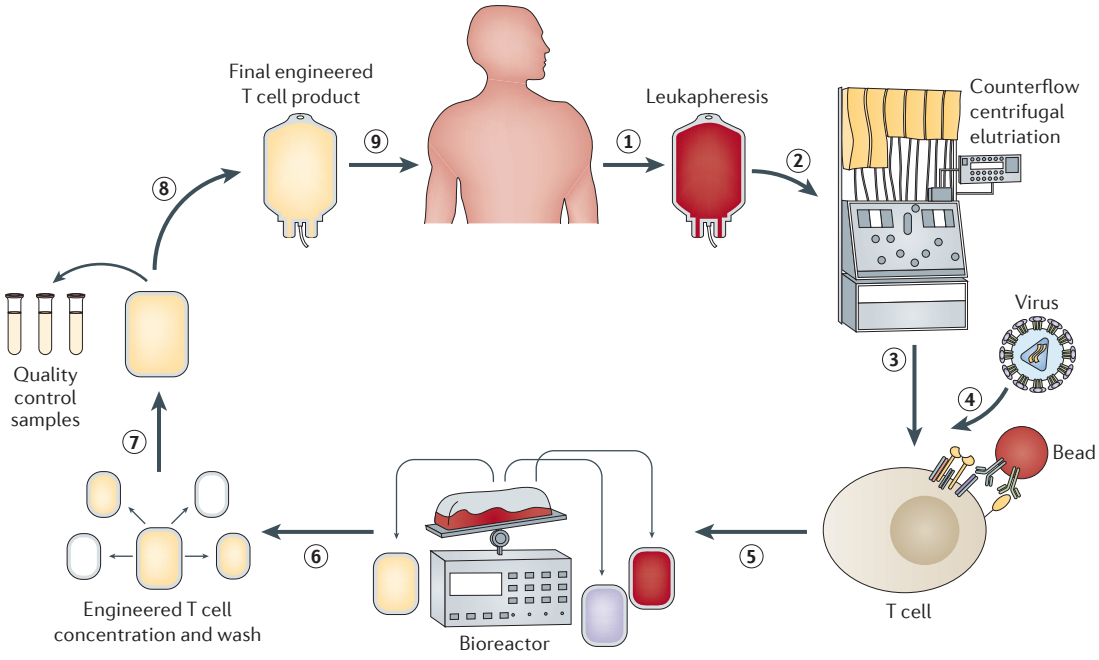

Figure 4 | **Engineered T cell manufacturing.** Leukocytes are generally collected by leukapheresis (step 1) and lymphocytes can be enriched (step 2) by counterflow centrifugal elutriation[204] or subsets selected according to cellular phenotype (not shown). The enriched lymphocytes are placed in culture and (step 3) stimulated with bead-based artificial antigen presenting cells[205,206], and viral vector (step 4) is added[207]. The culture is expanded in a bioreactor for several days (step 5) and then the T cell bulk product (step 6) is washed and concentrated, samples are removed for quality control release testing (step 7) and quality assurance review. The final formulation is cryopreserved (step 8), enabling easy shipment to distant infusion sites, where the final product bag (step 9) is thawed and infused. Manufacturing time is generally 5–10 days, and collection to infusion times can range from 2 to 4 weeks depending on patient clinical status and chemotherapy conditioning regimens.

Immune privileged sites
Body sites that resist immune infiltration and activation.

Immune activation threshold
The sum of minimum signals necessary for immune cell activation; specifically for T cell activation, effector function and proliferation.

Lymphodepletive preconditioning
Treatment regimen, usually chemotherapeutic, that results in lymphopenia and disruption in homeostasis resulting in the *in vivo* production of lymphocyte growth factors that can assist with engraftment following adoptive cellular immunotherapy.

effector T cell function. The ability to localize while retaining function is essential for solid tumour eradication, in particular when targeting tumours in immune privileged sites or within an immunosuppressive tumour microenvironment. Preliminary data from human clinical trials have shown mesothelin-specific CAR T cells to be well tolerated and potentially effective against ovarian cancer, mesothelioma and pancreatic cancer[99,100]. Importantly, despite broad, low-level mesothelin expression on benign tissue, on-target, off-tumour toxicities have not been observed to date. However, these studies were carried out with a CAR that comprised a mouse scFv, resulting in limited persistence of the CAR T cells, potentially owing to host rejection of CAR T cells based on xenogeneic epitopes; whether CAR T cells using a fully human scFv would have durable persistence and acceptable toxicity remains to be determined. Numerous phase I studies are being conducted to further demonstrate the safety and efficacy of mesothelin CAR T cells.

HER2 is a transmembrane receptor tyrosine kinase expressed on normal human gastrointestinal, respiratory, urinary tract, skin, breast and placental tissue. HER2 is also overexpressed in various solid tumours[101]. Millions of women with breast cancer and other tumour histiotypes have benefited from anti-HER2 therapy[102]; however, poor localization and penetration of monoclonal antibody have limited clinical response[103,104]. Furthermore, HER2 expression on some malignancies — for example, HER2$^+$ sarcomas — is below the monoclonal antibody-mediated immune activation threshold[105]. HER2-targeted T cells may overcome these limitations by actively trafficking to and infiltrating tumour microenvironments and triggering in response to low target density.

However, HER2-targeted T cell therapy also serves as an example of the challenge posed by low-level benign tissue target expression. Lethal pulmonary toxicity was observed in a patient with HER2$^+$ colon cancer who was treated with 10^{10} HER2-CAR T cells[106]. It is believed that low-level HER2 expression on pulmonary endothelium triggered this response. This type of reaction was not seen with HER2 monoclonal antibody therapy. This not only suggests that HER2-CAR T cells are able to be activated in response to lower levels of target, but also confirms that monoclonal antibody data are insufficient to predict the safety of T cell therapy. Subsequent HER2-CAR T cell trials have proceeded cautiously by using ultra-low doses of cells. In addition, lymphodepletive preconditioning, which removes endogenous competitors for T cell growth factors, was avoided, as that, along with other factors, may have exacerbated adverse effects[107]. Of note, despite these potential limitations, an antitumour immune response was still detected in patients with HER2$^+$ sarcoma treated with HER2-CAR T cells[107]. These findings are even more striking when one considers the relatively low expression of HER2 in these sarcomas. Preclinical studies demonstrate that HER2-CAR T cells have efficacy in clearing HER2$^+$ glioblastoma and medulloblastoma[108,109]. Alternatively, HER2-CAR T cells may be manufactured from cytomegalovirus (CMV)-specific autologous T cells, producing a bispecific product that will engage CMV$^+$ target cells with the TCR or HER2$^+$ target cells with the CAR, of potential benefit when CMV is also expressed in the tumour microenvironment. Preliminary clinical trial results have demonstrated safety and modest clinical responses associated with these bispecific CAR T cells[110].

A major thrust in the search for new cell targets lies in the discovery of methods to target neoantigens with TCRs or CARs that are particular to each tumour. T cell epitopes associated with impaired peptide processing (TEIPP) antigens are unique T cell epitopes resulting from impaired peptide processing. TEIPP antigens are important because they are derived from broadly expressed self antigens and, similarly to other antigens such as viral antigens, are not restricted by central tolerance[111]. TEIPP antigens do not require the cellular peptide transporter involved in antigen processing (TAP, which is composed of TAP1 and TAP2). Accordingly, tumours that have defects in TAP[112] remain susceptible to TEIPP-specific T cells[113] and are therefore targetable by engineered T cell immunotherapy despite immune evasion by downregulation of antigen presentation.

Table 2 | **Examples of engineered T cell receptor clinical trials**

Target	Indication	Clinical trials and refs*
MAGEA3	Various solid tumours	NCT02153905 and NCT02111850
		235
MAGEA4	Various solid tumours	NCT02096614
NY-ESO-1 with or without additional targets	Various solid tumours	NCT02366546, NCT02457650 and NCT02070406
		12,236,237
	Various malignancies	NCT01697527
	Melanoma	NCT01350401
	Various malignancies	NCT01967823
	Multiple myeloma	NCT01892293
		11
WT1	Myeloid malignancy	NCT01621724, NCT02550535 and NCT01640301
	Mesothelioma and NSCLC	NCT02408016
MART1	Metastatic melanoma	NCT02654821
		238
HPV16-E6	HPV-associated cancers	NCT02280811
Thyroglobulin	Metastatic thyroid cancer	NCT02390739
Melanoma antigen tyrosinase	Melanoma	NCT01586403
		239

CEA, carcinoembryonic antigen; HPV, human papillomavirus; MAGEA, melanoma-associated antigen; MART1, melanoma antigen recognized by T cells; NSCLC, non-small cell lung carcinoma; WT1, Wilms tumour protein 1. *Ongoing trials are indicated by NCT accession numbers and trials with published or presented results are denoted by references.

Neoantigens
Tumour-specific antigens that have not previously been seen by the immune system.

Central tolerance
Mechanism for developing lymphocytes in the thymus and bone marrow to be rendered non-reactive to self antigens.

Antibody-dependent, cell-mediated cytotoxicity
(ADCC). Lysis of a target cell bound by antibodies, which is mediated by an immune cell binding to the Fc portion of the antibodies.

Conceptual evolution in redirected T cell targeting in solid tumours. Clinical feedback has enabled re-evaluation of some basic tenets of CAR T cell targeting. Whereas previous approaches emphasized efficacy, minimization of off-tumour effects is now the primary driver of target selection when potent CAR T cells are used. CAR T cells are able to respond to minimal target expression, making target specificity particularly important. Off-tumour effects can be lethal and currently limit clinical applications, particularly with regard to solid tumour therapy. Although intracellular tumour markers have been classically excluded as potential targets, recent work forces their reconsideration. CARs, by definition, are designed with affinity to an extracellular ligand. However, human antibodies with affinity for an epitope of Wilms tumour protein 1 (WT1) presented by human leukocyte antigen (HLA) class I histocompatibility antigen, A-2α chain (HLA-A2) have been developed[114,115]. Further modifications of these antibodies have enhanced antibody-dependent, cell-mediated cytotoxicity (ADCC)[116]. Thus, whereas TCRs had the advantage of recognizing intracellular antigens presented by MHC to T cells, antibodies that can be incorporated into CAR constructs have now been generated. It is likely that antibodies to additional intracellular antigens presented by MHC will be generated in the future. Inclusion of these intracellular markers as potential targets could improve therapeutic specificity and therefore safety; however, the potential for off-target recognition of this class of CAR T cells remains to be tested.

As the repertoire of potential targets expands, better understanding of cancer biology may enable more precise targeting. Cancer stem cells (CSCs), which are subpopulations of tumour cells with 'stem cell-like' properties, have been identified in ovarian cancer[117], glioblastoma[118–120], multiple myeloma[7] and acute myeloid leukaemia[121,122], among others. Because these cells have been associated with disease progression and resistance to therapy, it has been hypothesized that targeting this subpopulation may provide more long-term benefit. Several varieties of CAR T cell have been shown to be capable of eliminating putative CSC subpopulations in preclinical models and one clinical trial[16,109,123–125]. Future strategies to target CSC subpopulations may maximize clinical effect while minimizing off-tumour effects.

Finally, new findings force us to rethink what it means for a T cell therapy to be 'specific' for a target. Two-step approaches are being used, wherein T cells are engineered to express a receptor with affinity for a non-specific molecule and this molecule is then fused to a targetable agent with high affinity for the tumour. For example, Kim *et al.*[126] created a fluorescein isothiocyanate (FITC)-specific CAR T cell, which bound to a fusion FITC–folate molecule. Folate receptor (FR)$^+$ cells are then 'painted' with the FITC–folate molecule, enabling FITC-CAR T cells to target FR$^+$ cells. Other preclinical models have been developed to target the Fcγ receptor (FcγR)[127]. The advantages of this approach are that the targetable agent may control response and enable simultaneous multivalent targeting by a single population of engineered T cells. One potential considerable challenge is achieving and maintaining sufficient concentration of both CAR T cells and the linker molecule at the tumour site. Alternatively, others have generated T cells specific for tumour antigen that, upon binding, produce cytokines that are intended to recruit endogenous immune cells and mediate tumour clearance. T cells redirected for universal cytokine killing (TRUCKs) have been engineered to express inducible or constitutive IL-12, which induces innate antitumour immune responses and alleviates immunosuppression in the tumour microenvironment[128,129] (FIG. 5a). By modifying the tumour stroma, TRUCKs have the ability to enhance tumour infiltration by endogenous immune cells[128]. Finally, despite a great deal of effort to define engineered T cell specificity *ex vivo*, the specificity of these cells may evolve upon their *in vivo* stimulation by exposure to tumour cells. After EGFRvIII$^+$ tumour cell clearance by EGFRvIII-CAR T cells, mice have been shown to be resistant to subsequent EGFRvIII$^-$ tumour challenge[73]. This demonstrates that engineered T cells have the ability to generate immunity to non-target tumour antigens after *in vivo* antitumour immune response[73,74]. Together, these findings serve as a reminder that an engineered T cell antitumour response is a dynamic process that relies on both cell design and host factors.

Building smarter redirected T cells

Novel gene transfer and editing. Current gene modification techniques used to produce engineered T cells must balance efficiency, safety and cost. Owing to robust efficiency, viral vector-based protocols are the most frequently used methods of T cell transduction[130] (FIG. 3). Both retroviral and lentiviral vectors are able to deliver moderate-sized payloads, which integrate into host genomes and consistently express the construct. Lentiviral vectors are preferred to retroviral vectors as they may integrate in non-dividing human primary cells and confer a decreased risk of insertional oncogenesis, at least as observed in haematopoietic stem cells[131–135]. Of note, to date, no lentivirus-transduced engineered T cell products have been reported to demonstrate insertional mutagenesis despite hundreds of patients being treated.

DNA transposons have been used to efficiently insert gene cassettes into the host genomic DNA[130,136,137]. Transposon-based systems, such as the Sleeping Beauty (SB) transposon system, have been developed to successfully produce engineered T cells of suitable quality for clinical investigations[138,139]. The safety and efficacy of transposon-engineered CART19 cells are currently under investigation[140] (NCT00968760). Alternative approaches, such as the PiggyBac transposon system, have also been used to generate several types of engineered T cell (for example, CART19 cells[141] and Epstein–Barr virus (EBV)-specific HER2-CAR T cells[142]). With viral and non-viral methods of integration, a theoretical risk of insertional oncogenesis remains.

Along with advances in electroporation techniques, the efficiency of non-integrating, non-viral methods of gene modification are showing promise as an alternative or complement to viral vector-based methods. Electroporation also enables provision of non-integrating constructs, such as mRNA, which eliminates the risk of

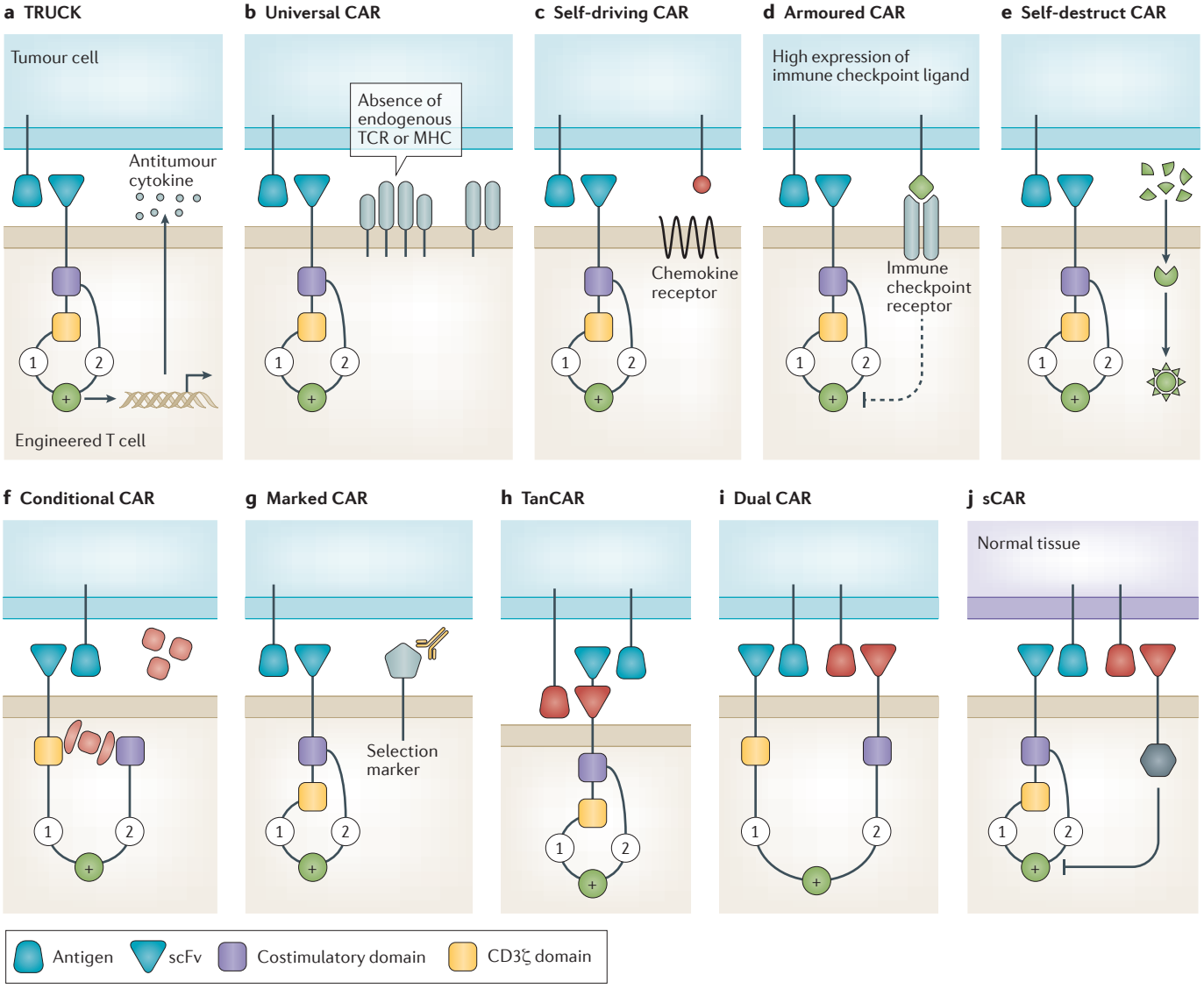

Figure 5 | **New chimeric antigen receptor models and concepts.** **a** | T cells redirected for universal cytokine killing (TRUCKs) co-express a chimeric antigen receptor (CAR) and an antitumour cytokine. Cytokine expression may be constitutive or induced by T cell activation (for example, interleukin-12 (IL-12)). Targeted by CAR specificity, localized production of pro-inflammatory cytokines recruits endogenous immune cells to tumour sites and may potentiate an antitumour response. **b** | Universal, allogeneic CAR T cells are engineered to no longer express endogenous T cell receptor (TCR) and/or major histocompatibility complex (MHC) molecules, thereby preventing graft-versus-host disease (GVHD) or rejection, respectively. **c** | Self-driving CARs co-express a CAR and a chemokine receptor, which binds to a tumour ligand (for example, C-C motif chemokine receptor 2 (CCR2)–C-C motif chemokine ligand 2 (CCL2)), thereby enhancing tumour homing. **d** | CAR T cells engineered to be resistant to immunosuppression (armoured CARs) may be genetically modified to no longer express various immune checkpoint molecules (for example, cytotoxic T lymphocyte-associated antigen 4 (CTLA4) or programmed cell death protein 1 (PD1)), with an immune checkpoint switch receptor, or may be administered with a monoclonal antibody that blocks immune checkpoint signalling. **e** | A self-destruct CAR may be designed using RNA delivered by electroporation to encode the CAR[98,143]. Alternatively, inducible apoptosis of the T cell (right part of panel **g**) may be achieved based on ganciclovir binding to thymidine kinase in gene-modified lymphocytes[208] or the more recently described system of activation of human caspase 9 by a small-molecule dimerizer[25,209]. **f** | A conditional CAR T cell is by default unresponsive, or switched 'off', until the addition of a small molecule to complete the circuit, enabling full transduction of both signal 1 and signal 2, thereby activating the CAR T cell[126,169]. Alternatively, T cells may be engineered to express an adaptor-specific receptor with affinity for subsequently administered secondary antibodies directed at target antigen[127]. **g** | Marked CAR T cells express a CAR plus a tumour epitope to which an existing monoclonal antibody agent binds. In the setting of intolerable adverse effects, administration of the monoclonal antibody clears the CAR T cells and alleviates symptoms with no additional off-tumour effects. **h** | A tandem CAR (TanCAR) T cell expresses a single CAR consisting of two linked single-chain variable fragments (scFvs) that have different affinities fused to intracellular co-stimulatory domain(s) and a CD3ζ domain. TanCAR T cell activation is achieved only when target cells co-express both targets. **i** | A dual CAR T cell expresses two separate CARs with different ligand binding targets; one CAR includes only the CD3ζ domain and the other CAR includes only the co-stimulatory domain(s). Dual CAR T cell activation requires co-expression of both targets on the tumour. **j** | A safety CAR (sCAR) consists of an extracellular scFv fused to an intracellular inhibitory domain (for example, CTLA4 or PD1). sCAR T cells co-expressing a standard CAR become activated only when encountering target cells that possess the standard CAR target but lack the sCAR target.

insertional oncogenesis. For these reasons, electroporation of engineered T cells is an emerging strategy for gene modification and interrogation of new tumour targets. As opposed to electroporation of DNA, electroporated mRNA does not require genomic integration for construct expression. Whereas integrated constructs have been observed for more than a decade after transfer of modified cells[17], electroporated mRNA rapidly degrades and is associated with transient expression[98,143]. In clinical application, transient expression of a construct may require repeated doses of adoptively transferred cells to achieve adequate effector function and may limit differentiation into a memory phenotype[98,144]. In humans, RNA-modified mesothelin-CAR T cells have been shown to be safe; however, repeated doses may be problematic if the engineered cells are themselves immunogenic owing to expression of non-self molecules such as scFv, if xenogeneic in origin. Although preliminary evidence suggests that these cells are effective at targeting mesothelin-positive tumours[99], one case of anaphylaxis has been described in the setting of infusions that were separated by 49 days[145]. Numerous active clinical trials are using mRNA-modified engineered T cells to target malignancy (TABLES 1,2).

Gene editing is one of the most exciting recent developments in the modernization of redirected T cell manufacturing. The overarching term gene editing refers to various techniques that confer particular advantages or disadvantages depending on application. What they share, however, is the ability to efficiently knock out and/or knock in genetic elements. Protein-based (zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs)) and RNA-based (CRISPR–Cas9) techniques are all, to varying degrees, effective at specific gene disruption or insertion. To produce engineered T cells resistant to tumour-mediated suppression, gene editing may be used to knock out inhibitory receptors and/or knock in an array of function-enhancing molecules.

Efficient gene editing of primary human T cells has been demonstrated using ZFNs, TALENs and CRISPR–Cas9 (REFS 146–151). Furthermore, the safety of gene-edited T cells in humans was demonstrated by the adoptive transfer of autologous T cells in which C-C motif chemokine receptor 5 (*CCR5*) was knocked out by ZFNs[151]. The manufacture of gene-edited human CAR T cells has been shown to be feasible with the production of TALEN or CRISPR–Cas9 gene-edited CAR T cells[148,149] (FIG. 5b). A preliminary description of the first use of gene-edited CAR T cells in humans was recently reported in an infant with CD19$^+$ ALL for whom autologous CAR T cells were unable to be produced[148]. Instead, engineered CAR T cells were produced from an unrelated donor by deleting the endogenous *TCR* to prevent graft-versus-host disease (GVHD). In addition, *CD52*, a lymphocyte marker present on the patient's malignant B cells, was deleted from the CAR T cells, enabling treatment with monoclonal antibody against CD52 to eliminate recipient lymphocytes while sparing the infused CD52$^-$ CAR T cells. The patient was heavily preconditioned with chemotherapy to delay donor CAR T cell rejection and thereby enhance CAR T cell persistence. The administration of donor-derived, gene-edited T cells has the potential to revolutionize the current manufacturing paradigm; a single donor could provide starting material to manufacture products for numerous recipients. Although promising, this exciting step forward will require further investigation in more patients to demonstrate the role of allogeneic CAR T cells in tumour control. Safe and effective use of allogeneic CAR T cells may require additional editing of endogenous molecules such as HLA[149,152].

Enhancing trafficking. Engineered T cell localization at target sites is crucial for clinical efficacy, particularly when targeting solid tumours[153]. Route of administration and effective trafficking to the tumour site each play an important part in granting T cell access to target tissue. Although T cells can migrate to nearly all body compartments, including immune privileged sites[63,100], accumulation of engineered T cells may be enhanced by local administration. In several preclinical solid tumour models, local administration of CAR T cells demonstrated superior accumulation at tumour sites and superior control of tumour growth compared with systemic administration[97,138,154]. Notably, in an orthotopic mouse model, intrapleurally injected mesothelin-CAR T cells outperformed systemically administered cells in clearance of intrathoracic and extrathoracic mesothelioma lesions[97]. The superior extrathoracic tumour clearance suggests that early exposure of engineered T cells to tumour immediately after transfer may enhance the activation of CAR T cells via early antigen exposure and may therefore improve their ability to mediate an antitumour response. Furthermore, engineered T cells can be modified to enhance trafficking (FIG. 5c). Chemokine receptor–ligand interactions have an important role in mediating endogenous immune cell trafficking. In fact, the efficacy of conventional chemotherapeutics is linked to the upregulation of chemokine ligands on tumour cells that is mediated by these drugs[155]. CAR T cells may be engineered to express chemokine receptors to enhance trafficking into tumour tissue and homing to tumour cells. For example, co-expression of the chemokine receptor CCR2b in CAR T cells targeting either GD2 or mesothelin has been shown to enhance tumour infiltration and antitumour effects in animal models[156,157].

Avoiding tumour suppression and escape. Malignancy may be refractory to engineered T cell therapy owing to immune escape or immunosuppression in the tumour microenvironment. Various CD19 mutations and alternative splicing have been associated with the development of CART19-resistant ALL[19]. In this setting multivalent targeting may prevent single-agent resistance. The combination of CD123- and CD19-targeted CAR T cells prevents the outgrowth of CD19$^-$ escape mutants in preclinical models[158]. The tumour microenvironment may also directly inhibit a potential antitumour immune response. By definition, the existence of a tumour is dependent on some degree of evasion or inhibition of endogenous immune control. Inhibition

Graft-versus-host disease
(GVHD). Immune reaction of a donor graft containing immune cells against the recipient host that is a by-product of a mismatch in human leukocyte antigens (HLAs). GVHD is a major cause of morbidity following allogeneic haematopoietic stem cell transplantation.

Allogeneic
Genetically distinct but from the same species.

Immune checkpoints
System of immune suppression.

Primary signal
The antigen-specific signal delivered to a T cell through the T cell receptor, which is complemented by co-stimulatory signals to achieve full-function T cell activation and effector function.

is achieved through various mechanisms, including cell–cell signalling and release of soluble cytokines. Importantly, like the endogenous immune system, adoptively transferred T cells are also susceptible to tumour-mediated immunosuppression[159]. Furthermore, chronic T cell activation induces upregulation of inhibitory ligands on the activated T cells, perhaps contributing to T cell exhaustion[160]. Various methods can be used to engineer T cells to be intrinsically resistant to tumour immunosuppression (FIG. 5d). The expression of a dominant-negative transforming growth factor-β (TGFβ) receptor type II confers T cell resistance to this tumour-produced, suppressive cytokine[161]. Others have transduced tumour-specific T cells with hybrid receptors comprising an IL-4 exodomain and an IL-7 endodomain[162]. Tumour-generated IL-4, a suppressive cytokine, produces an activating signal in these engineered T cells. The addition of programmed cell death protein 1 (PD1; also known as PDCD1) monoclonal antibody has been shown to enhance the function of CAR T cells in preclinical models, suggesting that, like endogenous immune cells, engineered T cells are subject to tumour immune suppression through immune checkpoints and would become more effective if disinhibited in this way[163]. Many groups are now attempting to generate CAR T cells resistant to PD1–PD1 ligand 1 (PDL1) or cytotoxic T lymphocyte-associated antigen 4 (CTLA4)–CD80/CD86 signalling[149,164]. Future T cell therapies are likely to incorporate multiple forms of immune checkpoint blockade to further enhance efficacy.

Improving safety: Boolean logic gates. While treatment-associated mortality is far below that seen with conventional treatments for relapsed or refractory cancers, serious adverse events have been observed following infusions of engineered T cells. Excessive and rapid tumour clearance has been associated with serious and occasionally fatal CRS. On-target, off-tumour activation of engineered T cells by a very low level of target on non-malignant tissue has been associated with dose-limiting toxicities[165] and death in some cases[106,124]. Finally, unexpected and fatal cross-reactivity seen with engineered TCR T cells demonstrates the current limitations of *in vitro* screening for cross-reactivity[166,167].

Molecular 'switches' enable greater control over the performance of engineered T cells *in vivo* and may improve safety. Cells may be engineered to express pro-death signals that can be induced with an exogenous element (off switch, see FIG. 5e). Examples of off switches or 'suicide genes' include Herpes simplex virus thymidine kinase (HSV-TK), which can be induced by treatment with ganciclovir, and inducible human caspase 9 (iCasp9), which can be induced by intravenous administration of FK506 binding protein (FK506BP). Deletion of CAR T cells in animal models has been achieved using both HSV-TK–ganciclovir[168] or iCasp9–FK506BP systems[169].

Alternatively, T cells may be engineered to conditionally activate only in the presence of an exogenous molecule, withdrawal of which terminates signalling (on switch) (FIG. 5f). On switches are currently under development as this technology is less mature. On switches may prove safer than off switches, as the default is no signalling. In addition, removal of the exogenous activator molecule does not necessarily lead to death of the engineered T cell. One can envision repeated dosing of the activator molecule, which can be tailored to the patient's tolerance of the treatment. The feasibility of producing CAR T cells with small-molecule-dependent signalling has been established in preclinical models[170,171]. In this system, the small-molecule-controlled switch redirects activity of the orthogonal receptor through the selective formation of immunological synapses in a temporally controlled manner. Furthermore, this system is readily adaptable to different antigen targets. Another type of flexible receptor targeting system has recently been described by Lim and colleagues[172]. This system, which is based on synthetic NOTCH receptors, enables conditional expression of a targeting receptor upon engagement with a tissue-specific ligand.

Engineered T cells may be marked with unique cell surface molecules to which approved therapeutic monoclonal antibodies bind (FIG. 5g). If this epitope is also expressed on tumour cells, treatment with these monoclonal antibodies could eliminate CAR T cell-mediated adverse effects while simultaneously treating the tumour. A fusion of CD34 and CD20 epitopes (RQR8)[148,173] and a truncated form of human EGFR polypeptide[174] have separately been expressed in CAR T cells. In the setting of intolerable adverse effects, these CAR T cells would be susceptible to elimination by rituximab (monoclonal CD20 antibody) or cetuximab (monoclonal EGFR antibody), respectively. Given the availability of such a wide array of inducible and specific methods of CAR T cell elimination, it is likely that more clinical trials will include such constructs in the future[25,169,173,174].

Deletion of CAR T cells may limit adverse effects, but will also terminate the antitumour clinical effect. Off-tumour toxicity can also be prevented by designing CAR T cells with enhanced specificity. To achieve this, CAR T cells have been designed to activate only in response to a particular combination of targets. For example, bispecific CARs have been generated such that the extracellular portion of the CAR contains two linked scFvs with different specificities (FIG. 5h). T cells expressing these tandem CARs (TanCAR) are activated only in the presence of both targets; a target cell positive for a single antigen is insufficient to trigger T cell activation and cell killing. TanCARs against $HER2^+CD19$ and $HER2^+IL13R\alpha2$ have been developed[175,176]. An alternative to this method is to combine one CAR that transmits only primary signal with a second CAR with distinct tumour antigen specificity that transmits only co-stimulation (FIG. 5i). In this approach, a single T cell expressing a CD3ζ-only CAR against the first target and a co-stimulatory domain-only CAR against a second target will become fully activated only in the presence of both targets. Such dual CARs against mesothelin plus FRα and HER2 plus mucin 1 (MUC1) have been shown to generate specific target cytotoxicity against dually expressed targets[177,178]. Lastly, extracellular scFv fused to inhibitory signalling domains can specifically inhibit CAR T

cell activation (FIG. 5j). These inhibitory signals enable protection of cells with a particular immunophenotype from CAR T cell killing[179]. Incorporation of all activation and inhibitory signals creates a complex computational algorithm for engineered TCR targeting and decision-making. Importantly, this has enabled reconsideration of targets previously thought to be undesirable owing to off-tumour toxicities. Furthermore, many of the same types of receptor design shown in FIG. 5 may be applied to the next generations of engineered TCRs to improve targeting and control. For example, a self-destruct or conditional switch may be inserted, along with the engineered TCR. A switch receptor, or armoured TCR, may be created by inserting a decoy receptor that binds to PDL1 on tumours, but provides an accessory signal to augment engineered TCR signalling[164,180]. These new molecular systems embedded in a cellular drug will soon enable highly specific immunophenotypes to be targeted, off-tumour effects to be minimized and safety to be enhanced in the clinic.

The future of cancer immunotherapy

The advent of kinase-targeted drug therapies and checkpoint blockade antibodies has increased survival in some patients with cancer. In the previous decade, patients with myeloma had an average survival of 2–3 years, and with the advent of improved therapies it is now 7–8 years, and still increasing[181,182,148,149]. Although CLL has remained incurable with standard treatments[151,183], the advent of effective targeted therapies such as ibrutinib and idelalisib has significantly extended survival[152,184].

Immune checkpoint therapies are a new class of cancer immunotherapy that constitutes a major advance in cancer treatment in the past decade, with reproducible benefit observed in 20–30% of patients with various previously incurable cancers[160]. However, there are serious adverse effects and sizeable costs associated with recurrent administration, and most patients do not currently benefit from these therapies. Thus, these therapies, which may require long-term maintenance dosing, present a considerable economic burden for patients and the economy.

In contrast, adoptive cell transfer therapy with engineered T cells has two characteristics that may complement the limitations of kinase-targeted and immune checkpoint therapies. First, engineered T cells require only one treatment for durable benefit[185]. Second, nearly all patients (>90%) with ALL respond to CAR T cells[8,10], a response rate not previously observed with other forms of cancer therapy. Although not yet tested clinically, preclinical models reveal a potent enhancement of anti-tumour efficacy with the combination of CAR T cells and immune checkpoint blockade[186] (NCT02650999). It is possible that the combination of these therapies could result in the long-term survival and eventual cure of several cancers after only a few treatments[187]. Even today, with early-generation manufacturing, the production and delivery of a one-time treatment delivering durable benefit is disruptive to health care financing and reimbursement models. The expanded availability of redirected T cell therapeutics in cancers beyond haematological malignancies is dependent on the development of automated cell engineering and potentially on the development of universal sources of allogeneic T cells.

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Acknowledgements
A.D.F. is supported by National Institutes of Health (NIH) grant 5T32HL007775-22; B.L.L. is supported by NIH grants 1RO1CA165206 and P30-CA016520-35. C.H.J. is supported by NIH grants 1RO1CA165206 and 5R01CA120409, by the Leukemia and Lymphoma Society and the Parker Foundation. The authors would like to acknowledge research assistance from K. Lamontagne, and the inspiration and support of their patients and their professional colleagues.

Competing interests statement
The authors declare competing interests: see Web version for details.