REVIEW ARTICLE
https://doi.org/10.1038/s43018-021-00241-5

nature cancer

Engineering-enhanced CAR T cells for improved cancer therapy

Michael C. Milone[1,2], Jie Xu[1,2,3], Sai-Juan Chen[3], McKensie A. Collins[1,2], Jiafeng Zhou[4], Daniel J. Powell Jr[1,2] and J. Joseph Melenhorst[1,2] ✉

Chimeric antigen receptor (CAR) T cell therapies have evolved from a research tool to a concept-shifting therapy with impressive responses in B cell malignancies. This Review summarizes the current state of the CAR T cell field, focusing on CD19- and B cell maturation antigen-directed CAR T cells—the most developed of the CAR T cell therapies. We discuss the many challenges to CAR T cell therapeutic success and innovations in CAR design and T cell engineering aimed at extending this therapeutic platform beyond hematologic malignancies.

Although antitumor immunity by T lymphocytes has been known for decades, translating it into anticancer therapies has been challenging. However, biological advances, such as the generation of single-chain antibody fragments (scFv)[1], the elucidation of pathways mediating the activation of functional memory T cells[2,3], and molecular cloning[4] have led to the engineering of chimeric antigen receptor (CAR) T cells, introducing a new era of cancer immunotherapy[5,6] and permitting the treatment of large groups of patients with genetically augmented patient-derived T cells.

The first generation of CAR T cells fused the scFv antibody fragment to T cell signaling domains comprising the immunoreceptor tyrosine-based activation motif, offering a relatively simple method of endowing T cells with major histocompatibility complex-independent recognition of antigens[7]. Over the following two decades, the CAR platform evolved into second- (two-domain) and third-generation (three-domain) CARs that incorporated additional signal transduction domains, including cytoplasmic domains from important T cell costimulatory receptors such as CD28, CD137 (4-1BB) and CD134 (OX-40) (reviewed in refs. [8,9]). These additional signaling domains promote both the persistence and antitumor activity of CAR T cells following adoptive transfer[3,10–14], and were essential to avoid the anergy observed with first-generation CARs[15].

The remarkable ability of a CAR to reprogram T cell specificity led to attempts at clinical translation. The earliest clinical application used a simple CAR design comprising a CD4 ectodomain fused to a CD3ζ cytoplasmic domain to treat human immunodeficiency virus-infected patients[16], and established both the safety of engineered CAR T cells and the potential for decade-long persistence of genetically modified T cells[17]. Subsequent studies evaluating first- and second-generation scFv-based CARs soon followed, leading to the demonstration of robust activity of CD19-specific CAR T (CART19) cells, and ultimately the regulatory approval of two CAR T cell therapies for hematologic malignancies in the United States (in 2017) and Europe (in 2018).

As preclinical models of adoptive T cell therapy are limited, correlative studies performed during their clinical development to determine the kinetics and quality of the infused CAR T cells, measure tumor cell dynamics and assess cytokine levels and repertoires during therapy have proven pivotal in improving our understanding of these complex therapies and enhancing their clinical application. These correlative studies have highlighted many factors that are essential to safely achieving both deep and durable clinical responses in otherwise treatment-refractory cancers. Here, we discuss the important role of correlative science in developing CAR T cell therapies, and highlight the challenges still faced during clinical application and the new technologies promising to address these complications to help extend this therapeutic modality beyond B cell malignancies.

Efficacy and toxicity of CD19-specific CAR T cell therapies

Normal and malignant B cells uniformly and exclusively express CD19 (ref. [18])—the dominant signaling moiety of a tetramolecular complex consisting of CD21, CD81 and CD225, which modulates B cell receptor signaling and mediates immunoglobulin-induced B cell activation[19]. Given CD19's broad expression within the B cell lineage from early pro-B cells to subsets of plasma cells (Fig. 1), as well as its generally uniform expression on B cell malignancies[20], this molecule became a prime target of CAR T cell approaches. The initial encouraging results in relatively small studies in non-Hodgkin's lymphoma (NHL)[13,21], chronic lymphocytic leukemia (CLL)[22–24] and acute lymphoblastic leukemia (ALL)[24,25] have since been confirmed in larger cohorts[26–36]. So far, the first patients with CLL treated with anti-CD19 CAR T cells have sustained remission beyond 9 years[37] and the first ALL patient to be treated with the same engineered product has been in remission for more than 7 years[25].

Generally, the overall response rate has been highest in B cell ALL (>80%), variable in lymphomas (~63–100%) and lower in CLL (50–70%)[28,35,38,39]. Patients with CLL who achieved remission with anti-CD19 CAR T cell treatment sustained their disease-free state[28,35,40]. In ALL, however, only 20–40% of patients sustained remission on this therapy[28,32,33,35,38,39]. Loss of CD19 expression is a major mechanism of resistance in ALL, accounting for around two-thirds of relapse cases and is a well-recognized phenomenon in lymphoma as well[41–43]. Loss of CAR T cell engraftment may account for most of the remaining cases of relapse[26]. Initial small trials[13,24] followed by larger ones[32,33,44] also confirmed the immense potential

[1]Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. [2]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. [3]Department of Hematology, Shanghai Institute of Hematology, Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China. [4]Immunotherapy Research Center for Hematologic Diseases of Hubei Province, Tianjin, People's Republic of China. ✉e-mail: mej@pennmedicine.upenn.edu

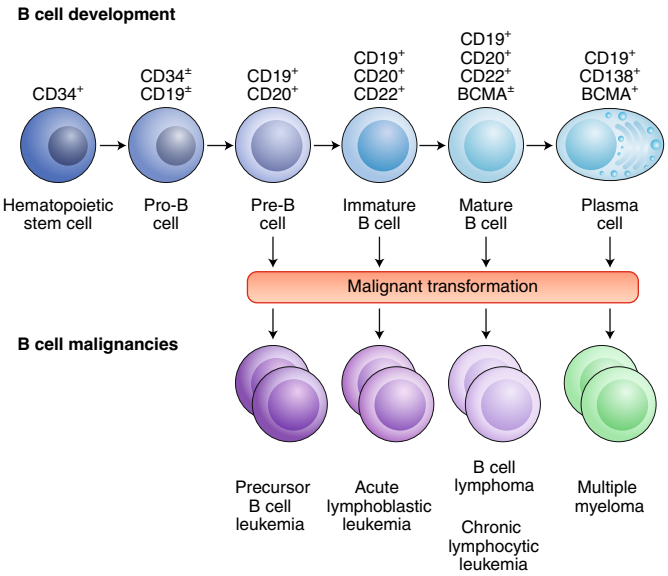

Fig. 1 | B cell malignancies at the different stages of B cell development. Normal B cell developmental lymphocytes (top) often share immunophenotypic characteristics with their malignant counterparts (bottom), reflecting the expansion of a dominant clone leading to the development of leukemia or lymphoma.

for this therapy in NHL. Both the CD28[33] and 4-1BB cosignaling anti-CD19 CAR T cells[45] induced complete remission in 40–50% of patients, most of whom remained disease free. Although clinical responses were generally sustained in NHL, most disease-free patients would display normal B cell recurrence and loss of detectable CAR T cells, suggesting that other mechanisms were responsible for long-term tumor control in NHL.

Interestingly, although patients with ALL and CLL generally achieved their best overall response within the first month following CAR T cell infusion, patients with lymphoma often continued to improve beyond the first month, with some patients not achieving their maximum response until 6 months post-CAR T cell treatment[32,33,44,46]. The reasons for these differences are not understood, and remain an important subject of study in the post-marketing phase.

Although CD19-specific CAR T cell therapies have shown remarkable clinical activity against B cell malignancies, these deep and durable responses do come at the cost of some unique adverse effects. Cytokine release syndrome (CRS) is the most frequently observed adverse event in CART19-treated patients. Most cases of CRS are mild or moderate in severity and manageable. However, the frequency of severe CRS across studies, reported in 19.8–38.8% of treated individuals[47], has been clouded by the use of diverse grading systems. Fortunately, a new consensus grading system for CRS was described recently, the adoption of which should greatly facilitate comparing its incidence across different CAR T cell products[48]. In addition to CRS, a somewhat unique and unexpected neurotoxicity has also been observed in CD19-specific CAR T cell-treated patients. This toxicity can range from mild delirium to severe encephalopathy. The incidence of neurotoxicity may depend on the disease and CAR design. Severe neurotoxicity was seldom reported in patients with CLL treated with the BBζ-signaling CAR[24,28,35,49], but was observed in every CAR T cell trial for ALL[26,29,33,38,39], more prominently with a CD28ζ signaling CAR[38,39]. Myelosuppression has also been observed in patients with lymphoma and leukemia treated with anti-CD19 CAR T cells[33,36,39,50]. Additionally, during the first 8 weeks post-infusion, febrile neutropenia and tumor lysis syndrome are commonly observed in patients with lymphoma treated with BBζ-based CAR T cells[44]. The majority of adverse events have been reversible through supportive care, cytokine inhibitors and glucocorticoid treatment[51].

Generating hypotheses with correlative studies

Defining the kinetics, homing and bioactivity of the cell therapy product and tumor response to treatment in each patient requires diligent monitoring, as these are critical components in the continued translational cycle from the bench to the bed and back again. Furthermore, the US Food and Drug Administration (FDA) mandates that sponsors observe study participants for delayed adverse events for as long as 15 years following the infusion of modified cells[52]. To this end, it is desirable to include a correlative studies laboratory in an organization that operates according to good clinical laboratory practice[53] (Fig. 2), to ensure that biospecimens from patients on cell therapy are handled by qualified personnel following experimental processes specified by standard operating procedure (SOP). Sample analytics and biobanking are two critical activities in such a laboratory, both of which should be carried out using rigorously validated, SOP-defined procedures. As most phase I trials are run in academic centers, some of the analytical methods would have to be developed and validated for novel, innovative therapies such as the CRISPR–Cas9-mediated disruption of endogenous genes in mature T cells, combined with lentiviral delivery of a tumor-targeting T cell receptor[54]. An example is the frequent monitoring of CAR T cell bioactivity in terms of changes in cytokine and soluble cytokine receptor levels[22,23,25,55–57] in serum early after infusion, given that high-grade toxicities may develop rapidly upon treatment.

The value of correlative studies is underscored by the identification of a rise in interleukin-6 (IL-6) levels in association with the onset of CRS in patients, which played a central role in prompting the evaluation of IL-6/IL-6 receptor blockade in severe CRS[25]. This insight proved life saving for many patients, and formed the foundation for co-developing anti-IL-6 and CD19 CAR T cell therapy, leading to their concurrent FDA approval for severe CRS[58] and B cell ALL, respectively. More extensive analyses of serum from patients in multiple trials have led to the discovery and validation of biomarkers of CRS and neurotoxicity, providing insight into the mechanisms that drive them[59] and potential paths to predicting these complications of CAR T cell therapy. Although not all studies agree on the precise cytokines[56] or biomarkers[57] to interrogate, they all focus on identifying predictive markers and developing algorithms to distinguish patients at increased risk of developing life-threatening toxicities.

Biobanked cells from patients have played a critical role in identifying mechanisms of resistance to CD19-specific CAR therapy. One of the earliest reports of CART19 in ALL revealed evidence of relapse in the context of loss of CD19 expression, which has been demonstrated to be the dominant resistance mechanism in ALL, occurring through various genetic mechanisms and rare iatrogenic causes[60–62]. Early loss of CAR T cells preceded by normal B cell recovery is another commonly observed event associated with relapse[26]. Analyses of the T cells used for manufacturing the CAR T cells, as well as the product itself, have revealed a number of associations that link CAR T cell quality to outcome. In particular, the presence of naive-like $CD27^+CD45RO^-$ cells in the apheresis product used for CART19 generation was shown to predict engraftment and clinical response in CLL[40]. The reinfusion of relapsing patients with leukemia with a murine scFv-based CAR has been associated with reduced expansion compared with first infusion[30,31,63], suggesting that an immune-mediated mechanism may underlie resistance to retreatment. Humanizing or developing a fully human scFv fragment might therefore enhance therapeutic success[63]. Recently, defects in death receptor signaling have been identified in a subset of ALL that is resistant to CD19-specific

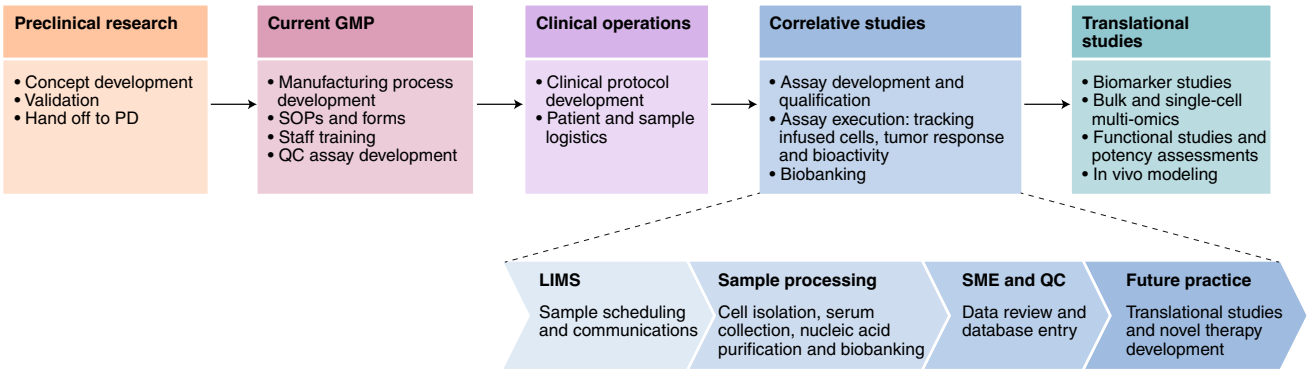

Fig. 2 | Operational pipeline for integrating correlative studies in translational science laboratories. Novel therapies that have been developed and preclinically validated in research laboratories are handed off to the process development (PD) team for scale-up and the development of a current good manufacturing practice (GMP) process. In collaboration with the GMP teams, SOPs and documentation forms are developed and GMP staff are trained in the new procedures. In parallel, the correlative studies laboratory ensures that all supportive assays, protocols and forms are in place, staff are trained, routine, qualified assays are developed and biobanking is ensured. This same team is also involved in protocol development, which is led by the clinical operations team with feedback from the study clinicians and the research laboratory that developed the new process. When a new clinical trial begins, the correlative studies laboratory starts receiving biospecimens from the clinic, manufacturing facility or collaborating laboratories, and logs these samples into the laboratory information management system (LIMS) to be processed as specified by standard operating procedures and examined using validated assays by qualified personnel. Aliquots are retained from each specimen for future translational studies. The data are reviewed by subject matter experts (SMEs) before being reviewed by the quality control (QC) manager and entered into a database. A staff statistician cleans and analyzes the data for reporting purposes (for example, to the FDA) or for scientific meetings and manuscript preparation.

CAR T cell therapy, providing additional resistance mechanisms beyond CD19 loss[64].

Correlative studies have also revealed differential kinetics of CAR T cells in responding and non-responding patients with CLL[28] and ALL[29,65,66], which led to the development of an in vitro, proliferation-based potency assay[40,67]. This correlation between clinical response and in vivo CART19 cell proliferation was not evident in trials of the same product when used for NHL[32,44,68], in contrast with a CD28-costimulated CAR[33], suggesting that the costimulatory domain dominated the differential expansion kinetics of CART19 for NHL.

Correlative studies sometimes also provide unexpected observations that can lead to new ideas. The rather dramatic expansion of an ultra-low dose of CAR T cells (1.4×10^7) followed by the eradication of a leukemic mass in one patient[23] suggested that the proliferative response was key to the antitumor response[10,11].

In aggregate, correlative studies followed by mechanistic investigations based on samples and data from patients treated with CD19-specific CAR T cells in clinical studies continue to improve our understanding of therapy-related toxicities and mechanisms of escape.

Clinical impact of T cell biology and CAR engineering

Although CART19 therapy has been efficacious in ALL and NHL, many factors contributing to patient response remain poorly understood. As patient-derived T cells are used to target a tumor-associated cell-surface protein, the immune system is repurposed to treat the malignancy. Thus, the therapeutic efficacy still depends on T cell memory and effector functions. This also includes T cell fitness, which is affected by the malignancy and previous therapies, and, most importantly, the ability of the CAR-redirected T cells to sustain the antitumor response, because most tumors exist in actively growing and dormant phases, which can last from several years to decades[69–71]. By harnessing T cells, this form of immunotherapy abides by similar target cell quiescence–reactivation principles to induce a cure. Naive and memory T cells retain the ability to proliferate vigorously in response to cognate antigen recognition, in contrast with their effector progeny that have lost that ability and instead directly lyse the tumor. Two studies recently confirmed that this therapy depends on a functional, self-renewing T cell pool, demonstrating that in CLL the advanced age of the patient population in combination with effector-memory skewing limited CAR T cell functionality (Fig. 3)[40,72]. Furthermore, response to therapy in CLL can be predicted based on the presence of a pool of more functional early memory cells[40]. CAR T cells and other therapies that rely on immune system activation may therefore have limited effect in malignancies that terminally skew T cell differentiation or occur in aged populations where T cells are less functional at baseline. That baseline functionality of the T cell pool plays a substantial role in dictating response rates was confirmed in a separate study, which revealed that $CD8^+$ T cell dysfunction at apheresis and the rapid expression of immune checkpoint molecules after infusion marked CAR T cells from non-responding and partially responding patients with ALL[66]. Therefore, CAR T cells are subject to inhibition via endogenous immune checkpoint pathways such as programmed cell death protein 1 (ref. [73]). Inhibitory receptor–ligand interactions normally dampen T cell functions to prevent an overactive immune response and sustain a memory T cell pool. In CAR T cells, this can result in failure to eliminate the tumor and loss of T cell persistence. Whereas checkpoint blockade can improve responses, other immune-suppressive factors in the microenvironment can impair CAR T cell function. Immune-suppressive cytokines, metabolic competition and high inhibitory ligand expression levels all serve to modulate the function of cell-based therapies[73–77].

Enhancing CAR T cell potency by genome engineering

Although the natural basis of CAR T cell efficacy, as laid out in the previous sections, presents the foundation of immunogene therapies with CAR T cells (and probably other systems that depend on sustained tumor control), CAR T cell engineering may also impact cell function, as recently reported[67,78]. CAR T cells produced with lentivirus display quasi-random integration of the vector throughout the genome, introducing the potential for genomic activation or disruption events[79,80]. Although the majority of CAR T cells

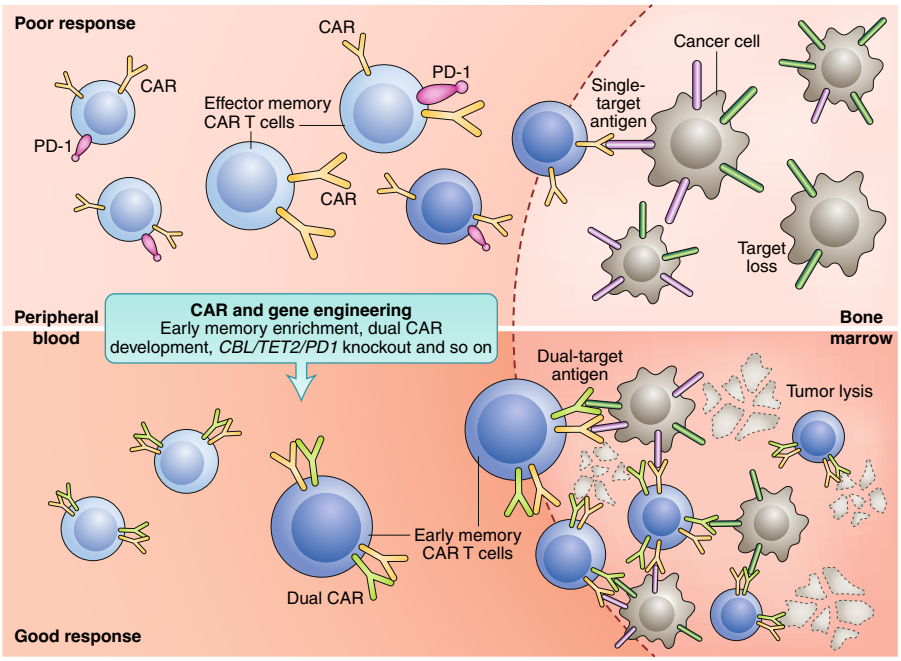

Fig. 3 | Current strategies to overcome the hurdles of poor response to autologous CAR T cell therapy. Several factors, such as low frequencies of early memory CAR T cells in the infusion product, overexpression of checkpoint inhibitory molecules on the apheresis T cells and loss of target antigen on the tumor, have been shown to contribute to the lack of efficacy of CAR T cells in many patients. Optimizing the manufacturing process using laboratory-based engineering approaches, such as memory T cell enrichment, dual CAR development and specific gene editing, is essential to improve the quality of CAR T cell product, thereby enhancing its capacity for tumor clearance and in vivo persistence. PD-1, programmed cell death protein 1.

generated in this process are polyclonal[67,79], the CAR T cell population undergoes rapid changes after infusion due to, among other factors, selective expansion of CAR T cell clones for reasons that are currently poorly understood[67,79,81]. In most patients, a multitude of clones contribute to the antitumor response[79,81]. Two recently published reports concern clonal CD8$^+$ CAR T cell expansion in two patients, in whom the CAR was shown by sequencing vector integration sites to have integrated into the *CBL* and *TET2* gene loci. In the case of the *TET2* integration, the patient's CAR T cell population underwent delayed expansion accompanied by tumor clearance, complete remission status and contraction of the clonal population[67]. The *CBL*-integrated clone underwent a similar, albeit less dramatic, expansion process[78]. *CBL* knockdown had previously been associated with decreased T cell activation thresholds, reduced reliance on costimulation and decreased sensitivity to programmed cell death protein 1 inhibition, which could represent mechanisms for the therapeutic effect[82–85]. These cases highlight how lentiviral integrations can substantially impact CAR T cell growth, persistence and effector function. Further insight into the fate of CAR T cells was provided by analyzing the CAR vector integration site landscape in the infusion product and post-infusion aliquots of 58 patients with CLL and ALL, demonstrating that CAR-mediated gene disruptions frequently occur in proliferation-augmenting pathways[79]. These findings suggest that such gene disruptions may be as important as T cell quality and CAR design in the outcomes observed with CAR T cell therapy[79].

Additionally, targeted CAR integrations have revealed locus-specific regulation and protective effects. For instance, CAR expression from the T cell receptor-α constant (*TRAC*) locus optimized CAR expression and protected cells from exhaustion compared with integration in other sites[86]. The genomic landscape of the CAR transgene cassette can therefore play an important role in how individual CAR T cells function. Unique cases such as the *TET2* and *CBL* loci integration events are informative not only on how genome regulation can influence CAR expression and function, but also in terms of novel regulators of these functions. The identification of *TET2* disruption as an enhancer of T cell persistence has sparked a wide array of research focused on knocking out *TET2* to improve CAR function and determine the mechanisms underlying this selective advantage.

Natural killer cells have also been engineered to express a B cell targeting CAR combined with constitutive secretion of IL-15 (ref. [87]). Preclinical studies have similarly demonstrated a beneficial effect of CAR T cells co-expressing IL-15 (ref. [88]). Based on these findings, several clinical trials have been launched to evaluate T cells engineered to express this cytokine in conjunction with a tumor-targeting CAR. However, IL-15 was separately demonstrated to drive antigen-independent growth of T cells, resulting in a pre-leukemic disorder in mice[89,90]. Therefore, the addition of a safety switch to the CAR and IL-15 construct should allow for control of the infused cells, as indicated in the design of one of these trials (NCT03721068), which targets GD2 in brain cancers using anti-GD2 CAR with IL-15 and the iCaspase 9 safety switch. Moreover, preclinical studies have shown augmented antitumor efficacy of IL-18 co-expressing T cells in a CD19-redirected T cell model[91]. Similar combination therapies have been shown to jointly blunt tumor function and boost T cell potency[92–95]. Next-generation CAR T cell therapies incorporating such engineering approaches are expected to further raise the therapeutic index.

Tumor-redirected T cells encounter numerous inhibitory signals in the tumor bed, the most notorious of which is transforming growth factor-β[96]. CAR T cells engineered to express a dominant-negative transforming growth factor-β receptor showed augmented potency against a solid tumor model[97], leading to the development of an ongoing clinical trial to target prostate cancers with a prostate-specific maturation antigen-specific CAR T cell (NCT03089203).

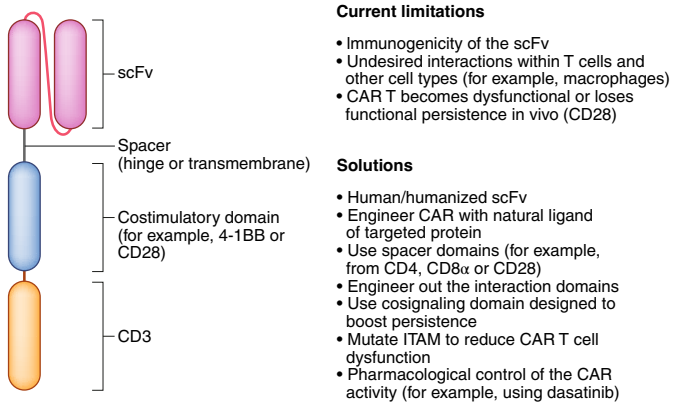

Fig. 4 | CAR design limitations that affect clinical responses following CAR T cell treatment, and potential solutions. Most CARs are made up of a TAA-binding scFv fragment (for example, CD19 fused in-frame with a T cell signaling domain) enhanced with a costimulatory domain (for example, CD28 or 4-1BB) that is separated from the scFv by a spacer sequence. The design of this synthetic receptor affects various aspects of its in vivo performance, and ultimately clinical responses. Additionally, small molecules such as dasatinib may tone dysfunction-inducing CAR signaling[190]. ITAM, immunoreceptor tyrosine-based activation motif.

Tumor resistance to CAR T cell therapy

Extensive clinical data have revealed mechanisms by which tumor cells escape CAR T cell targeting, and have informed engineering advances to overcome this. The anti-CD19 CAR was shown to require minute quantities of target antigen to display full effector function[98]; therefore, the tumor cells could only escape this pressure via antigen loss[25,60,61,99–101] or antigen masking[62]. Routine analyses revealed that a pediatric patient with ALL treated with murine anti-CD19 CAR relapsed with the original disease two months post-treatment[25]. In the ensuing months and years, similar patterns of relapse were observed from routine correlative studies, which were later confirmed by clinical pathology[25,60,61,99]. The molecular basis of these and similar cases of antigen loss-related anti-CD19 CAR T cell therapy were shown to be related to the acquisition of open reading frame-disrupting mutations in the target antigen, compounded by altered messenger RNA splicing in tumor cells[60,61]. Others similarly observed antigen-negative relapse in CD19-directed CAR T cell therapies[30,38,99]. Additionally, mixed-lineage leukemia-rearranged leukemias displayed lineage switch-related relapse with loss of CD19 protein expression[99,100], further illustrating how the immense immune pressure exerted by CD19-specific CAR T cells mediates Darwinian selection of the malignant cell pool. These examples demonstrate the impact of T cell and tumor cell physiology on clinical responses to single antigen-directed CAR T cell therapies. This knowledge has been used to prevent relapse through a bispecific CAR T cell that recognizes two antigens present on the tumor surface. This anti-CD19/anti-CD22 bispecific CAR T cell has been used successfully to treat an adult patient with ALL who has remained disease free for more than 1 year post-therapy[102]. Antigen loss has now also been observed in a patient on CD22-targeting CAR treatment[103], whereas others have shown that downregulation was sufficient to evade CART22 treatment[104].

CAR design

As described above, many factors independent of the CAR itself impact therapeutic efficacy. Correlative studies by various groups targeting the same tumor-associated antigen (TAA) (for example, CD19) with an scFv derived from the same monoclonal antibody (for example, FMC63) but different spacer domains, cosignaling domains and so on have allowed the identification of several pain points and success stories of chimeric receptors (Fig. 4). First, it has become obvious that costimulation has to be engineered into the CAR, as even the transduction of memory T cells could not rescue a first-generation CAR[105]. Hence, around the time that CD28 costimulation was discovered as an essential component to memory T cell formation and effector differentiation[2], second-generation CARs were developed that included this domain[106]. However, comparative clinical studies to demonstrate the differential impact of CD28 and other cosignaling domains on effector and memory function in vivo are lacking and would be useful, because despite the CAR-contained CD28 driving a profound effector differentiation, it can also render the T cells dysfunctional with loss of persistence[107].

Early data also revealed the profound impact of the spacer domain on CAR T cell function (reviewed in ref. [108]). Most early-generation CARs, including those in first-generation CAR designs[5,6], used scFv derived from mouse antibodies. T cells discern minute differences between cancerous and normal cells, and a single difference in amino acid residues can induce a robust immune response against this non-self entity[109–111]. This same selective threat elimination machinery deletes recombinant proteins containing minimal sequence divergence from the native protein just as efficiently as foreign threats[112,113]. It should therefore come as no surprise that suicide genes[114] and CARs incorporating non-human sequences are readily targeted by the immune system[30,31,115–117]. Moreover, the poor expansion of re-infused CAR T cells[5,6,26,31,56,118] was correlated with the detection of patient-derived T cell epitopes in the CAR[30,31]. The field is therefore moving away from incorporating non-human tumor-targeting moieties into the CAR[63]. That being said, the remarkable response rates with a non-human CAR in multiple myeloma recently suggest that deep molecular remissions are possible (see below).

Extending CD19 CAR T cell therapy beyond CD19$^+$ malignancies

Although multiple myeloma derives from plasma cells (the terminal stage of B cell differentiation), myeloma precursor cells may express CD19. Pilot studies suggested a potential benefit to targeting CD19 in myeloma[119], yet little or no activity was apparent in the vast majority of treated patients, indicating that an alternative target antigen is needed to address this disease with CAR T cell therapy[98]. Myeloma cells uniformly express B cell maturation antigen (BCMA) (Fig. 1), leading to the development of BCMA-specific CAR T cells. Currently, 90 relevant clinical trials are listed on ClinicalTrials.gov, with a few moving forward towards their commercial roll-out (see Tables 1 and 2 for the constructs and trials furthest along in their development).

BCMA has been targeted by various groups using a diverse array of chimeric receptors (reviewed in refs. [120,121]). Although human anti-BCMA CARs gained traction in myeloma[122–131], non-human-derived BCMA CARs with an anti-BCMA murine[132–135] (or alpaca) immunoglobulin[115,136–138] chain are further along in clinical trials (Table 2).

Lymphodepletion using cyclophosphamide and fludarabine before adoptive T cell transfer further boosts CAR T cell expansion[139] by depleting cytokine sinks[140] and immune-suppressive cells[141,142]. Although response rates vary widely among different BCMA-specific CAR T cell products, the biggest challenge remains the durability of response, with patients appearing to ultimately progress regardless of the product[136].

The mechanisms that underlie myeloma resistance to BCMA CAR T therapy are coming to light through correlative analysis of biobanked specimens from early-phase clinical trials. Comparisons are difficult to make across trials, institutions and therapies, even though they all target the same myeloma-associated antigen, as differences in the cell manufacturing process, vector used, CAR design and trial participant selection criteria, among other factors, are

Table 1 | Summary of BCMA-targeted CAR structures

Manufacturer	CAR name	Gene delivery system	Species of antigen-binding domain	Structure of antigen-binding domain	Hinge and transmembrane domain	Signaling domain	Satety switch
National Cancer Institute	CAR-BCMA	Retroviral vector	Mouse	scFv	CD8α	CD28-CD3ξ	No
bluebird bio/ Celgene	Idecabtagene vicleucel/ bb2121	Lentiviral vector	Mouse	scFv	CD8α	4-1BB-CD3ξ	No
	bb21217	Lentiviral vector	Mouse	scFv	CD8α	4-1BB-CD3ξ	No
Hrain Biotechnology	BCMA CAR T	Retroviral vector	Mouse	scFv	NA	4-1BB-CD3ξ	EGFRt
Nanjing Legend/ Janssen	Ciltacabtagene autoleucel/ LCAR-B38M	Lentiviral vector	Alpaca	VHH	CD8α	4-1BB-CD3ξ	No
University of Pennsylvania	CART-BCMA	Lentiviral vector	Human	scFv	CD8α	4-1BB-CD3ξ	No
Memorial Sloan Kettering Cancer Center	MCARH171	Retroviral vector	Human	scFv	CD8α	4-1BB-CD3ξ	EGFRt
Memorial Sloan Kettering Cancer Center	JCARH25	Lentiviral vector	Human	scFv	CD28	4-1BB-CD3ξ	No
Fred Hutchinson Cancer Research Center	FCARH143	Lentiviral vector	Human	scFv	NA	4-1BB-CD3ξ	EGFRt
CARsgen Therapeutics	CT053	Lentiviral vector	Human	scFv	NA	4-1BB-CD3ξ	No
IASO Biotherapeutics	CT103A	Lentiviral vector	Human	scFv	CD8α	4-1BB-CD3ξ	No
Poseida Therapeutics	P-BCMA-101	piggyBac DNA modification system	Human	Centyrin	NA	4-1BB-CD3ξ	Yes (activated by rimiducid)

EGFRt, truncated epidermal growth factor receptor; VHH, variable heavy-chain domain; NA, not applicable.

likely to affect outcomes. Modulation of BCMA expression may also play a role. Early studies preselected patients based on the expression of BCMA. Although to date no notable association between baseline BCMA expression and clinical response to BCMA CAR T therapy has been reported in the published literature, several studies have observed a reduction in BCMA expression following therapy, which may be contributing to resistance[122]. The mechanism of BCMA downregulation in myeloma is not entirely understood, but this protein is shed naturally from the cell surface by the γ-secretase protease complex[143–145]. The resulting increased concentrations of soluble BCMA could also block CAR binding to the native, cell-bound protein, thereby limiting the clinical impact of BCMA CAR T cells further. The preliminary results of a clinical trial that included patients who had failed previous BCMA-targeted therapy, and combined a γ-secretase inhibitor (JSMD194) with a low dose of BCMA CAR T cells, showed a 100% response rate[146].

T cell-intrinsic mechanisms similar to those seen with CD19-specific CAR T cells in ALL and CLL may also be contributing to resistance. In this latter setting, patient T cells expressing a fully human, BBζ-signaling CAR exhibited the most dramatic expansion kinetics in complete responders, whereas non-responders exhibited little expansion in the first month after infusion. This led to the discovery of an early memory T cell subset in apheresed (that is, pre-CAR engineering) T cells that is associated with responses in CLL[40]. Similarly, data from a phase I study of BCMA-specific CAR T cell therapy showed that the expansion and persistence of CAR T cells in non-responders were substantially lower than in responders[68,122,147]. Again, the frequency of naive-like, early memory T cells within the apheresis product used to generated the CAR T cells showed a correlation with early engraftment. Although prospective studies using selected subsets of T cells are necessary to confirm the role of these T cells in outcome, these data suggest that some resistance to therapy may be intrinsic to the T cell product.

CAR T cell therapy for solid tumors

Although CAR T cells can mediate deep and durable cancer remission in B cell malignancies, achieving comparable clinical responses in non-hematopoietic solid cancers remains a daunting task. Nevertheless, a complete response to CAR T cell therapy of recurrent multifocal glioblastoma was achieved using multiple intracavitary and intraventricular infusions of autologous T cells genetically redirected to IL-13 receptor α2 (ref. [148]), laying the foundation for additional investigations into how to apply effective CAR T cell therapy in this and other non-hematopoietic solid cancers[5,6,149–151]. CAR T cell trials have established that deep, durable remissions with CAR-engineered cells correlate with a minimal proportion of early memory T cells in pre- and post-CAR engineering T cells. Critical features include early memory T cell differentiation in responding patients and an absence or low levels of T cell dysfunction, glycolysis, effector cell and exhaustion[40]. These findings were validated in functional studies and additional cohorts of leukemias, but also myeloma and NHL[68]. CAR T cells targeting solid tumor antigens may have a different set of requirements to achieve efficacy than those targeting B-lineage malignancies. In addition to identifying

Table 2 | Summary of BCMA CAR-engineered autologous T cell monodrug clinical trials with a focus on treating relapse and refractory multiple myeloma

Manufacturer	Name of product	Clinical trial registered number	Year data updated	Number of patients evaluated	Enrollment based on BCMA expression	Number of lines of previous therapies	Disease burden at time of infusion	Conditioning therapy	Infusion dose	Efficacy					Reference(s)
										Overall response rate (%)	CR to stringent CR (%)	VGPR rate (%)	Median OS (months)	Median PFS (months)	
National Cancer Institute	CAR-BCMA	NCT02215967	2018	16	Yes	Average = 9.5 (range = 3–19)	Relapsed/refractory cases with BCMA uniformly expressed on tumor cells, including extramedullary diseases, and 40% of patients carried high-risk cytogentics	Cyclophosphamide and fludarabine	9×10^6 CAR^+ T cells per kg	81	13	50	NA	7.8	Ref. [133]
bluebird bio/ Celgene	Idecabtagene vicleucel/ bb2121	NCT02658929	2020	62	Yes	Above 3	44% of relapsed/ refractory cases had ≥50% bone marrow $CD138^+$ plasma cells	Cyclophosphamide and fludarabine	50×10^6, 150×10^6, 450×10^6 or 800×10^6 CAR^+ T cells in total	76	39	26	34.2	8.8	Ref. [134]
	bb21217	NCT03274219	2020	46	Yes	Average = 6 (range = 3–17)	57% of relapsed/ refractory cases were triple refractory	Cyclophosphamide and fludarabine	150×10^6, 300×10^6 or 450×10^6 CAR^+ T cells in total	55	18	30	NA	NA	Ref. [135]
Hrain Biotechnology	BCMA CAR T	NCT03093168	2019	44	No	Above 2	19.6% had extramedullary plasmacytoma	Cyclophosphamide and fludarabine	9×10^6 CAR^+ T cells per kg	80	41	18	Not reached	15	Ref. [115]
Nanjing Legend/ Janssen	Ciltacabtagene autoleucel/ LCAR-B38M	NCT03090659	2018	57	Yes	Average = 3 (range = 1–9)	51% of relapsed/ refractory cases had ≥40% tumor BCMA expression (patients with extramedullary involvement were included) and 37% of patients had stage III diesase	Cyclophos phamide	0.07×10^6–2.1×10^6 CAR^+ T cells per kg	88	68	5	Not reached	15	Ref. [138]
		NCT03090659 and ChiCTR-ONH-17012285	2019	17	Yes	Average = 4.6 (range = 3–11)	88% of relapsed/ refractory cases had >70% tumor BCMA expression and 29% had extramedullary disease, while 38% patients carried high-risk cytogentics	Cyclopho sphamide with or without fludarabine	0.21×10^6–1.52×10^6 CAR^+ T cells per kg	88	76	12	Not reached	12	Ref. [136]
		NCT03548207	2020	97	No	Average = 6 (range = 3–18)	87.6% of relapsed/ refractory cases were triple refractory	Cyclopho sphamide and fludarabine	0.5×10^6–1.0×10^6 CAR^+ T cells per kg	95	56	32	Not reached	Not reached	Ref. [139]

Continued

Table 2 | Summary of BCMA CAR-engineered autologous T cell monodrug clinical trials with a focus on treating relapse and refractory multiple myeloma (Continued)

Manufacturer	Name of product	Clinical trial registered number	Year data updated	Number of patients evaluated	Enrollment based on BCMA expression	Number of lines of previous therapies	Disease burden at time of infusion	Conditioning therapy	Infusion dose	Efficacy					Reference(s)
										Overall response rate (%)	CR to stringent CR (%)	VGPR rate (%)	Median OS (months)	Median PFS (months)	
University of Pennsylvania	CART-BCMA	NCT02546167	2019	25	No	Average = 7 (range = 3–13)	A median of 65% had myeloma cells on bone marrow biopsy, 28% had extramedullary disease and 96% carried high-risk cytogentics	Cyclopho sphamide or no condi tioning therapy	10×10^6–500×10^6 CAR^+ T cells in total	48	25	20	17	2, 2 and 4 in three cohorts, resp ectively	Ref. [123]
Memorial Sloan Kettering Cancer Center	MCARH171	NCT03070327	2018	11	Yes	Average = 6 (range = 4–14)	82% had high-risk cytogenetics	Cyclopho sphamide with or without fludarabine	72×10^6, 137×10^6, 475×10^6 or 818×10^6 CAR^+ T cells in total	64	NA	NA	NA	NA	Ref. [124]
Memorial Sloan Kettering Cancer Center	Orvac abtagene autoleucel/ JCARH25	NCT03430011	2018	8	No	Average = 10 (range = 4–15)	50% had high-risk cytogenetics	Cyclopho sphamide and fludarabine	50×10^6 or 150×10^6 CAR^+ T cells in total	100	38	25	NA	NA	Ref. [125]
			2020	44	No	Average = 6 (range = 3–8)	NA	Cyclopho sphamide and fludarabine	300×10^6, 450×10^6 or 600×10^6 CAR^+ T cells in total	91	39	25	Not reached	Not reached	Ref. [126]
Fred Hutchinson Cancer Research Center	FCARH143	NCT03338972	2018	11	Yes	Average = 8 (range = 6–11)	The median percentage of bone marrow plasma cells was 58% (range = 20% to >80%) and 100% had high-risk cytogenetics	Cyclopho sphamide and fludarabine	50×10^6 or 150×10^6 CAR^+ T cells in total	100	36	46	NA	NA	Ref. [127]
CARsgen Thera peutics	CT053	NCT03716856, NCT03302403 and NCT03380039	2020	24	Yes	Average = 4.5 (range = 2–11)	41.7% had extramedullary involvement	Cyclopho sphamide and fludarabine	50×10^6, 100×10^6, 150×10^6 or 180×10^6 CAR^+ T cells in total	88	79	NA	NA	18.8	Ref. [128]
		NCT03975907	2020	12	No	Average = 6 (range = 3–7)	14.2% had extramedullary disease and 35.7% had high-risk cytogenetics	Cyclopho sphamide and fludarabine	100×10^6 or 150×10^6 CAR^+ T cells in total	100	42	25	NA	NA	Ref. [130]
		NCT03915184	2020	10	No	Average = 6 (range = 3–11)	93% were triple refractory, 36% had extramedullary disease and 64% had high-risk cytogenetics	Cyclophosphamide and fludarabine	150×10^6–300×10^6 CAR^+ T cells in total	100	40	10	NA	NA	Ref. [129]

Continued

Table 2 | Summary of BCMA CAR-engineered autologous T cell monodrug clinical trials with a focus on treating relapse and refractory multiple myeloma (Continued)

Manufacturer	Name of product	Clinical trial registered number	Year data updated	Number of patients evaluated	Enrollment based on BCMA expression	Number of lines of previous therapies	Disease burden at time of infusion	Conditioning therapy	Infusion dose	Efficacy				Reference(s)
										Overall response rate (%)	CR to stringent CR (%)	VGPR rate (%)	Median OS (months)	Median PFS (months)	
IASO Biotherapeutics	CT103A	ChiCTR1800018137	2019	16	NA	Above 3	25% relapsed after a previous murine BCMA CAR T therapy and 31.3% had extramedullary disease and/or plasma cell leukemia	Cyclophosphamide and fludarabine	1×10^6, 3×10^6, 6×10^6 or 8×10^6 CAR^+ T cells per kg	100	75 (out of 8 cases beyond 6 months)	25 (out of 8 cases beyond 6 months)	NA	NA	Ref. [131]
Poseida Therapeutics	P-BCMA-101	NCT03288493	2020	34	No	Average = 7 (range = 3–18)	NA	Cyclophosphamide and fludarabine	0.75×10^6–15×10^6 CAR^+ T cells per kg	57	NA	NA	NA	NA	Ref. [132]

CR, complete response; OS, overall survival; PFS, progression-free survival; VGPR, very good partial response.

appropriate target antigens, these requirements include the need for CAR T cells to: (1) traffic to sites of disease; (2) migrate through tumor endothelial and stromal barriers before infiltrating into tumors; (3) broadly attack cancer cells in the face of heterogeneous antigen expression; and (4) thrive in a harsh tumor microenvironment (TME) characterized by hypoxia, oxidative stress, nutrient deprivation and acidic pH, as well as many immunosuppressive soluble cytokines and factors, overexpression of inhibitory molecules with coordinated expression of inhibitory receptors on T cells, and the presence of an array of immune cells with immunosuppressive function, including regulatory T cells, tumor-associated macrophages, myeloid-derived suppressor cells and tumor-associated neutrophils (Fig. 3). Ultimately, CAR T cell therapy may achieve greater efficacy in patients harboring solid tumors once approaches are developed that address each of these barriers together.

An expanding cadre of tumor-specific antigens and TAAs that could be targeted using CAR T cell therapy in non-hematopoietic solid cancers have been identified, including mesothelin, folate receptor alpha, human epidermal growth factor receptor 2 (HER2), IL-13 receptor α2, epidermal growth factor receptor variant III (EGFRvIII), claudin 18.2, mucin 1, cell-surface associated (MUC1), glypican-2, carbonic anhydrase IX and others. Nevertheless, identification of an antigen with restricted expression on solid cancer cells has been challenging. Ideally, CAR T cells should be highly specific for a tumor-restricted antigen, expressed uniformly and at high levels on cancer cells, but not on vital healthy tissue. The importance of antigen exclusivity was demonstrated in CAR T cell trials targeting TAAs such as HER2 and carbonic anhydrase IX, which are expressed by both cancer cells and normal tissues, and which resulted in severe toxicity[6,152]. The need for consistent antigen expression was illustrated in a clinical trial targeting mutant EGFRvIII, a CAR target antigen with highly restricted but heterogeneous expression in glioblastoma. Although intravenous T cell infusion resulted in CAR T cell trafficking to the brain with accompanied antigen-directed activity against $EGFRvIII^+$ cancer cells, the heterogeneous EGFRvIII expression and potential antigen loss resulted in outgrowth of antigen-negative disease[150]. In some cases, targeting antigens with more restricted and uniform expression in tumors, or those preferentially expressed on organs that are not essential for patient survival, such as follicle-stimulating hormone receptor[153], may pave the way toward broader and safer antitumor activity. Nevertheless, heterogeneous TAA expression is common in solid tumors, highlighting the need to develop multi-antigen targeting approaches or strategies that improve epitope spreading and engagement of endogenous antitumor immunity. Evidence already exists for epitope spreading and bolstering of endogenous immunity in clinical trials and in preclinical models of CAR T cells in solid tumors[154,155], suggesting that antigen spreading may be necessary to improve activity. As an alternative approach to address both antigen heterogeneity and the threat of antigen loss, so-called universal immune receptors (UIRs) were created (reviewed in ref. [156]). These CARs do not directly recognize the tumor antigen, but rather recognize a tag, such as biotin[157], on an antigen-targeted ligand (for example, an antibody or scFv fragment) that serves as an immunologic bridge between the CAR and TAA. UIRs allow the modified T cells to recognize multiple distinct TAAs simultaneously or sequentially, thus addressing both the heterogeneity and TAA loss observed with monospecific CARs, with the added benefit of dose-dependent control of T cell activity. Clinical trials of UIR T cells are ongoing (for example, NCT03680560, NCT03266692 and NCT03189836). Another approach, referred to as dual or tandem CARs, allows CAR T cells to recognize two or more distinct antigens rather than one. Proof of this principle has been established in solid tumor models using a HER2/MUC1 bispecific CAR for breast cancer cells in vitro[158], a HER2/IL-13 receptor α2 bispecific CAR for the treatment of a glioma xenograft in vivo[159], and an EGFR/epithelial cell

adhesion molecule/HER2 tri-specific against Raji lymphoma cells engineered to express these TAAs[160]. Alternatively, diversification of TAAs recognized by single CAR T cell products for solid tumor treatment may be achieved using SynNotch systems for the conditional expression of a second CAR following engagement of a primary CAR with a cognate TAA, thereby allowing for potential localized expression of a CAR specific for a distinct antigen at the site of primary target encounter[161]. An alternative approach would be through bicistronic vectors for the engineered co-expression of a CAR specific for one antigen and a soluble bispecific T cell engager specific for a second antigen[162].

Although for hematopoietic cancers intravenous infusion of CAR T cells may target cancer cells in natural immune cell environments such as the blood, lymph nodes and bone marrow, it remains challenging to deliver CAR T cells targeting solid tumors to distant tumor deposits. In some cases, direct intratumoral or regional delivery of T cells may facilitate and improve T cell infiltration and antitumor activity, particularly for compartmentalized cancers[148,163,164]. Lymphodepleting chemotherapy as a preconditioning regimen may also augment CAR T cell accumulation in solid tumors after intravenous infusion. Following intravenous administration of indium-111-labeled tumor-infiltrating lymphocytes to patients with metastatic melanoma, the cells rapidly accumulated in the lungs, liver and spleen before progressively localizing in tumor deposits[165]. In these trials, tumor-infiltrating lymphocyte accumulation was enhanced with previous lymphodepletion and associated with an improved clinical response to treatment[165,166]. Still, the natural trafficking of T cells to tumors requires that they respond to chemokines produced in the TME[167], and that tumor-derived chemokines be matched to the expression of the appropriate chemokine receptors on the infused T cells to permit trafficking[168]. Although most CAR T cells do not naturally express cognate receptors for the chemokines produced by tumors, it is possible to engineer matched chemokine receptor expression to achieve enhanced infiltration and killing of solid tumors[169–171]. CAR T cells may also be outfitted to produce chemokine ligands, such as CCL19 and other factors, to foster chemokine receptor-dependent recruitment of endogenous T cells and dendritic cells to tumor sites when infused without previous lymphodepletion[172].

CAR T cells trafficking to solid tumor sites also encounter formidable physical barriers that can both block T cell infiltration and disable T cell function. Major barriers include the fibrotic tumor stroma, comprised of extracellular matrix (ECM) and cancer-associated fibroblasts (CAFs), and the abnormal vasculature at the tumor site. Solid malignancies, such as pancreatic, ovarian and breast cancers, often contain fibrotic tumor stroma that may impede effective delivery of drugs including CAR T cells. CAR T cells naturally express low levels of enzymes that degrade ECM components, but engineering the expression of heparanase was shown to improve their capacity to degrade ECM proteoglycans, thereby promoting CAR T cell entry into stroma-rich tumors and antitumor activity[173].

CAFs contribute to ECM remodeling, modulate tumor angiogenesis and promote metastasis, with CAF depletion fostering endogenous antitumor immunity in an autochthonous model of pancreatic ductal adenocarcinoma[174]. Thus, engineering CAR T cells against fibroblast activation protein, which is expressed by CAFs and myofibroblasts, was shown to target stromal CAFs and inhibit cancer progression without notable toxicity in multiple solid tumors[175]. However, fibroblast activation protein-targeted CARs also recognized multipotent bone marrow stromal cells, resulting in lethal bone toxicity and cachexia in other tumor models[176].

CAR T cells can also be designed to target and disrupt the tumor vasculature to allow T cell infiltration and restrict the flow of blood and nutrients to solid tumors. For instance, targeting vascular endothelial growth factor receptor 2 using CARs can augment T cell infiltration and inhibit the progression of different types of vascularized syngeneic solid tumors[177,178]. CARs specific for prostate-specific membrane antigen can ablate prostate-specific membrane antigen-positive vessels and limit tumor progression in vivo through indirect loss of tumor cells (related to the disruption of the vasculature[179]), and CARs targeting the angiogenic integrin $\alpha_v\beta_3$ on the vascular endothelium can disrupt tumor vessels and suppress tumor outgrowth[180]. CAR T cells may also be combined with antivasculature agents, including antivascular endothelial growth factor or prostaglandin E_2 antibodies[181], antitumor endothelial marker 1/endosialin immunotoxin[182] or agents targeting molecules on the tumor endothelium, such as Fas ligand, which establishes a tumor endothelial death barrier and kills incoming effector CD8 T cells[181]. Together, these findings provide rationale for further investigation and the use of stroma-disrupting strategies as both preparative and combinatorial regimens to augment T cell entry into solid tumors in TAA-targeted CAR T cell trials.

In the stroma and tumor bed, CAR T cells contend with overexpression of inhibitory checkpoint ligands with coordinated expression of inhibitory receptors on T cells, immunosuppressive soluble cytokines and factors, various immunosuppressive cell types and a hypoxic and nutrient-deprived environment. Both tumor cells and immune cells in the TME can regulate CAR T cell activation through the expression of inhibitory signals that block T lymphocyte activation and function, thereby circumventing otherwise effective immune control of tumor progression.

Future prospects

Over the past decade, an astounding series of proof-of-concept trials have taken place, with validation of early results in phase II trials[39,44,183–186] leading to the approval of CD19-specific CAR T cell therapies for select B cell malignancies. Separately, insight into the biology of CRS has led to biomarker-driven trials (NCT02906371) and the discovery and validation of a novel biomarker profile of this potentially lethal toxicity[59]. Additional observations from routine and translational studies have revealed mechanisms of resistance and response, as well as identification of the natural basis of successful and failed CAR T cell therapy[40,66,68]. Novel therapies started to incorporate small molecules, which proved to augment T cell function and simultaneously inhibit the malignant population[35,93,95,187]. Combination trials also targeted more than one surface protein, either on the same target cell (as with CD19 and CD22) or on precursors and progeny of the tumor (as with CD19 and CD20, CD22 or BCMA)[185,188,189]. In the next few years, we are likely to witness increased efficacy of CAR T cells for solid tumors—a major current focus in this field. However, a better understanding and monitoring of the tumor will be essential for CAR T cell therapy to be offered to patients in the early stages of their disease, before genomic instability and evolution of the tumor complicate treatment.

Received: 28 February 2020; Accepted: 28 June 2021;
Published online: 19 August 2021

References

1. Bird, R. E. et al. Single-chain antigen-binding proteins. *Science* **242**, 423–426 (1988).
2. June, C. H., Ledbetter, J. A., Gillespie, M. M., Lindsten, T. & Thompson, C. B. T-cell proliferation involving the CD28 pathway is associated with cyclosporine-resistant interleukin 2 gene expression. *Mol. Cell Biol.* **7**, 4472–4481 (1987).
3. Brentjens, R. J. et al. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. *Nat. Med.* **9**, 279–286 (2003).
4. Mullis, K. B. The unusual origin of the polymerase chain reaction. *Sci. Am.* **262**, 56–65 (1990).
5. Kershaw, M. H. et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin. Cancer Res.* **12**, 6106–6115 (2006).
6. Lamers, C. H. et al. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. *J. Clin. Oncol.* **24**, e20–e22 (2006).

7. Eshhar, Z., Waks, T., Gross, G. & Schindler, D. G. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc. Natl Acad. Sci. USA* **90**, 720–724 (1993).
8. Sadelain, M.Chimeric antigen receptors: a paradigm shift in immunotherapy. *Ann. Rev. Cancer Biol.* **1**, 447–466 (2017).
9. Filley, A. C., Henriquez, M. & Dey, M.CART immunotherapy: development, success, and translation to malignant gliomas and other solid tumors.*Front. Oncol.* **8**, 453 (2018).
10. Milone, M. C. et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. *Mol. Ther.* **17**, 1453–1464 (2009).
11. Carpenito, C. et al. Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. *Proc. Natl Acad. Sci. USA* **106**, 3360–3365 (2009).
12. Zhao, Y. et al. A herceptin-based chimeric antigen receptor with modified signaling domains leads to enhanced survival of transduced T lymphocytes and antitumor activity. *J. Immunol.* **183**, 5563–5574 (2009).
13. Savoldo, B. et al. CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. *J. Clin. Invest.* **121**, 1822–1826 (2011).
14. Friedmann-Morvinski, D., Bendavid, A., Waks, T., Schindler, D. & Eshhar, Z. Redirected primary T cells harboring a chimeric receptor require costimulation for their antigen-specific activation. *Blood* **105**, 3087–3093 (2005).
15. Brocker, T. Chimeric Fv-ζ or Fv-ε receptors are not sufficient to induce activation or cytokine production in peripheral T cells. *Blood* **96**, 1999–2001 (2000).
16. Mitsuyasu, R. T. et al. Prolonged survival and tissue trafficking following adoptive transfer of CD4ζ gene-modified autologous $CD4^+$ and $CD8^+$ T cells in human immunodeficiency virus-infected subjects. *Blood* **96**, 785–793 (2000).
17. Scholler, J. et al. Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. *Sci. Transl. Med.* **4**, 132ra153 (2012).
18. Uckun, F. et al. Detailed studies on expression and function of CD19 surface determinant by using B43 monoclonal antibody and the clinical potential of anti-CD19 immunotoxins. *Blood* **71**, 13–29 (1988).
19. Ishiura, N. et al. Differential phosphorylation of functional tyrosines in CD19 modulates B-lymphocyte activation. *Eur. J. Immunol.* **40**, 1192–1204 (2010).
20. LeBien, T. W. & Tedder, T. F. B lymphocytes: how they develop and function. *Blood* **112**, 1570–1580 (2008).
21. Kochenderfer, J. N. et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood* **116**, 4099–4102 (2010).
22. Kalos, M. et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci. Transl. Med.* **3**, 95ra73 (2011).
23. Porter, D. L., Levine, B. L., Kalos, M., Bagg, A. & June, C. H. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N. Engl. J. Med.* **365**, 725–733 (2011).
24. Brentjens, R. J. et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. *Blood* **118**, 4817–4828 (2011).
25. Grupp, S. A. et al. Chimeric antigen receptor–modified T cells for acute lymphoid leukemia. *N. Engl. J. Med.* **368**, 1509–1518 (2013).
26. Maude, S. et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N. Engl. J. Med.* **371**, 1507–1517 (2014).
27. Kochenderfer, J. N. et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J. Clin. Oncol.* **33**, 540–549 (2015).
28. Porter, D. L. et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci. Transl. Med.* **7**, 303ra139 (2015).
29. Lee, D. W. et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet* **385**, 517–528 (2015).
30. Turtle, C. J. et al. CD19 CAR-T cells of defined $CD4^+$:$CD8^+$ composition in adult B cell ALL patients. *J. Clin. Invest.* **126**, 2123–2138 (2016).
31. Turtle, C. J. et al. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of $CD8^+$ and $CD4^+$ CD19-specific chimeric antigen receptor-modified T cells. *Sci. Transl. Med.* **8**, 355ra116 (2016).
32. Schuster, S. J. et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. *N. Engl. J. Med.* **377**, 2545–2554 (2017).
33. Neelapu, S. S. et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N. Engl. J. Med.* **377**, 2531–2544 (2017).
34. Kochenderfer, J. N. et al. Lymphoma remissions caused by anti-CD19 chimeric antigen receptor T cells are associated with high serum interleukin-15 levels. *J. Clin. Oncol.* **35**, 1803–1813 (2017).
35. Turtle, C. J. et al. Durable molecular remissions in chronic lymphocytic leukemia treated with CD19-specific chimeric antigen receptor-modified T cells after failure of ibrutinib. *J. Clin. Oncol.* **35**, 3010–3020 (2017).
36. Locke, F. L. et al. Phase 1 results of ZUMA-1: a multicenter study of KTE-C19 anti-CD19 CAR T cell therapy in refractory aggressive lymphoma. *Mol. Ther.* **25**, 285–295 (2017).
37. Melenhorst, J. J. et al. Long-term remission of CLL sustained by pauciclonal anti-CD19 chimeric antigen receptor T (CTL019) cell clones. *Blood* **132** (Suppl. 1), 699 (2018).
38. Park, J. H. et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *N. Engl. J. Med.* **378**, 449–459 (2018).
39. Maude, S. L. et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N. Engl. J. Med.* **378**, 439–448 (2018).
40. Fraietta, J. A. et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nat. Med.* **24**, 563–571 (2018).
41. Neelapu, S. S. et al. A comparison of two-year outcomes in ZUMA-1 (axicabtagene ciloleucel) and SCHOLAR-1 in patients with refractory large B cell lymphoma. *Blood* **134** (Suppl. 1), 4095 (2019).
42. Baird, J. H. et al. CD22-Directed CAR T-cell therapy induces complete remissions in CD19-directed CAR-refractory large B-cell lymphoma. *Blood* **137**, 2321–2325 (2021).
43. Bukhari, A. et al. Rapid relapse of large B-cell lymphoma after CD19 directed CAR-T-cell therapy due to CD-19 antigen loss. *Am. J. Hematol.* **94**, E273–E275 (2019).
44. Schuster, S. J. et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N. Engl. J. Med.* **380**, 45–56 (2019).
45. Schuster, S. J. et al. Sustained disease control for adult patients with relapsed or refractory diffuse large B-cell lymphoma: an updated analysis of Juliet, a global pivotal phase 2 trial of tisagenlecleucel. *Blood* **132**, 1684 (2018).
46. Wang, M. et al. KTE-X19 CAR T-cell therapy in relapsed or refractory mantle-cell lymphoma. *N. Engl. J. Med.* **382**, 1331–1342 (2020).
47. Jin, Z. et al. The severe cytokine release syndrome in phase I trials of CD19-CAR-T cell therapy: a systematic review. *Ann. Hematol.* **97**, 1327–1335 (2018).
48. Lee, D. W. et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol. Blood Marrow Transplant.* **25**, 625–638 (2019).
49. Ajina, A. & Maher, J. Strategies to address chimeric antigen receptor tonic signaling. *Mol. Cancer Ther.* **17**, 1795–1815 (2018).
50. Maus, M. V. et al. Society for immunotherapy of cancer (SITC) clinical practice guideline on immune effector cell-related adverse events. *J. Immunother. Cancer* **8**, e001511 (2020).
51. Neelapu, S. S. et al. Chimeric antigen receptor T-cell therapy—assessment and management of toxicities. *Nat. Rev. Clin. Oncol.* **15**, 47–62 (2018).
52. *Long Term Follow-Up After Administration of Human Gene Therapy Products: Guidance for Industry* (US Food and Drug Administration, 2020); https://www.fda.gov/media/113768/download
53. DAIDS Guidelines for Good Laboratory Practice Standards (National Institute of Allergy and Infectious Diseases, 2019); https://www.niaid.nih.gov/sites/default/files/gclp.pdf
54. Stadtmauer, E. A. et al. CRISPR-engineered T cells in patients with refractory cancer. *Science* **367**, eaba7365 (2020).
55. Hay, K. A. et al. Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy. *Blood* **130**, 2295–2306 (2017).
56. Turtle, C. J. et al. CD19 CAR-T cells of defined $CD4^+$:$CD8^+$ composition in adult B cell ALL patients. *J. Clin. Invest.* **126**, 2123–2138 (2016).
57. Davila, M. L. et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci. Transl. Med.* **6**, 224ra225 (2014).
58. Kadauke, S. et al. Early administration of tocilizumab (Toci) for the prevention of grade 4 cytokine release syndrome (CRS) after CD19-directed CAR T-cell therapy (CTL019). *Cytotherapy* **21**, e2–e3 (2019).
59. Teachey, D. T. et al. Identification of predictive biomarkers for cytokine release syndrome after chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Cancer Discov.* **6**, 664–679 (2016).
60. Sotillo, E. et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. *Cancer Discov.* **5**, 1282–1295 (2015).
61. Orlando, E. J. et al. Genetic mechanisms of target antigen loss in CAR19 therapy of acute lymphoblastic leukemia. *Nat. Med.* **24**, 1504–1506 (2018).
62. Ruella, M. et al. Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. *Nat. Med.* **24**, 1499–1503 (2018).

63. Maude, S. L. et al. Efficacy and safety of CTL019 in the first US phase II multicenter trial in pediatric relapsed/refractory acute lymphoblastic leukemia: results of an interim analysis. *Blood* **128**, 2801 (2016).
64. Singh, N. et al. Impaired death receptor signaling in leukemia causes antigen-independent resistance by inducing CAR T cell dysfunction.*Cancer Discov.* **10**, 552–567 (2020).
65. Mueller, K. T. et al. Cellular kinetics of CTL019 in relapsed/refractory B-cell acute lymphoblastic leukemia and chronic lymphocytic leukemia. *Blood* **130**, 2317–2325 (2017).
66. Finney, O. C. et al. CD19 CAR T cell product and disease attributes predict leukemia remission durability. *J. Clin. Invest.* **129**, 2123–2132 (2019).
67. Fraietta, J. A. et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. *Nature* **558**, 307–312 (2018).
68. Wang, M. et al. Identification and validation of predictive biomarkers to CD19- and BCMA-specific CAR T-cell responses in CAR T-cell precursors. *Blood* **134** (Suppl. 1), 622 (2019).
69. Aguirre-Ghiso, J. A. Models, mechanisms and clinical evidence for cancer dormancy. *Nat. Rev. Cancer* **7**, 834–846 (2007).
70. White, E. & DiPaola, R. S. The double-edged sword of autophagy modulation in cancer. *Clin. Cancer Res.* **15**, 5308–5316 (2009).
71. Sosa, M. S., Bragado, P. & Aguirre-Ghiso, J. A. Mechanisms of disseminated cancer cell dormancy: an awakening field. *Nat. Rev. Cancer* **14**, 611–622 (2014).
72. Palma, M. et al. T cells in chronic lymphocytic leukemia display dysregulated expression of immune checkpoints and activation markers. *Haematologica* **102**, 562–572 (2017).
73. Chong, E. A. et al. PD-1 blockade modulates chimeric antigen receptor (CAR)-modified T cells: refueling the CAR. *Blood* **129**, 1039–1041 (2017).
74. Ren, J. et al. Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition. *Clin. Cancer Res.* **23**, 2255–2266 (2017).
75. John, L. B. et al. Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells. *Clin. Cancer Res.* **19**, 5636–5646 (2013).
76. Newick, K. et al. Augmentation of CAR T-cell trafficking and antitumor efficacy by blocking protein kinase A localization. *Cancer Immunol. Res.* **4**, 541–551 (2016).
77. Renner, K. et al. Metabolic hallmarks of tumor and immune cells in the tumor microenvironment. *Front. Immunol.* **8**, 248 (2017).
78. Shah, N. N. et al. Clonal expansion of CAR T cells harboring lentivector integration in the *CBL* gene following anti-CD22 CAR T-cell therapy. *Blood Adv.* **3**, 2317–2322 (2019).
79. Nobles, C. L. et al. CD19-targeting CAR T cell immunotherapy outcomes correlate with genomic modification by vector integration.*J. Clin. Invest.* **130**, 673–685 (2020).
80. Bushman, F. D. Retroviral insertional mutagenesis in humans: evidence for four genetic mechanisms promoting expansion of cell clones. *Mol. Ther.* **28**, 352–356 (2020).
81. Sheih, A. et al. Clonal kinetics and single-cell transcriptional profiling of CAR-T cells in patients undergoing CD19 CAR-T immunotherapy. *Nat. Commun.* **11**, 219 (2020).
82. Naramura, M., Kole, H. K., Hu, R. J. & Gu, H. Altered thymic positive selection and intracellular signals in Cbl-deficient mice. *Proc. Natl Acad. Sci. USA* **95**, 15547–15552 (1998).
83. Peer, S., Baier, G. & Gruber, T. Cblb-deficient T cells are less susceptible to PD-L1-mediated inhibition. *Oncotarget* **8**, 41841–41853 (2017).
84. Schmitz, M. L. Activation of T cells: releasing the brakes by proteolytic elimination of Cbl-b. *Sci. Signal.* **2**, pe38 (2009).
85. Stromnes, I. M. et al. Abrogating Cbl-b in effector CD8$^+$ T cells improves the efficacy of adoptive therapy of leukemia in mice. *J. Clin. Invest.* **120**, 3722–3734 (2010).
86. Eyquem, J. et al. Targeting a CAR to the *TRAC* locus with CRISPR/Cas9 enhances tumour rejection. *Nature* **543**, 113–117 (2017).
87. Liu, E. et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. *N. Engl. J. Med.* **382**, 545–553 (2020).
88. Lanitis, E. et al. Optimized gene engineering of murine CAR-T cells reveals the beneficial effects of IL-15 coexpression.*J. Exp. Med.* **218**, e20192203 (2021).
89. Mishra, A. et al. Aberrant overexpression of IL-15 initiates large granular lymphocyte leukemia through chromosomal instability and DNA hypermethylation. *Cancer Cell* **22**, 645–655 (2012).
90. Fehniger, T. A. et al. Fatal leukemia in interleukin 15 transgenic mice follows early expansions in natural killer and memory phenotype CD8$^+$ T cells. *J. Exp. Med.* **193**, 219–232 (2001).
91. Hu, B. et al. Augmentation of antitumor immunity by human and mouse CAR T cells secreting IL-18. *Cell Rep.* **20**, 3025–3033 (2017).
92. Fraietta, J. A. et al. Ibrutinib enhances chimeric antigen receptor T-cell engraftment and efficacy in leukemia. *Blood* **127**, 1117–1127 (2016).
93. Gill, S. I. et al. Prospective clinical trial of anti-CD19 CAR T cells in combination with ibrutinib for the treatment of chronic lymphocytic leukemia shows a high response rate. *Blood* **132**, 298 (2018).
94. Gauthier, J. et al. Feasibility and efficacy of CD19-targeted CAR T cells with concurrent ibrutinib for CLL after ibrutinib failure. *Blood* **135**, 1650–1660 (2020).
95. Kater, A. P. & Melenhorst, J. J. CAR-T and ibrutinib vs CLL: sequential or simultaneous? *Blood* **135**, 1611–1612 (2020).
96. Gorelik, L. & Flavell, R. A. Immune-mediated eradication of tumors through the blockade of transforming growth factor-β signaling in T cells. *Nat. Med.* **7**, 1118–1122 (2001).
97. Kloss, C. C. et al. Dominant-negative TGF-β receptor enhances PSMA-targeted human CAR T cell proliferation and augments prostate cancer eradication. *Mol. Ther.* **26**, 1855–1866 (2018).
98. Garfall, A. L. et al. Chimeric antigen receptor T cells against CD19 for multiple myeloma. *N. Engl. J. Med.* **373**, 1040–1047 (2015).
99. Gardner, R. et al. Acquisition of a CD19-negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T-cell therapy. *Blood* **127**, 2406–2410 (2016).
100. Jacoby, E. et al. CD19 CAR immune pressure induces B-precursor acute lymphoblastic leukaemia lineage switch exposing inherent leukaemic plasticity. *Nat. Commun.* **7**, 12320 (2016).
101. Zhang, Z. et al. Point mutation in *CD19* facilitates immune escape of B cell lymphoma from CAR-T cell therapy. *J. Immunother. Cancer* **8**, e001150 (2020).
102. Jia, H. et al. Haploidentical CD19/CD22 bispecific CAR-T cells induced MRD-negative remission in a patient with relapsed and refractory adult B-ALL after haploidentical hematopoietic stem cell transplantation. *J. Hematol. Oncol.* **12**, 57 (2019).
103. Shalabi, H. et al. Sequential loss of tumor surface antigens following chimeric antigen receptor T-cell therapies in diffuse large B-cell lymphoma.*Haematologica* **103**, e215–e218 (2018).
104. Fry, T. J. et al. CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. *Nat. Med.* **24**, 20–28 (2018).
105. Louis, C. U. et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. *Blood* **118**, 6050–6056 (2011).
106. Finney, H. M., Akbar, A. N. & Lawson, A. D. Activation of resting human primary T cells with chimeric receptors: costimulation from CD28, inducible costimulator, CD134, and CD137 in series with signals from the TCRζ chain. *J. Immunol.* **172**, 104–113 (2004).
107. Long, A. H. et al. 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. *Nat. Med.* **21**, 581–590 (2015).
108. Hong, M., Clubb, J. D. & Chen, Y. Y.Engineering CAR-T cells for next-generation cancer therapy. *Cancer Cell* **38**, 473–488 (2020).
109. Den Haan, J. et al. Identification of a graft versus host disease-associated human minor histocompatibility antigen. *Science* **268**, 1476–1480 (1995).
110. Den Haan, J. M. et al. The minor histocompatibility antigen HA-1: a diallelic gene with a single amino acid polymorphism. *Science* **279**, 1054–1057 (1998).
111. Bevan, M. J. The major histocompatibility complex determines susceptibility to cytotoxic T cells directed against minor histocompatibility antigens. *J. Exp. Med.* **142**, 1349–1364 (1975).
112. Lamberth, K. et al. Post hoc assessment of the immunogenicity of bioengineered factor VIIa demonstrates the use of preclinical tools. *Sci. Transl. Med.* **9**, eaag1286 (2017).
113. Mahlangu, J. N. et al. Changes in the amino acid sequence of the recombinant human factor VIIa analog, vatreptacog alfa, are associated with clinical immunogenicity. *J. Thromb. Haemost.* **13**, 1989–1998 (2015).
114. Berger, C., Flowers, M. E., Warren, E. H. & Riddell, S. R. Analysis of transgene-specific immune responses that limit the in vivo persistence of adoptively transferred HSV-TK-modified donor T cells after allogeneic hematopoietic cell transplantation. *Blood* **107**, 2294–2302 (2006).
115. Xu, J. et al. Exploratory trial of a biepitopic CAR T-targeting B cell maturation antigen in relapsed/refractory multiple myeloma. *Proc. Natl Acad. Sci. USA* **116**, 9543–9551 (2019).
116. Jensen, M. C. et al. Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans. *Biol. Blood Marrow Transplant.* **16**, 1245–1256 (2010).
117. Wagner, D. L. et al. Immunogenicity of CAR T cells in cancer therapy. *Nat. Rev. Clin. Oncol.* **18**, 379–393 (2021).
118. Lamers, C. H. et al. Immune responses to transgene and retroviral vector in patients treated with ex vivo-engineered T cells. *Blood* **117**, 72–82 (2011).
119. Hajek, R., Okubote, S. A. & Svachova, H. Myeloma stem cell concepts, heterogeneity and plasticity of multiple myeloma. *Br. J. Haematol.* **163**, 551–564 (2013).
120. Mikkilineni, L. & Kochenderfer, J. N.CAR T cell therapies for patients with multiple myeloma. *Nat. Rev. Clin. Oncol.* **18**, 71–84 (2021).
121. D'Agostino, M. & Raje, N. Anti-BCMA CAR T-cell therapy in multiple myeloma: can we do better? *Leukemia* **34**, 21–34 (2020).

122. Cohen, A. D. et al. B cell maturation antigen-specific CAR T cells are clinically active in multiple myeloma. *J. Clin. Invest.* **129**, 2210–2221 (2019).
123. Mailankody, S. et al. Clinical responses and pharmacokinetics of MCARH171, a human-derived BCMA targeted CAR T cell therapy in relapsed/refractory multiple myeloma: final results of a phase I clinical trial. *Blood* **132**, 959 (2018).
124. Mailankody, S. et al. JCARH125, anti-BCMA CAR T-cell therapy for relapsed/refractory multiple myeloma: initial proof of concept results from a phase 1/2 multicenter study (EVOLVE). *Blood* **132**, 957 (2018).
125. Mailankody, S. et al. Orvacabtagene autoleucel (orva-cel), a B-cell maturation antigen (BCMA)-directed CAR T cell therapy for patients (pts) with relapsed/refractory multiple myeloma (RRMM): update of the phase 1/2 EVOLVE study (NCT03430011). *J. Clin. Oncol.* **38** (Suppl. 15), 8504–8504 (2020).
126. Green, D. J. et al. Fully human BCMA targeted chimeric antigen receptor T cells administered in a defined composition demonstrate potency at low doses in advanced stage high risk multiple myeloma. *Blood* **132**, 1011 (2018).
127. Hao, S. et al. Two-year follow-up of investigator-initiated phase 1 trials of the safety and efficacy of fully human anti-BCMA CAR T cells (CT053) in relapsed/refractory multiple myeloma. *Blood* **136**, 27–28 (2020).
128. Kumar, S. K. et al. Results from lummicar-2: a phase 1b/2 study of fully human B-cell maturation antigen-specific CAR T cells (CT053) in patients with relapsed and/or refractory multiple myeloma. *Blood* **136**, 28–29 (2020).
129. Chen, W. et al. Results from lummicar-1: a phase 1 study of fully human B-cell maturation antigen-specific CAR T cells (CT053) in Chinese subjects with relapsed and/or refractory multiple myeloma. *Blood* **136**, 49–50 (2020).
130. Li, C. et al. Efficacy and safety of fully human BCMA targeting CAR T cell therapy in relapsed/refractory multiple myeloma. *Blood* **134**, 929 (2019).
131. Costello, C. L. et al. Phase 1/2 study of the safety and response of P-BCMA-101 CAR-T cells in patients with relapsed/refractory (r/r) multiple myeloma (MM) (PRIME) with novel therapeutic strategies. *Blood* **136**, 29–30 (2020).
132. Brudno, J. N. et al. T cells genetically modified to express an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of poor-prognosis relapsed multiple myeloma. *J. Clin. Oncol.* **36**, 2267–2280 (2018).
133. Lin, Y. et al. Idecabtagene vicleucel (ide-cel, bb2121), a BCMA-directed CAR T cell therapy, in patients with relapsed and refractory multiple myeloma: updated results from phase 1 CRB-401 study. *Blood* **136**, 26–27 (2020).
134. Alsina, M. et al. Updated results from the phase I CRB-402 study of anti-BCMA CAR-T cell therapy bb21217 in patients with relapsed and refractory multiple myeloma: correlation of expansion and duration of response with T cell phenotypes. *Blood* **136**, 25–26 (2020).
135. Fu, W. Sr et al. Efficacy and safety of CAR-T therapy with safety switch targeting BCMA for patients with relapsed/refractory multiple myeloma in a phase 1 clinical study. *Blood* **134**, 3154 (2019).
136. Wang, B. -Y. et al. Long-term follow-up of a phase 1, first-in-human open-label study of LCAR-B38M, a structurally differentiated chimeric antigen receptor T (CAR-T) cell therapy targeting B-cell maturation antigen (BCMA), in patients (pts) with relapsed/refractory multiple myeloma (RRMM). *Blood* **134** (Suppl. 1), 579 (2019).
137. Zhao, W.-H. et al. A phase 1, open-label study of LCAR-B38M, a chimeric antigen receptor T cell therapy directed against B cell maturation antigen, in patients with relapsed or refractory multiple myeloma. *J. Hematol. Oncol.* **11**, 141 (2018).
138. Madduri, D. et al. Results from CARTITUDE-1: a phase 1b/2 study of JNJ-4528, a CAR-T cell therapy directed against B-cell maturation antigen (BCMA), in patients with relapsed and/or refractory multiple myeloma (R/R MM). *Blood* **134**, 577 (2019).
139. Haas, A. R. et al. Phase I study of lentiviral-transduced chimeric antigen receptor-modified T cells recognizing mesothelin in advanced solid cancers. *Mol. Ther.* **27**, 1919–1929 (2019).
140. Gattinoni, L. et al. Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8$^+$ T cells. *J. Exp. Med.* **202**, 907–912 (2005).
141. Baba, J. et al. Depletion of radio-resistant regulatory T cells enhances antitumor immunity during recovery from lymphopenia. *Blood* **120**, 2417–2427 (2012).
142. Kodumudi, K. N., Weber, A., Sarnaik, A. A. & Pilon-Thomas, S. Blockade of myeloid-derived suppressor cells after induction of lymphopenia improves adoptive T cell therapy in a murine model of melanoma. *J. Immunol.* **189**, 5147–5154 (2012).
143. Pont, M. J. et al. γ-Secretase inhibition increases efficacy of BCMA-specific chimeric antigen receptor T cells in multiple myeloma. *Blood* **134**, 1585–1597 (2019).
144. Green, D. J. et al. Response to Bcma CAR-T cells correlates with pretreatment target antigen density and Is improved by small molecule inhibition of gamma secretase. *Blood* **134** (Suppl. 1), 1856 (2019).
145. Laurent, S. A. et al. γ-Secretase directly sheds the survival receptor BCMA from plasma cells. *Nat. Commun.* **6**, 7333 (2015).
146. Cowan, A. J. et al. Efficacy and safety of fully human Bcma CAR T cells in combination with a gamma secretase inhibitor to increase Bcma surface expression in patients with relapsed or refractory multiple myeloma. *Blood* **134** (Suppl. 1), 204 (2019).
147. Raje, N. et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. *N. Engl. J. Med.* **380**, 1726–1737 (2019).
148. Brown, C. E. et al. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. *N. Engl. J. Med.* **375**, 2561–2569 (2016).
149. Kandalaft, L. E., Powell, D. J. & Coukos, G. A phase I clinical trial of adoptive transfer of folate receptor-alpha redirected autologous T cells for recurrent ovarian cancer. *J. Transl. Med.* **10**, 157 (2012).
150. O'Rourke, D. M. et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci. Transl. Med.* **9**, eaaa0984 (2017).
151. Castellarin, M., Watanabe, K., June, C. H., Kloss, C. C. & Posey, A. D. Driving cars to the clinic for solid tumors. *Gene Ther.* **25**, 165–175 (2018).
152. Morgan, R. A. et al. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol. Ther.* **18**, 843–851 (2010).
153. Urbanska, K., Stashwick, C., Poussin, M. & Powell, D. J. Jr. Follicle-stimulating hormone receptor as a target in the redirected T-cell therapy for cancer. *Cancer Immunol. Res.* **3**, 1130–1137 (2015).
154. Beatty, G. L. et al. Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. *Cancer Immunol. Res.* **2**, 112–120 (2014).
155. Evans, R. A. et al. Lack of immunoediting in murine pancreatic cancer reversed with neoantigen. *JCI Insight* **1**, e88328 (2016).
156. Minutolo, N. G., Hollander, E. E. & Powell, D. J. Jr. The emergence of universal immune receptor T cell therapy for cancer. *Front. Oncol.* **9**, 176 (2019).
157. Urbanska, K. et al. A universal strategy for adoptive immunotherapy of cancer through use of a novel T-cell antigen receptor. *Cancer Res.* **72**, 1844–1852 (2012).
158. Wilkie, S. et al. Dual targeting of ErbB2 and MUC1 in breast cancer using chimeric antigen receptors engineered to provide complementary signaling. *J. Clin. Immunol.* **32**, 1059–1070 (2012).
159. Hegde, M. et al. Tandem CAR T cells targeting HER2 and IL13Rα2 mitigate tumor antigen escape. *J. Clin. Invest.* **126**, 3036–3052 (2016).
160. Balakrishnan, A. et al. Multispecific targeting with synthetic ankyrin repeat motif chimeric antigen receptors. *Clin. Cancer Res.* **25**, 7506–7516 (2019).
161. Roybal, K. T. et al. Engineering T cells with customized therapeutic response programs using synthetic notch receptors. *Cell* **167**, 419–432.e16 (2016).
162. Choi, B. D. et al. CAR-T cells secreting BiTEs circumvent antigen escape without detectable toxicity. *Nat. Biotechnol.* **37**, 1049–1058 (2019).
163. Song, D. G. et al. In vivo persistence, tumor localization, and antitumor activity of CAR-engineered T cells is enhanced by costimulatory signaling through CD137 (4-1BB). *Cancer Res.* **71**, 4617–4627 (2011).
164. Tchou, J. et al. Safety and efficacy of intratumoral injections of chimeric antigen receptor (CAR) T cells in metastatic breast cancer. *Cancer Immunol. Res.* **5**, 1152–1161 (2017).
165. Fisher, B. et al. Tumor localization of adoptively transferred indium-111 labeled tumor infiltrating lymphocytes in patients with metastatic melanoma. *J. Clin. Oncol.* **7**, 250–261 (1989).
166. Pockaj, B. A. et al. Localization of 111indium-labeled tumor infiltrating lymphocytes to tumor in patients receiving adoptive immunotherapy. Augmentation with cyclophosphamide and correlation with response. *Cancer* **73**, 1731–1737 (1994).
167. Harlin, H. et al. Chemokine expression in melanoma metastases associated with CD8$^+$ T-cell recruitment. *Cancer Res.* **69**, 3077–3085 (2009).
168. Brown, C. E. et al. Tumor-derived chemokine MCP-1/CCL2 is sufficient for mediating tumor tropism of adoptively transferred T cells. *J. Immunol.* **179**, 3332–3341 (2007).
169. Moon, E. K. et al. Expression of a functional CCR2 receptor enhances tumor localization and tumor eradication by retargeted human T cells expressing a mesothelin-specific chimeric antibody receptor. *Clin. Cancer Res.* **17**, 4719–4730 (2011).
170. Craddock, J. A. et al. Enhanced tumor trafficking of GD2 chimeric antigen receptor T cells by expression of the chemokine receptor CCR2b. *J. Immunother.* **33**, 780–788 (2010).
171. Di Stasi, A. et al. T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. *Blood* **113**, 6392–6402 (2009).
172. Adachi, K. et al. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. *Nat. Biotechnol.* **36**, 346–351 (2018).

173. Caruana, I. et al. Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. *Nat. Med.* **21**, 524–529 (2015).
174. Feig, C. et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc. Natl Acad. Sci. USA* **110**, 20212–20217 (2013).
175. Wang, L. C. et al. Targeting fibroblast activation protein in tumor stroma with chimeric antigen receptor T cells can inhibit tumor growth and augment host immunity without severe toxicity. *Cancer Immunol. Res.* **2**, 154–166 (2014).
176. Tran, E. et al. Immune targeting of fibroblast activation protein triggers recognition of multipotent bone marrow stromal cells and cachexia. *J. Exp. Med.* **210**, 1125–1135 (2013).
177. Chinnasamy, D. et al. Gene therapy using genetically modified lymphocytes targeting VEGFR-2 inhibits the growth of vascularized syngenic tumors in mice. *J. Clin. Invest.* **120**, 3953–3968 (2010).
178. Chinnasamy, D. et al. Local delivery of interleukin-12 using T cells targeting VEGF receptor-2 eradicates multiple vascularized tumors in mice. *Clin. Cancer Res.* **18**, 1672–1683 (2012).
179. Santoro, S. P. et al. T cells bearing a chimeric antigen receptor against prostate-specific membrane antigen mediate vascular disruption and result in tumor regression. *Cancer Immunol. Res.* **3**, 68–84 (2015).
180. Fu, X., Rivera, A., Tao, L. & Zhang, X. Genetically modified T cells targeting neovasculature efficiently destroy tumor blood vessels, shrink established solid tumors and increase nanoparticle delivery. *Int. J. Cancer* **133**, 2483–2492 (2013).
181. Motz, G. T. et al. Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. *Nat. Med.* **20**, 607–615 (2014).
182. Guo, Y. et al. Tumour endothelial marker 1/endosialin-mediated targeting of human sarcoma. *Eur. J. Cancer* **90**, 111–121 (2018).
183. Spiegel, J. Y. et al. Outcomes in large B-cell lymphoma progressing after axicabtagene ciloleucel (Axi-cel): results from the U.S. Lymphoma CAR-T Consortium. *J. Clin. Oncol.* **37** (Suppl. 15), 7517–7517 (2019).
184. Munshi, N. C. et al. Idecabtagene vicleucel (ide-cel; bb2121), a BCMA-targeted CAR T-cell therapy, in patients with relapsed and refractory multiple myeloma (RRMM): initial KarMMa results. *J. Clin. Oncol.* **38**, 8503 (2020).
185. Yan, Z. et al. A combination of humanised anti-CD19 and anti-BCMA CAR T cells in patients with relapsed or refractory multiple myeloma: a single-arm, phase 2 trial. *Lancet Haematol.* **6**, e521–e529 (2019).
186. Locke, F. L. et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1–2 trial. *Lancet Oncol.* **20**, 31–42 (2019).
187. Gill, S. I. et al. Prospective clinical trial of anti-CD19 CAR T cells in combination with ibrutinib for the treatment of chronic lymphocytic leukemia shows a high response rate. *Blood* **132** (Suppl. 1), 298 (2018).
188. Shah, N. N. et al. Bispecific anti-CD20, anti-CD19 CAR T cells for relapsed B cell malignancies: a phase 1 dose escalation and expansion trial. *Nat. Med.* **26**, 1569–1575 (2020).
189. Pan, J. et al. Sequential CD19-22 CAR T therapy induces sustained remission in children with r/r B-ALL. *Blood* **135**, 387–391 (2020).
190. Weber, E. W. et al. Pharmacologic control of CAR-T cell function using dasatinib. *Blood Adv.* **3**, 711–717 (2019).

Acknowledgements

This work was supported by funding from the National Institutes of Health (R01CA241762 and R01GM138908 to J.J.M.), Stand up to Cancer Convergence 2.0 Grant (to J.J.M.), Parker Institute for Cancer Immunotherapy (J.J.M. and M.A.C.), National Natural Science Foundation of China (81970189), National Visiting Scholar Program of the China Scholarship Council and Guang-Ci Visiting Scholar Program of Ruijin Hospital affiliated to Shanghai Jiao Tong University (to J.X.).

Author contributions

J.J.M. developed the concept of the paper. All authors contributed to the first draft of the manuscript and approved the final version.

Competing interests

J.J.M., D.J.P. Jr and M.C.M. are inventors on several patents issued and pending in the field of CAR T cell therapy for cancer, which are assigned to the University of Pennsylvania. Under the University of Pennsylvania's policies, J.J.M., D.J.P. Jr and M.C.M. either currently, or may in the future, receive royalties from the licensing of these patent rights. M.C.M. is an inventor on issued and pending patents related to CAR technology, and receives royalties from the licensing of this IP. He is also a founder and equity holder in Cabaletta Bio. J.X., S.-J.C. and M.A.C. have no conflicts of interest. J.Z. receives research funding from and is chairman of the Medical and Scientific Advisory Board of IASO Biotherapeutics. D.J.P. Jr is or has provided consultation for Iovance Biotherapeutics, Bellicum Pharmaceuticals, Neon Therapeutics, and Tmunity Therapeutics, and holds patents in the areas of tumor-infiltrating lymphocytes and gene-engineered T cells. J.J.M. receives research funding from IASO Biotherapeutics, consult for Simcere of America, Shanghai Unicar Therapy, Johnson & Johnson, Poseida, and IASO Biotherapeutics, and is on the Medical and Scientific Advisory Board of IASO Biotherapeutics and Poseida Therapeutics. J.J.M. further holds patents related to CAR T cell manufacturing and biomarkers.

Additional information

Correspondence should be addressed to J.J.M.

Peer review information *Nature Cancer* thanks Terry Fry and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.