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Perspective

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CAR T cell therapy for patients with solid tumours: key lessons to learn and unlearn

Steven M. Albelda [1,2]

Abstract

Chimeric antigen receptor (CAR) T cells have been approved for use in patients with B cell malignancies or relapsed and/or refractory multiple myeloma, yet efficacy against most solid tumours remains elusive. The limited imaging and biopsy data from clinical trials in this setting continues to hinder understanding, necessitating a reliance on imperfect preclinical models. In this Perspective, I re-evaluate current data and suggest potential pathways towards greater success, drawing lessons from the few successful trials testing CAR T cells in patients with solid tumours and the clinical experience with tumour-infiltrating lymphocytes. The most promising approaches include the use of pluripotent stem cells, co-targeting multiple mechanisms of immune evasion, employing multiple co-stimulatory domains, and CAR ligand-targeting vaccines. An alternative strategy focused on administering multiple doses of short-lived CAR T cells in an attempt to pre-empt exhaustion and maintain a functional effector pool should also be considered.

Sections

[1]Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. [2]Pulmonary and Critical Care Division, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. e-mail: albelda@pennmedicine.upenn.edu

Introduction

Chimeric antigen receptor (CAR)-expressing T cells targeting the B cell antigen CD19 are highly effective therapies in patients with relapsed and/or refractory (R/R) forms of certain B cell malignancies (especially acute lymphoblastic leukaemia and lymphoma) that are able to provide very durable responses. Autologous CD19-targeted CAR T cells are now approved by most major regulatory bodies including the FDA (in the USA), the EMA (in Europe) and the NMPA (in China). These CAR T cells have thus changed clinical practice[1–4]. CAR T cells targeting B cell maturation antigen (BCMA), although not yet curative, have also shown strong antitumour activity and have been approved by the same agencies for clinical use in patients with R/R multiple myeloma[5]. Autologous tumour-infiltrating lymphocytes (TILs) expanded ex vivo have shown robust antitumour activity in selected patients with advanced-stage solid tumours, especially in those with melanoma[6,7]. However, the development of CAR T cells with clinical utility in such patients remains elusive[8–10], for reasons that are largely unknown, making this a tantalizing, but as-yet unfulfilled goal. The key question is 'why?'. Numerous reviews have discussed the possible reasons for the limited activity of CAR T cells in patients with solid tumours and highlight a number of potentially challenging issues, including: (1) insufficiently specific target antigens (creating the risk of on-target/off-tumour toxicity); (2) poor trafficking; (3) short persistence; (4) loss of effector function; and (5) tumour antigen heterogeneity. Accordingly, the list of key characteristics of an ideal CAR T cell to treat patients with solid tumours is long (Box 1).

The goal of this Perspective is not to add another comprehensive review to the long list that are already published, nor to discuss the issues related to CAR T cell antigen selection or toxicity. For this purpose, the reader is referred to a number of excellent reviews[2,3,10–19]. Instead, I reanalyse and reflect upon some of the currently available data and suggest possible research directions that might lead to success. I first define the key characteristics of CAR T cells with the potential to be effective against solid tumours and then discuss how successful the current strategies tested in clinical trials have been in terms of achieving these specific characteristics. However, analysing the performance of CAR T cells in patients with solid tumours is challenging, owing to the very limited data from imaging and/or biopsy samples. The current knowledge base therefore relies primarily on data extrapolated from mouse models, which have several limitations that are also considered. I then identify certain themes from previously successful trials and propose some lessons that need to be learned from the experience with TIL therapy, and just as importantly, some lessons from previous clinical trials testing CD19-targeted CAR T cells in patients with haematological cancers that might need to be unlearned. I discuss particularly promising approaches that might improve antitumour activity and propose a potential alternative strategy that focuses on using multiple doses of short-lived CAR T cells to pre-empt CAR T cell exhaustion and thus maintain a functional effector pool. Rather than providing detailed references for every point, the reader is referred to comprehensive review articles from the past few years. However, I highlight specific articles that are particularly relevant, recent or not discussed in review articles.

Why have CAR T cells failed in solid tumours?

Surprisingly, we do not know why CAR T cells have been so ineffective against solid tumours compared with their successes in patients with B cell malignancies. Most clinical trials testing CAR T cells in patients with solid tumours have provided data on feasibility and safety, along with estimates of the number of CAR T cells present in the blood and antitumour activity[3,8,10,20,21]. In general, CAR T cell production has been feasible, the CAR T cells have been well tolerated (with a few exceptions[22,23]), and CAR T cells have routinely been identified in blood samples (primarily using sensitive quantitative PCR techniques). However, the numbers of CAR T cells present in blood samples consistently peaks at 7–14 days after infusion and usually falls to low levels by day 28 (refs. 24–26). Furthermore, the numbers of CAR T cells typically detected in blood samples from patients with solid tumours participating in clinical trials are usually about fivefold to tenfold lower than those seen in the most successful trials testing CD19-targeted CAR T cells. When tested, prior lymphodepletion tends to confer a modest increase in the numbers of CAR T cells present in the blood[25,27]. However, owing to limited trafficking of CAR T cells into tumours (see below), whether the number of CAR T cells in the blood correlates with the number present in the tumour remains unclear.

In terms of clinical responses, antitumour activity has generally been limited[8,10,20], albeit with a few exceptions. Interesting responses at the case report level have been described using multiple doses of an IL-13Ra-targeted CAR in a patient with glioblastoma[28] and using GD2-specific CAR T cells in four paediatric patients with pontine or midline glioma[29]. More encouraging results have been provided by two reports from studies involving larger numbers of patients, which indicate clear antitumour effects (similar in magnitude to those reported with CD19-targeted CAR T cells in patients with lymphoma) using a claudin18.2-targeted CAR in patients with gastrointestinal tumours[30] and a GD2-specific CAR in patients with neuroblastoma[31].

Unfortunately, our understanding of why the vast majority of trials testing CAR T cells have only shown minimal clinical activity in patients with solid tumours is limited owing to the scarcity of data assessing CAR T cell trafficking using serial imaging and/or analysis of post-infusion biopsy samples. Furthermore, even fewer studies have evaluated the functional activity of CAR T cells isolated from tumours after infusion. We are thus lacking answers to basic questions as to, for example: (1) how many CAR T cells get into the tumours; (2) whether they proliferate and/or persist once they arrive; and (3) how long they are able to remain functional. Without answers to these key questions, it is difficult to know where and how to make the necessary improvements.

Animal models: between a rock and a hard place

Owing to the lack of clinical data, the bulk of our knowledge of the in vivo activity of CAR T cells is primarily derived from two types of mouse models. The majority of published studies involve the administration of human-derived CAR T cells injected into immunodeficient (usually Nod-SCID *Il2Rγ*-knockout (NSG)) mice bearing tumours derived from human cancer cell lines. The other, less widely used approach is to inject mouse-derived CAR T cells (often after lymphodepletion) into immunocompetent mice bearing syngeneic tumours (mostly in subcutaneous locations). To quote the British statistician, George E. P. Box: "all models are wrong, but some are useful"[32]. This statement is certainly applicable to these preclinical models (Table 1). The advantages and disadvantages of such models are summarized in greater detail in an excellent review published in 2022 (ref. 33).

The general feeling among researchers in the field has been that the model in which human T cells are injected into NSG mice is the most informative owing to the many differences between mouse and human T cells (Table 1), and that preclinical studies using the same human CAR T cells as used in a clinical trial would be more relevant. However, a syngeneic model testing mouse-derived CAR T cells

Box 1

Key characteristics of a CAR T cell for use in patients with solid tumours

If one were to describe an ideal chimeric antigen receptor (CAR) T cell with the best chance of being successful in the treatment of patients with solid tumours, it would include the following characteristics.

After cancer diagnosis

The CAR construct chosen would preferably target an antigen expressed at high levels and exclusively by all the tumour cells. If this pattern of expression is not possible, the CAR should target an antigen that is overexpressed by all tumour cells but have selective activity for cells that express high levels of the target antigen to avoid activity against non-malignant cells that express low levels of the antigen, thus avoiding on-target off-tumour effects. An ideal CAR would target more than one tumour antigen in order to avoid resistance that arises from antigen escape. The target would also ideally have an oncogenic role in order to reduce the risk of immune-edited resistance variants emerging. The CAR construct signalling domains should ensure the persistence of active CAR T cells and not cause tonic signalling that would be likely to lead to rapid exhaustion.

Before injection into the patient

The successful CAR T cell would need to be successfully manufactured. Although new modifications are being evaluated, under most current protocols this involves surviving apheresis, activation, genetic modification, freezing and storage without undergoing necrosis, activation-induced cell death, fratricide or becoming contaminated. Large numbers of highly functional CAR T cells would need to be generated from limited numbers of autologous T cells within a rapid turnaround time.

After injection into the patient

After intravenous injection, successful CAR T cells must first avoid killing vascular cells, especially in the lungs, so that immediate toxicity does not occur[22]. The injected cells must overcome the natural tendency to home to secondary lymphoid organs and bone marrow, and instead travel, arrest and transmigrate through tumour blood vessels.

After reaching the tumour

CAR T cells that are able to bind with and transmigrate through tumour blood vessels must then migrate through the tumour stroma, overcoming substantial physical barriers (including perivascular cells and extracellular matrix). CAR T cells must also be able to survive within a harsh and immunosuppressive tumour microenvironment and maintain their cytolytic capacity.

The preferred candidate must then be able to directly interact with and kill tumour cells, ideally repeatedly. This objective will require the CAR T cells to overcome unfavourable chemokine gradients, limited synapse formation owing to low levels of tumour cell ICAM1 expression, and any mechanisms of intrinsic tumour cell resistance. In addition to targeting tumour cells expressing the cognate antigen, the CAR T cell would ideally also induce a bystander effect by secreting cytotoxic factors or stimulating the endogenous immune system to kill any tumour cells that do not express the antigen.

Finally, the CAR T cell must proliferate within the tumour in order to produce additional effector CAR T cells and ideally also persist systemically at low levels for an indefinite period of time in order to maintain tumour surveillance. Considering the many challenges that a CAR T cell would need to overcome, the lack of success seen thus far is perhaps not surprising.

provides the best method of taking into account the extremely important effects of an intact tumour microenvironment (TME), the endogenous immune system, mismatches between mouse and human cytokines, growth factors and adhesion molecules (for example, mouse and human IFNs are species-specific), and the effects of the non-malignant bone marrow and secondary lymphoid organs on CAR T cell function. As examples, studying the ability of CAR T cells to induce an endogenous immune bystander effect[34], activation of TME[35] or the effect of a CAR construct capable of secreting IL-18 (ref. 36) were only possible using immunocompetent models.

A potential solution to some of these issues could be the increased use of humanized mice[37], that is, immunodeficient mice that have been reconstituted with a human immune system using $CD34^+$ stem cells or fetal tissues. However, these models remain challenging for a number of reasons, including incomplete reconstitution, high costs, the potential for spontaneous rejection of injected human tumour cells, xenoreactivity and difficulties in ensuring that the CAR T cells, bone marrow cells and cancer cells are all HLA-matched, as they would be in a patient. In addition, the non-haematopoietic cells are fundamentally still of mouse origin, which can lead to all of the issues described in the previous paragraph.

Lastly, the quantity of cells tested in mouse models of solid tumours, which typically ranges from 10^6 to 10^7 CAR T cells, is another crucial aspect. Direct extrapolation would translate to administering 10^{10}–10^{11} CAR T cells to patients, which substantially exceeds the numbers used in clinical trials[38], would probably make manufacturing challenging, and would possibly have toxic effects. This disparity in cell quantities makes translating promising preclinical findings to clinical settings difficult, and might contribute to the limited success of CAR T cell therapies in patients with solid tumours.

Understanding the reasons for failure of CAR T cells in solid tumours

Trafficking

Results from animal models. Two fundamental questions in CAR T cell research and development that need to be answered are where the T cells go after intravenous injection, and, specifically, how effective they are at getting into tumours. These questions

Table 1 | Comparison of preclinical models for testing CAR T cells

Feature	Human T cells in immunodeficient mice	Mouse T cells in syngeneic mice
T cell characteristics	The T cells can be allowed to become less activated before injection because they can be maintained longer in culture; this approach results in less AICD during expansion and longer persistence	Requires highly activated T cells with limited persistence; the T cells undergo higher levels of AICD during expansion
Expansion	Activated and rested T cells can proliferate to a greater degree	Activated T cells have only modest expansion capacity
Storage	Relatively easy to manufacture and freeze.	More difficult to manufacture the T cells and cannot be frozen in a way that retains function
Lymphodepletion	Mice are already maximally lymphodepleted, which falsely enhances the extent of engraftment (although human homeostatic cytokines are not present)	Lymphodepletion is required in order to mimic the clinical situation; this can have direct effects on tumours
TME features	Abnormal TME: NSG mice have no T_{reg} cells and only rudimentary myeloid cells, including very few DCs	Has a fully intact TME, as well as MDSCs, DCs and T_{reg} cells
Lymphatic system	Only residual lymph nodes and small spleens	Intact lymph nodes and spleens
	Abnormal 'empty' bone marrow	Intact bone marrow
Host immunological characteristics	Lack of physiological growth factors and cytokines	Growth factors and cytokines that reflect mouse physiology
	Many mouse cytokines and growth factors do not crossreact with human T cells and vice versa (for example, human IFNγ will not stimulate mouse TME cells); a lack of T cell growth factors could limit the extent of T cell expansion	Full match between endogenous growth factors, cytokines and T cells
	No endogenous immune system to activate	Enables the effects of CAR T cells on the endogenous immune system to be studied
	GVHD eventually develops, thus limiting the time window for observation	No GVHD

AICD, activation-induced cell death; CAR, chimeric antigen receptor; DCs, dendritic cells; GVHD, graft versus host disease; MDSCs, myeloid-derived suppressor cells; NSG, Nod-SCID Il2Rγ-knockout; TME, tumour microenvironment; T_{reg}, $CD4^+$ regulatory T cells.

can be addressed using two techniques: tumour biopsy sampling and imaging.

Tumour biopsy sampling followed by immunohistochemical, molecular or flow cytometry analyses provides information at the highest possible level of resolution and has been employed extensively in preclinical studies in which biopsy sampling can be performed over time and in replicate animals. However, the availability of investigative biopsy samples obtained from patients with solid tumours is much more restricted owing to the associated risks, discomfort and costs for the patient. Additional limitations include being restricted to a single time point per patient, necrosis (especially in post-treatment samples), and the limited amount of material obtained through fine-needle sampling methods (which can lead to sampling bias). A solution to these limitations could be CAR T cell imaging.

The topic of CAR T cell imaging, typically using PET or single-photon emission CT (SPECT) imaging, has been reviewed in detail elsewhere[39–42]. Direct radiolabelling of the CAR T cells enables highly sensitive detection but usually involves isotopes with relatively short half-lives (<3 days), enabling only short-term data collection. Genetic alteration of the T cells with genes encoding luciferases or other enzymes, such as herpes simplex thymidine kinase[43], the iodine symporter[44], prostate-specific membrane antigen (PSMA)[45] or dihydrofolate reductase[46], to enable optical, SPECT or PET imaging is generally less sensitive and might induce an immune response, but has the advantage of enabling serial imaging and data collection over longer periods of time.

A number of preclinical studies have investigated the distribution of human T cells encoding one or more genes that enable detection using imaging in NSG mice after intravenous injection, using both biopsy sampling and CAR T cell imaging[40]. The results are generally consistent and demonstrate that after intravenous injection, human T cells first accumulate in the lungs with peak retention at 2–4 h after injection. T cells then localize to the liver and spleen and, to a lesser extent, to the bone marrow and lymph nodes at 12–24 h after injection. Initial tumour-specific uptake is usually very limited, although the numbers of CAR T cells present within tumours gradually increases over time with more intratumoural CAR T cells being visualized several weeks after injection[47]. This accumulation presumably reflects intratumoural proliferation, although this effect has not been investigated in detail.

Fewer studies have investigated the accumulation of adoptively transferred mouse CAR T cells. However, similar patterns of initial uptake have been found in the lungs, then the liver, spleen, and secondary lymph nodes and bone marrow[47–49]. Compared with human CAR T cells, intratumoural accumulation tends to occur slightly earlier. However, unlike human CAR T cells, the number of transferred mouse T cells peaks at ~7–10 days after injection and then the cells disappear[48]. The trafficking and persistence of mouse T cells can be increased to some extent with use of lymphodepletion prior to injection[35,49]; however, the intratumoural proliferation and increases in CAR T cell numbers seen over time with human T cells generally do not occur[50].

In summary, preclinical data from both types of models demonstrate that very few of the injected CAR T cells initially enter tumours. Mouse T cells injected into syngeneic mice persist for only a few weeks. However, human CAR T cells injected into immunodeficient mice seem to proliferate over a period of weeks resulting in intratumour accumulation.

Results from human clinical trials. What happens to CAR T cells injected into patients with solid tumours? Some limited data are available from patients injected with TILs expanded ex vivo that were labelled with ^{111}In-oxine (half-life 2.8 days), predominantly conducted by the National Cancer Institute Surgical Branch in the 1980s and 1990s[51–55].

One other report describes the localization of CAR T cells injected into patients with ovarian cancer[56]. Similar to the patterns seen in both types of mouse model, these human data suggest an initial accumulation in the lungs and secondary lymphoid organs, followed by very inefficient trafficking to tumours over the following 24–48 h. A study published in June 2023 described the retention of ^{111}In-labelled CAR T cells after intratumoural injection for 48 h with no cells detected systemically in patients with head and neck squamous cell carcinoma[57].

Imaging studies using genetic labelling techniques (such as the genetic tag HSVtk[42]) are now starting to be conducted, although these have not yet been validated or approved for clinical use. Obviously, the ability to monitor CAR T cell localization using imaging is an important unmet need that will yield crucial information about the fate of the CAR T cells over extended time periods.

Why is tumour trafficking so limited? This topic has been discussed in detail in several reviews[17,58–63]. In brief, trafficking into tumours first requires that circulating T cells recognize the chemokines secreted by, and bound to, the surfaces of endothelial cells (which are primarily in the tumour stroma and not cancer cell-rich areas). This initial endothelial recognition is followed by rolling adhesion mediated by selectins and then firm adhesion by integrins. Driven by chemokines (especially CXCL9, CXCL10 and CXCL11, and CCL5), T cells then transmigrate into the tumour stroma (controlled by T cell CCRs such as CXCR3 and CCR5). Here, perivascular cells, extracellular matrix proteins and mesenchymal stromal cells (primarily fibroblasts) provide barriers. Some T cells migrate through the stroma and an even smaller number eventually move into cancer cell-rich areas, guided by tumour-derived chemokines, where they can kill tumour cells, a process requiring binding to ICAM1 on tumour cells[64,65]. This process is extremely inefficient, resulting in very few T cells successfully interacting with tumour cells. Many suggested reasons for this lack of efficiency exist including chemokine–CCR mismatches, deficits in adhesion receptors and the extracellular matrix acting as a barrier (Fig. 1).

An additional important, but under-appreciated issue is probably 'misdirection' of CAR T cells towards lymphoid tissues and away from solid tumours. CAR T cell manufacturing has thus far primarily been guided by data generated from trials testing CD19-targeted products in patients with B cell leukaemia or lymphoma that emphasize the importance of lymph node and/or bone marrow trafficking, expansion and persistence of antitumour activity. T cells with high levels of CCR7 and CD62L expression are known to preferentially traffic to lymph nodes or bone marrow[58,66,67]. Thus, the goal of most current protocols is to produce CAR T cells predominantly of a central memory cell ($CD62L^{high}$ and $CCR7^{high}CD45RO^{+}$) phenotype as opposed to an effector memory cell ($CD62L^{low}$ and $CCR7^{low}CD45RO^{+}$) phenotype (Fig. 2), which generally does not favour trafficking to tumours. Approaches designed to address CAR T cell misdirection are discussed in detail below.

Two additional caveats should be considered. First, cryopreservation might affect CD62L expression and thus affect trafficking: CD62L expression has been reported to decrease somewhat after thawing of cells[68]. Second, CD62L has been suggested to promote antitumour activity through other mechanisms, particularly in the context of T cell receptor (TCR)-directed adoptive T cell transfer[69]. Specifically, CD62L might guide the trafficking of T cells towards high endothelial cell venules within the tertiary lymphoid structures present in some solid tumours[58,70,71]. These lymphoid structures could potentially facilitate T cell infiltration and enable improved antitumour immune responses.

Persistence

In terms of CAR T cell persistence in clinical trials involving patients with solid tumours, almost every trial conducted thus far has assessed the presence of CAR T cells only in blood samples, using quantitative PCR and/or flow cytometry. Data from these studies are quite congruent. In almost every solid tumour trial, the numbers of CAR T cell DNA transcripts in the blood range from 10^3 to 10^4 copies per microgram DNA[24–26] and CAR T cells are only detectable for about a month after infusion, with a peak typically seen at 10–14 days. By contrast, most successful trials testing CD19-targeted CAR T cells in patients with leukaemia have found high numbers of CAR T cells in the blood, typically 10^5–10^6 copies per microgram DNA, often with persistence ranging from months to years[1]. An interesting exception to this general observation is provided by the successful trial involving paediatric patients with neuroblastoma, in whom very high levels of circulating GD2-targeted CAR T cells in the blood were noted (peak mean values of ~2×10^5 copies per microgram DNA, with persistence for up to 2 years)[31]. Limited data suggest that circulating CAR T cell levels in patients with solid tumours can be increased somewhat using lymphodepletion[25,27].

In contrast to the analysis of blood samples, only a few studies have investigated the presence and/or persistence of CAR T cells within tumour tissues. The limited available data were obtained from tumour biopsy samples (as opposed to imaging), although these investigations are limited by the lack of consistent biopsy sampling, variability in both the timing and location of sampling, the small size of the samples, and difficulties in obtaining repeat biopsy samples from the same patient. As examples, data are plotted from several clinical trials involving patients with solid tumours in which biopsy samples were obtained from at least some of the patients after the intravenous administration of: (1) mesothelin-targeted CAR T cells to patients with mesothelioma, pancreatic or ovarian cancer[25]; (2) EGFRvIII CAR T cells to patients with glioblastoma[24]; (3) PSMA-targeted CAR T cells armoured with a dominant-negative *TGFβR2* allele to patients with prostate cancer[26]; or (4) CD19-targeted CAR T cells to patients with lymphoma[72] (Fig. 3). Similar biopsy results were published in March 2023 from another trial in which patients with lymphoma received CD19-targeted CAR T cells[73]. In the first three trials, CAR T cells were detected using quantitative PCR to calculate the number of copies per microgram DNA. In the trial testing CD19-targeted CAR T cells, in situ hybridization (ISH) was used to determine the number of CAR T cells present in the biopsy samples. Despite the limitations of these data, and the many differences between the various studies, results are similar and show that few intratumoural CAR T cells are consistently identified and that these often have limited persistence. The numbers of CAR T cells tend to be highest at early time points (7–14 days) after intravenous administration. Although CAR T cells were not directly identified, a report from the ZUMA-1 study (testing the CD19-targeted CAR T cell axicabtagene ciloleucel in patients with lymphoma) that examined baseline tumour biopsy samples showed that responses are more common in tumours that are more inflamed at baseline[74].

In summary, the results of both preclinical and clinical studies show very inefficient trafficking of CAR T cells to tumours after intravenous injection, and most of this trafficking is likely to occur (although not well studied) soon after injection. The limited data available from human studies (Fig. 3) suggest that the few CAR T cells that enter solid tumours have limited persistence and do not proliferate extensively. In this important aspect, the available clinical data more closely resemble that from immunocompetent mouse models exposed to mouse-derived CAR T cells

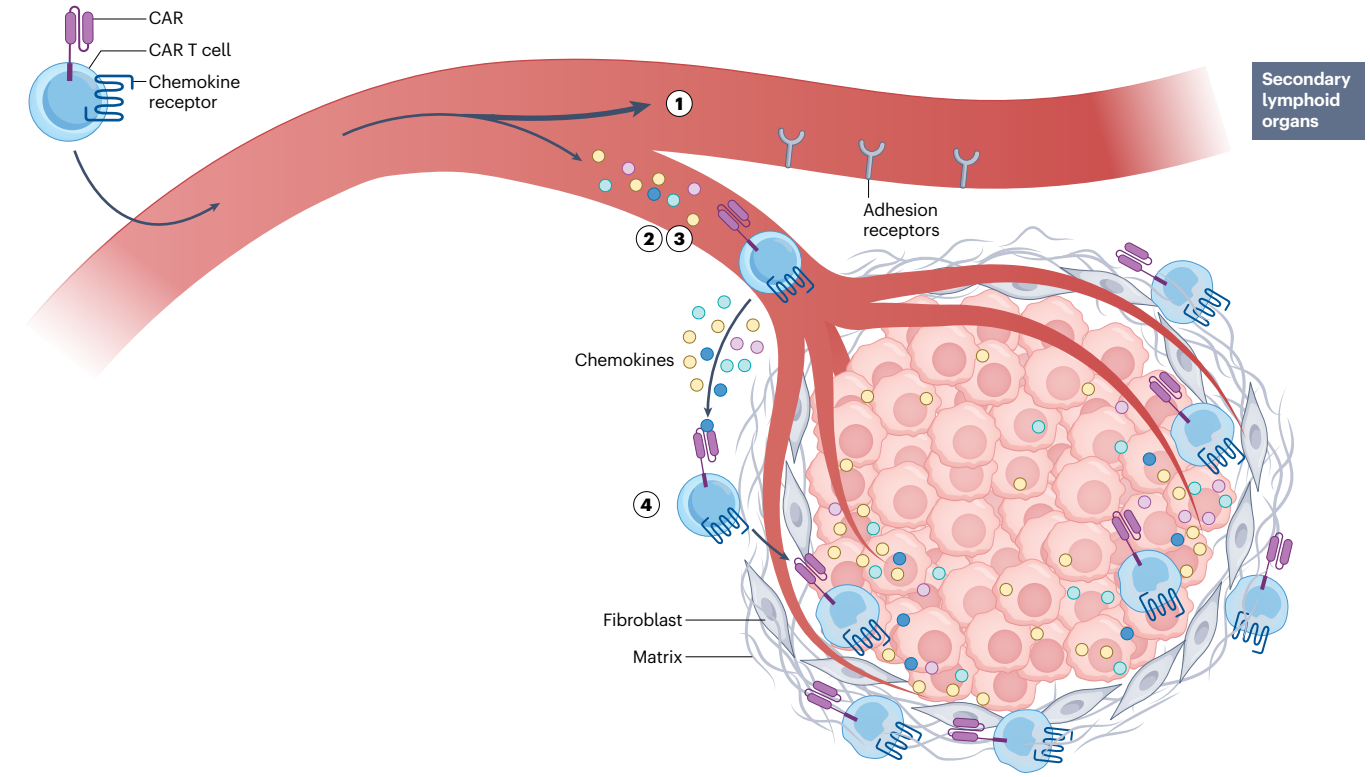

Fig. 1 | Targeting barriers to the activity of CAR T cells. Schematic diagram of the potential barriers to targeting solid tumours using chimeric antigen receptor (CAR) T cells. After intravenous injection, these barriers include: (1) misdirection of the T cells towards lymphoid tissues and away from tumours; (2) a lack of expression of adhesion receptors (selectin ligands and ICAM1) on tumour blood vessels; (3) mismatch between the chemokine receptors expressed on CAR T cells and the chemokines produced by solid tumours; and (4) extracellular matrix barriers and stromal cells (such as fibroblasts).

as opposed to immunodeficient mice exposed to human-derived CAR T cells.

Functionality of intratumoural CAR T cells

A consistent finding from preclinical studies investigating human-derived CAR T cells in NSG mice is that their cytotoxic activity is initially high, but becomes progressively reduced over time[18,47,75–78]. This loss of activity also appears to occur with intratumoural mouse CAR T cells[48], although these have been less extensively studied given that their persistence is generally shorter. This acquired and also reversible loss of CAR T cell activity over time has been reproduced in vitro simply by repetitively stimulating human CAR T cells with antigen-expressing tumour cells[79]. Both genomic and epigenomic changes have been associated with this hypofunctional state[18,77,80,81]. However, many of the epigenetic changes are reversible when T cell stimulation is removed.

The reasons for the induction of this progressive hypofunctional state are not completely understood. The multiple proposed factors present within the TME have been described in detail elsewhere[16–18,77,82], and include low pH, hypoxia, nutrient deprivation owing to low levels of key amino acids and glucose, high levels of reactive oxygen species (ROS), the presence of immunosuppressive mediators (such as TGFβ, PGE2, adenosine and IL-10), and inhibitory intercellular interactions with myeloid-derived suppressor cells and $CD4^+$ regulatory T cells. Postulated T cell intrinsic factors include 'regulatory shutdown' mediated by immune checkpoints (such as PD-1, CTLA4, TIM3, TIGIT and LAG3) and inhibitory intracellular signalling pathways (such as DGK, NR4A, SHP1 and cbl-b). A variety of epigenetic changes have also been implicated[76,79]. Furthermore, CAR-specific issues, such as the extent of tonic signalling, can also lead to hypofunction[76].

Unfortunately, only limited data assessing the phenotypes and/or functional capacities of injected human CAR T cells within solid tumours in clinical trials are available. Flow cytometry data on CAR T cells present in blood samples obtained on day 14 from three patients in a trial testing receptor tyrosine kinase-like orphan receptor 1 (ROR1)-targeted CAR T cells indicate upregulation of inhibitory receptors and a marked reduction in cytokine production after CD3–CD28 stimulation[35]. Elsewhere, the immunohistochemical phenotypes of a limited number of CAR T cells visualized within lymphoma biopsies obtained at about 10 days after injection was investigated[71] (Fig. 3d). These authors report that most of the CAR T cells detected at this early time point have phenotypic evidence of either ongoing or prior activation. Similarly, in a report from a first-in-human study testing EGFRvIII-targeted CAR T cells in patients with glioblastoma, the authors state: "In all four subjects who had their tumors resected within 2 weeks of CART-EGFRvIII infusion, we detected CAR^+ cells by RNAscope ISH. These T cells were composed of a mixture of $CD8^+$ and $CD8^-$ T cells, and many had an activated phenotype"[24]. However, activation data were not provided. I am unaware of any studies that isolated and directly measured the functional activity of CAR-expressing TILs from solid tumour biopsy samples, which is not surprising given their low numbers.

Nevertheless, given the available preclinical data on CAR T cell function and the extensive literature describing the loss of cytolytic function of endogenous TILs in patients with cancer[77], intratumoural CAR T cells in patients would also seem likely to become hypofunctional within days to weeks of infiltrating a tumour. Additional clinical data regarding intratumoural CAR T cell function would greatly aid the field.

In summary, the fundamental objective of T cell-based cancer therapy is to supply a sufficient quantity of polyfunctional CAR T cells that are capable of continuously targeting and eliminating tumour cells over time (functional persistence). Current evidence suggests that the key problem with CAR T cell therapy in patients with solid tumours is that too few of the administered cells can infiltrate the tumours and remain functional (Fig. 4). In preclinical models testing human CAR T cells, many cells are highly functional at early time points, although very few of these cells are located within the tumour (Fig. 4a). As the number of intratumoural CAR T cells increases, they become progressively hypofunctional leading to only a short window of time in which adequate numbers of sufficiently functional CAR T cells are available (Fig. 4a). This situation is similar with mouse-derived CAR T cells in preclinical models (Fig. 4b) and is exacerbated by the relative lack of persistence compared with human-derived cells.

Tumour heterogeneity and antigen spreading

Unlike B cell malignancies or multiple myeloma (which have almost uniformly high levels of CD19 or BCMA expression, respectively), antigen expression in solid tumours is almost always lower and more heterogeneous. Thus, unless some sort of bystander or antigen spreading effect is induced by the CAR T cells or multiple antigens are targeted, the likelihood of successful treatment is low[83,84]. Although initially postulated as a potential feature of CAR T cells[2], a general consensus is emerging that CAR T cells alone do not engender substantial bystander effects[85,86]. For example, in a preclinical study using a syngeneic mouse model, mesothelin-targeted CAR T cells were able to entirely eradicate tumours comprising 100% mesothelin-expressing cells, although when the tumour contained only 10% mesothelin-negative cells, only a temporary slowing of tumour growth ocurred[34]. Thus, the fact that the eligibility criteria for most trials testing CAR T cells in patients with solid tumours did not stipulate that a high percentage of tumour cells had to express the target antigen is of some concern: sometimes the level of target expression was not even evaluated. In addition to baseline tumour heterogeneity, data from trials testing CD19-targeted CAR T cells in B cell malignancies[87,88], and even from one trial involving patients with solid tumours[24], indicate that highly active CAR T cells are able to exert selection pressures that enable disease relapse owing to the outgrowth of antigen-negative (or antigen-mutated) tumour cells.

Lessons we can learn and unlearn from adoptive T cell transfer

Proof-of-concept has been established that adoptive T cell transfer can be highly effective in certain situations. As described previously, CD19-targeted CAR T cells can cure certain B cell leukaemias and lymphomas, and BCMA-targeted CAR T cells can produce durable remissions in patients R/R multiple myeloma[8]. Patients with certain solid tumours can now be successfully treated using adoptive T cell transfer, with the most prominent successes being with TIL therapy in those with melanoma[6,7,89]. Promising results have also been achieved using TILs in patients with non-small-cell lung cancer[90]. A phase I trial testing TCR-engineered T cells targeting E7 in patients with HPV-associated epithelial tumours has also provided promising evidence of efficacy[91]. What can we learn or unlearn from these successes to achieve more success with CAR T cells in solid tumours?

T cells optimized for lymph node trafficking might not be optimal for solid tumours

As discussed previously, virtually all CAR T cell manufacturing techniques have the goal of producing naive-like or central memory-like cells (Fig. 2) that will preferentially migrate to the lymph nodes and bone marrow[66]. This strategy has a strong rationale when applied to adoptively transferred TILs or TCR-transduced T cells because the bone marrow and lymph nodes are the natural environment in which dendritic cells (DCs) expressing T cell cognate peptide antigens can optimally present antigens, activate T cells and induce expansion into effector cells[92–94]. This approach also seems reasonable for CAR T cells targeting haematological cancers (or other cancers with a predilection for accumulation

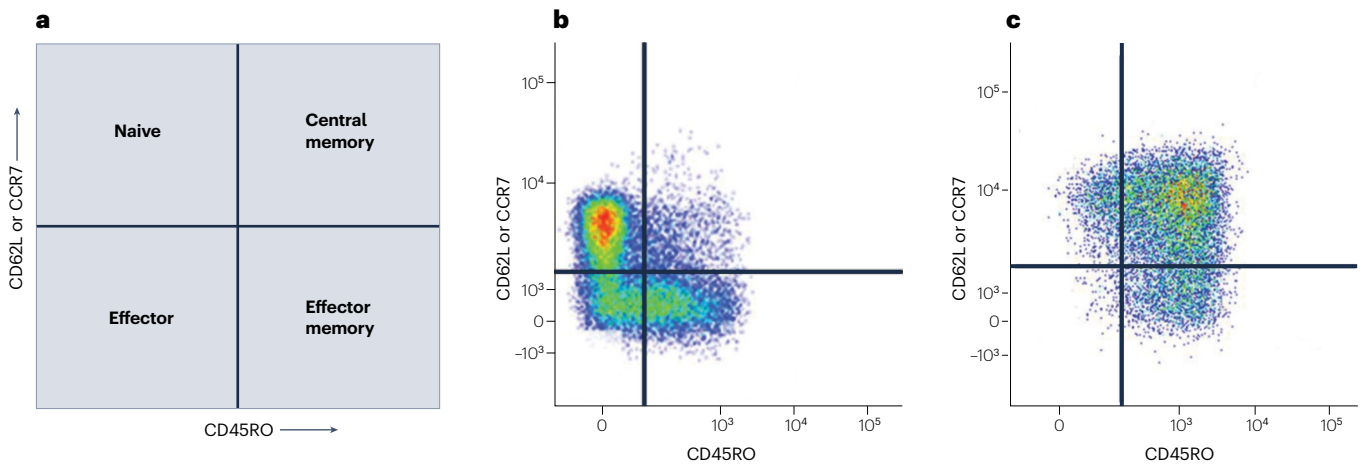

Fig. 2 | Injected CAR T cells are highly enriched in central memory T cells compared to circulating T cells. The differentiation status of T cells is defined by expression of the lymph node homing receptors CD62L or CCR7 and CD45RO, which designate previous antigen experience (memory cells) (panel **a**). Peripheral blood T cells (panel **b**) are primarily naive or effector cells with a small proportion of effector memory cells. By contrast, CAR T cells used for injection (panel **c**) are primarily central memory cells.

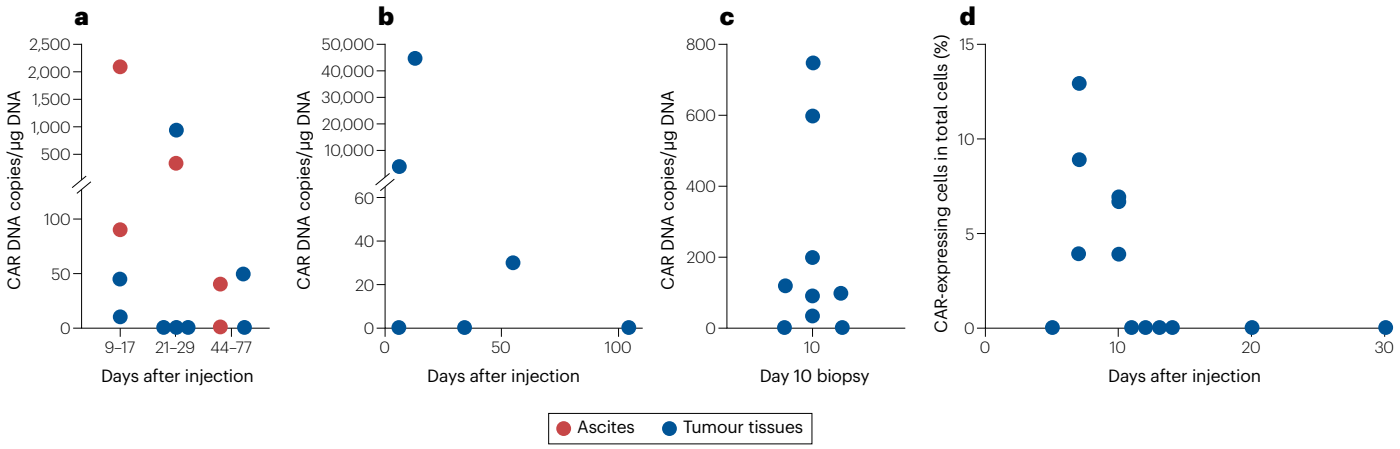

Fig. 3 | CAR T cell persistence in selected trials. The figure shows the presence of chimeric antigen receptor (CAR) T cells within solid tumours replotted using data from four clinical trials in which biopsy samples were obtained from at least some patients after intravenous administration of: mesothelin-targeted CAR T cells to patients with mesothelioma, pancreatic or ovarian cancers (panel **a**)[25]; EGFRvIII-targeted CAR T cells to patients with glioblastoma (panel **b**)[24]; PSMA-targeted CAR T cells armoured with a dominant-negative *TGFβR2* allele to patients with prostate cancer (panel **c**)[26]; and CD19-targeted CAR T cells to patients with lymphoma (panel **d**)[72]. Compared to the experience with CAR T cells in patients with B cell leukaemia, relatively low numbers of intratumoural CAR T cells were identified, and persistence was limited.

in bone marrow, such as neuroblastoma) as the presence of high numbers of CD19-positive tumour cells or BCMA-expressing myeloma cells often seen in lymph nodes and/or bone marrow appears to support the activation and proliferation of CD19 or BCMA-targeted CAR T cells. In this regard, the observation that the CD19-targeted CAR T cell products with the highest clinical efficacy in patients with chronic lymphocytic leukaemia or B cell lymphoma are those with the highest proportion of naive-like and central memory ($CCR7^+$) cells is not surprising[95–97].

In contrast to effector or effector memory cells, which preferentially traffic to peripheral sites, the high expression of CD62L and CCR7 on naive or central memory T cells enables preferential trafficking to secondary lymph nodes and bone marrow[66,67]. Cells of these phenotypes might be suboptimal when used to develop CAR T cells targeting solid tumours. Solid tumour-directed CAR T cells are designed to target intact antigens that are expressed on the surfaces of solid tumour cells. Accordingly, when such cells enter the bone marrow or lymph nodes, they will not encounter their antigen and will therefore not be activated to proliferate or differentiate into effector cells unless the tumour has metastasized to those locations. Support for a potential benefit of effector-like cells has been provided by data showing that effector-like CAR T cells are more effective than memory-like T cells in solid tumour models[98,99]. Thus, generating CAR T cells using standard protocols that are designed to optimize lymph node trafficking might be a key lesson to unlearn for solid tumours.

DC interactions are likely to be important

An important difference between TILs (or TCR-transduced T cells) and CAR T cells is that the former can be extensively activated by DCs, which can provide optimal co-stimulatory signals. This co-stimulation can occur in lymph nodes and in bone marrow, although evidence also exists supporting an important role of activated DCs within tumours[100]. Finding ways to harness the power of DC activation (to enhance the ability of CAR T cells to proliferate and persist) is a lesson that we should attempt to learn from trials involving TILs.

Long-term persistence might not be needed

A general consensus exists that the successes of CAR T cells in patients with certain haematological cancers are closely linked with CAR T cell persistence[19,101,102]. Some data from trials testing TIL infusions indicate that T cell persistence is also associated with response[103–105]; however, the importance of persistence has not yet been established in clinical trials testing CAR T cells in patients with solid tumours, for a number of reasons. Firstly, given the lack of clinical success in patients with solid tumours, along with uniformly short persistence, establishing correlations between CAR T cell persistence and efficacy, as has been done in trials involving TILs[105] and with CAR T cells in leukaemias and lymphomas[1,96,106,107], has not been feasible. Secondly, the relationship between persistence in blood and persistence in solid tumours has not been validated[45]. Thirdly, and perhaps most importantly, data from solid tumour models indicate that CAR T cell persistence is associated with increasing levels of T cell hypofunction (Fig. 4).

The conventional approach to this conundrum has been to continue with the haematological cancer paradigm but also develop ways to modify the CAR T cells to make them more persistent and less susceptible to hypofunction (Fig. 5). Two trials with results published in the past few years have shown some encouraging success in patients with solid tumours using this approach[30,31]. However, another approach to consider would be to unlearn the lesson of persistence and instead administer fresh CAR T cells using a multiple-dosing strategy. As injected CAR T cells become exhausted over time, they could be replaced by a new dose of highly functional CAR T cells. Given the limited persistence and rapid loss of function seen with traditional lentivirally engineered CAR T cells in patients with solid tumours, this approach could potentially enable larger numbers of functional CAR T cells to accumulate over extended time periods and might avoid the need for repeat lymphodepletion (Fig. 5). Of course, reductions in the immunogenicity of the CAR components would be necessary in order to avoid rejection of cells that are administered multiple times[23].

Targeting multiple tumour antigens is important

In addition to differences in their mechanisms of activation, another important difference between TILs and CAR T cells is that the former are polyclonal in nature and thus probably target more than one antigen. Data from a study published in 2022 indicate a correlation between the number of neoepitopes recognized by the infused product and response in patients with melanoma[108]. This is a lesson that should be learned. Any successful CAR T cell therapy for patients with solid tumours will need to target multiple tumour-specific targets, optimally trigger some sort of bystander effect and/or induce antigen spreading to the endogenous immune system. For example, recruitment of DCs by CAR T cells engineered to secrete FLT3 ligand, along with DC activation using 4-1BB agonism, promoted DC recruitment and activation and led to enhanced endogenous T cell responses and antitumour activity in mouse models[85].

How does the field best move forward?

Given these challenges and the lessons to be potentially learned and unlearned, I propose that researchers in the field consider moving forward by optimizing two different strategies to achieve greater success in patients with solid tumours (Fig. 5). Strategy 1, the current direction of the field, is based on the conventional 'memory cell' paradigm developed for use in patients with haematological malignancies. Strategy 2 is more speculative, and involves a somewhat less-conventional approach employing a different 'short-lived effector cell' paradigm.

Strategy 1: The memory cell paradigm

The goals of this approach are to maximize CAR T cell trafficking and persistence while minimizing the extent of CAR T cell hypofunction. This objective has been achieved in patients with haematological cancers by administering CAR T cells with a maximal ability to proliferate and persist (those with a naive, stem or memory phenotype) with the hope of long-term engraftment. Lymphodepletion with high-dose chemotherapy is used to enhance engraftment as part of the strategy. CAR T cell persistence and prevention of hypofunction have been addressed by introducing specific genetic alterations to the T cells (by either adding additional genes or using genome editing techniques (usually CRISPR) to suppress possible inhibitory factors), allowing T cells to 'rest' and thus prevent chronic stimulation, or altering the TME directly or by using armoured CAR T cells capable of secreting cytokines and/or other proteins[18].

A major challenge associated with this strategy is the need to introduce multiple genetic alterations, a task that is often difficult to accomplish in primary T cells. Using lentiviral vectors, expressing one, or possibly two, additional transgenes is currently possible by linking them to the CAR using ribosome 2A self-cleaving peptides; however, the packaging capabilities of this approach are limited. Using retroviral systems (which have a larger cargo limit than lentiviruses), co-expression of up to four separate transgenes is feasible using skip peptides while also maintaining adequate titre and transgene expression (although insertional mutagenesis caused by retroviruses might be

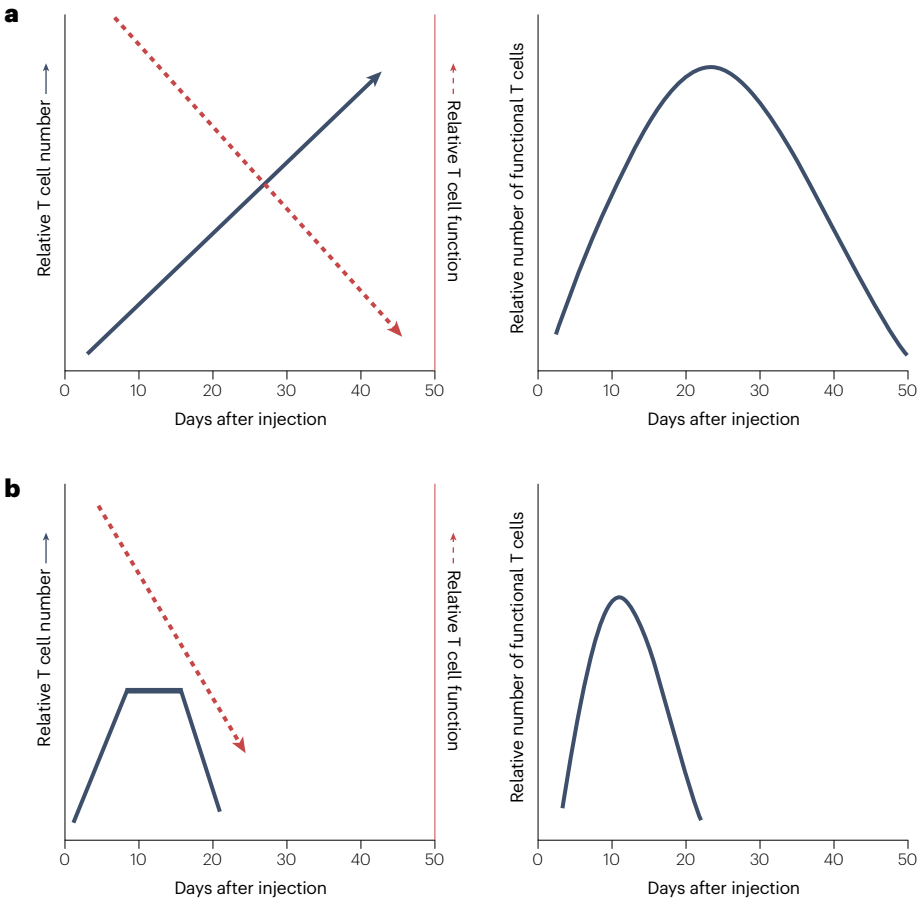

Fig. 4 | Lack of functional intratumoural CAR T cells limits antitumour activity. This schematic figure indicates the relative number of human (panel **a**) or mouse (panel **b**) chimeric antigen receptor (CAR) T cells present within tumours (left panels, black lines) and the relative functional capacity of the CAR T cells (left panels, red lines). At early time points, the CAR T cells are highly functional but very few reside within the tumour. Over time, the number of CAR T cells present within tumours increases, although they become progressively hypofunctional. This pattern results in a limited time window in which adequate numbers of functional CAR T cells are present within the tumours (right panels).

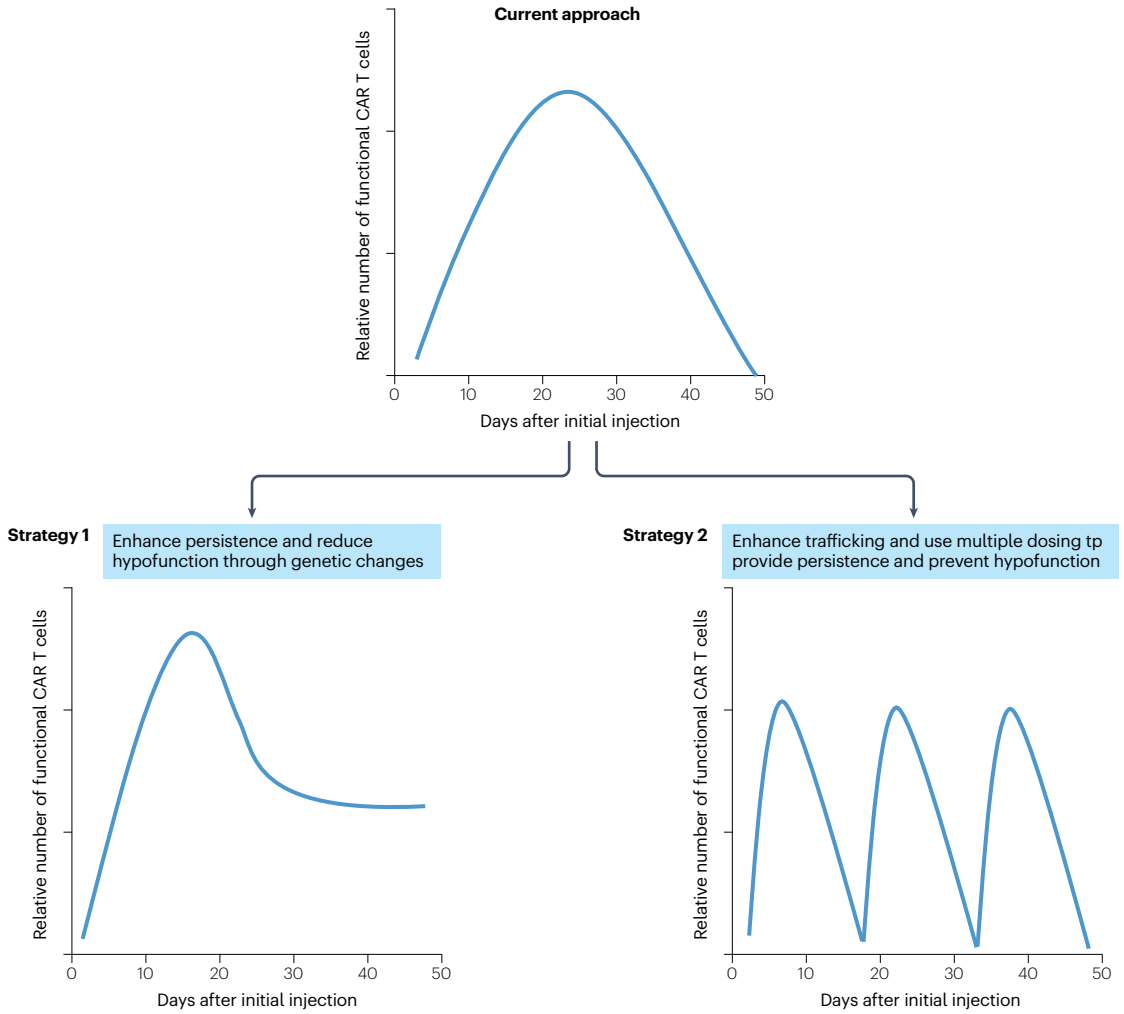

Fig. 5 | Possible strategies to improve the activity of CAR T cells in patients with solid tumours. Current approaches result in a limited time window (indicated by the area under the curve (AUC)) in which adequate numbers of functional chimeric antigen receptor (CAR) T cells are present within tumours (top panel). Strategy 1 aims to increase the AUC by increasing CAR T cell persistence and preventing hypofunction. Strategy 2 aims to increase the AUC by not focusing on persistence, but instead providing multiple doses of highly active CAR T cells to replace existing CAR T cells that have become hypofunctional.

more of an issue). T cells can be transduced with multiple lentiviruses, although because the efficiency of each vector is <100%, the final product ends up being a mixture of cells expressing variable proportions of the desired transgenes. In the past years, a number of groups have been able to knockdown one or more genes using standard CRISPR technology, although once again owing to incomplete knockdowns as more than one gene is targeted, the resulting product becomes a mixture of cell types and each knockdown increases the possibility of introducing off-target genetic alterations[109,110]. However, a number of more advanced CRISPR techniques, such as base editing or prime editing (in which DNA breaks are not induced), have been developed, and these enable highly efficient gene editing with very low off-target mutation rates[111]. In a study published in June 2023, investigators used base editing to inactivate three genes and generate universal, off-the-shelf CAR T cells[112].

The development of allogeneic T cells derived from induced pluripotent stem cells (iPSCs) could provide an alternative method of improving CAR T cell trafficking and persistence by allowing multiple genetic changes to be made[113–118]. iPSCs would first be modified using various CRISPR guides enabling the knockdown of selected genes to render the cells hypo-immunogenic (such as *B2M* and/or *TRA* or *TRB* (encoding TCRα or TCRβ, respectively)) and then with guides enabling the knockdown of selected key inhibitors of T cell function. After selecting clones with all the intended alterations (and no unintended alterations), additional genes could be added to enhance trafficking or function. After each change, cells can be sorted and/or cloned to achieve high levels of the desired genetic alterations. In the final stage, the modified iPSC product could be transduced with the CAR of choice for a particular patient's tumour and then be differentiated to the desired T cell phenotype.

Although the ultimate goal of this approach is clear, many technical challenges in using iPSCs to develop mature allogeneic CAR T cells exist, including reducing immune rejection by recipient cells and safety issues related to gene editing. The difficulties associated with differentiating iPSCs into functioning $\alpha\beta^+$ CAR T cells should not be

underestimated given that T cell maturation involves a complex differentiation pathway that includes time-sensitive activation of TCR and Notch signalling. However, progress is being made, especially with T cell-derived iPSCs[115,118,119]. Despite these hurdles, at least one clinical trial using allogeneic CD19-targeted CAR T cells derived from iPSCs to treat patients with B cell malignancies is recruiting patients (NCT04629729). Initial results from a meeting abstract suggest acceptable safety and some efficacy in heavily pretreated patients[120].

Strategy 2: The short-lived effector cell paradigm

Investigators have thus far been largely unable to generate large numbers of persistent and functional CAR T cells in patients with solid tumours. This lack of functionally persistent CAR T cells indicates a need for alternative approaches, such as those focused on enhancing initial CAR T cell trafficking and effector activity and the use of multiple dosing strategies to promote persistence and bypass hypofunction. One way this could be achieved is to administer repeat injections of traditional lentivirally transduced CAR T cells.

Although not well studied, some data supporting the superiority of more short-lived, more effector-like T cells in mouse models of solid tumours are available. In our original study of mesothelin-targeted CAR T cells[121], my group found that cells with a CD28–CD3ζ cytoplasmic domain (that were thus more effector-like) had better antitumour activity than those with a 4-1BB–CD3z cytoplasmic domain. As mentioned previously, two studies comparing more effector-like versus more memory-like CAR T cells also showed enhanced antitumour activity of effector-like cells[98,99].

Given that persistence is not a goal, strategy 2 could also potentially be implemented using mRNA-transduced CAR T cells (either ex vivo or in situ)[122–131]. This paradigm was initially tested in CAR T cells in solid tumours by my group[123,132] and others (reviewed in detail elsewhere[128]) to assess toxicity, with the rationale that if any adverse effects were noted, the CAR expressed on transduced T cells would be short-lived. After safety had been established, subsequent trials were conducted using CAR T cells engineered using lentiviral transduction methods[25], following the successful paradigm established in prior trials testing CD19-targeted CAR T cells. Interestingly, in our mesothelin mRNA–CAR trials, which did not use lymphodepletion[132], we detected CAR T cells in the blood after each injection and the clinical responses observed were superior to those seen with lentivirally engineered CAR T cells[25]. Considering the failures of 'long-lasting' CAR T cells over the past decade, this approach might be worth re-evaluating. However, fully human CAR T cells would need to be developed for future clinical testing: one patient developed a severe immune reaction against the mouse single-chain antibody segments used in our mesothelin CAR construct after multiple injections[23].

The technology used in the production of mRNA–CAR T cells has advanced considerably since the initial trials. Areas of improvement include mRNA optimization, purification and the use of lipid nanoparticles[128,133] for both ex vivo and in situ delivery. The lack of restrictions in the size of the transgenes (as is seen with viral packaging) is an obvious advantage of this approach, enabling the possibility of multiplexing. More than one mRNA can be introduced via electroporation and expressed at the same time, enabling the introduction of multiple genetic alterations[134,135]. Multiplexing is also possible using mRNA packaged in lipid nanoparticles[136].

A potential limitation of mRNA–CAR T cells prepared using this approach, however, is the logistical issue of having to administer multiple doses of CAR T cells over time versus the 'one and done' approach in strategy 1. This disadvantage could be offset by the avoidance of lymphodepletion (which is expensive and time-consuming, carries risks of infection and bleeding, and is thus not feasible in all patients). Although patients would need to return for repeat infusions, these would be analogous to the administration of chemotherapy or immune-checkpoint inhibitors, which are typically given every 4–6 weeks, sometimes for many years.

Finally, some reports from the past few years describe the in situ generation of functional CAR T cells after intravenous injections of pseudotyped lentiviruses[137] or lipid nanoparticles capable of the targeted delivery of CAR–mRNAs[138]. Development of this technology could be paradigm-changing, as it would avoid the need for the expensive and time-consuming generation of ex vivo CAR T cells, would not require lymphodepletion, and could be highly amenable to repeated dosing in a variety of medical settings.

Specific approaches

Regardless of the strategy used, the key limiting issues described above will need to be successfully addressed. Many of the solutions proposed will apply to both strategies. However, certain approaches would probably be more applicable to one or the other paradigm (Table 2).

Trafficking

An obvious way to increase the number of functional CAR T cells within solid tumours would be to improve the trafficking of highly active, freshly injected cells into tumours. Thus, enhancing CAR T cell trafficking into solid tumours should be a major goal in both strategies that could be achieved using several approaches[60–62] (Fig. 1).

Firstly, a mismatch often exists between the chemokine receptors (CCRs) expressed on the CAR T cells and the chemokines produced by solid tumours. Activated $CD8^+$ T cells (including CAR T cells) primarily express CXCR3 (which binds to the IFN-induced chemokines CXCL9, CXCL10 and CXCL11), CXCR4 (binding CXCL12 (also known as SDF)), and CCR5 (binding CCL5 (also known as RANTES)). Unfortunately, many tumours do not secrete these chemokines but instead produce myeloid cell-attracting chemokines such as CCL2 (also known as MCP1), CXCL1, CXCL2 and/or CXCL8 (also known as IL-8)[139]. This mismatch can theoretically be addressed by modifying the CAR T cells to express myeloid cell-attracting CCRs or other CCRs that are usually not present on activated T cells, such as CCR2, CCR4, CXCR2 or CX3CR1 directly[62], by upregulating CCRs[140], and by altering the TME to produce cytokines or other agents that will attract CXCR3-expressing CAR T cells[141]. As examples, my group demonstrated that expression of CCR2b on CAR T cells can augment the trafficking of mesothelin-targeted T cells leading to improved antitumour activity[142] and that administration of an oncolytic tumour-homing vaccinia virus expressing CXCL11 markedly enhances CAR T cell trafficking into tumours[143]. Other TME-activating agents that have been investigated include stimulatory RNAs[144], cytokines such as IL-12 (refs. 145,146), chemokines such as CCL19 (ref. 147), or innate immune stimulants such as poly-IC[148], the bacterial protein flagellin[149] and oxaliplatin[35].

Secondly, deficits relating to adhesion receptors might impair CAR T cell infiltration into solid tumours. Tumour blood vessels are often both leaky and dysfunctional, and are known to under-express selectins[59], which are key mediators of T cell rolling and ultimately migration into tumours. Expression of the key β2 integrin ligand, ICAM1, can be downregulated on tumour vessels in some cancers[58,64]. These deficits have not been targeted extensively, although one promising approach involves altering T cell fucosylation status using the enzyme FUT7 to form the tetrasaccharide sialyl-Lewis X (sLeX)

Table 2 | Approaches to augment CAR T cell efficacy in patients with solid tumours

Problem	Potentially useful in both	Strategy 1 (conventional 'memory cell paradigm')	Strategy 2 (non-conventional 'effector cell paradigm')
Trafficking	Introduce chemokine or adhesion receptors Engineer T cells to express enzymes capable of digesting the TME Reduce the numbers of fibroblasts or matrix Alter T cell adhesion properties during expansion	Same as in column 2	Use effector rather than central memory or naive/stem cell-like T cells Equip cells with a CD28 cytoplasmic domain
Persistence	Strategy-specific	Use 4-1BB or other optimized cytoplasmic domains Introduce multiple co-stimulatory domains Use mostly undifferentiated cells where possible (expose cells to IL-7 and/or IL-15 as opposed to IL-2 during expansion) CAR T ligand vaccination	Can be achieved using multiple injections Could use mRNA CAR T cells generated ex vivo or possibly in situ
Hypofunction	PD-1–CD28 switch receptors Knockdown of intracellular inhibitors such as SHP2, NR4A and DGK Protect from agents promoting an immunosuppressive TME such as PGE2, adenosine or TGFβ (by introducing dominant-negative *TGFBR2* alleles) Inhibition of cell-surface inhibitors such as PD-1, CTLA4 and FAS Metabolic/ROS-mediated protection (catalase) Activate the TME Introduce changes in the microbiota	Introduce CAR T cell resting periods to avoid tonic signalling Use induced pluripotent stem cells that enable multiple genetic changes to be introduced Multiple co-stimulatory domains can promote the secretion of cytokines (IL-2 or IL-18) that will stimulate T cells	Less of an issue as CAR T cells will be replaced before severely hypofunctional, although some approaches might be useful Could use multiplexed mRNA to introduce multiple changes that will be present while the CAR is expressed Use of CD28 cytoplasmic domain can protect from immunosuppression by TGFβ
Heterogeneity	CAR T cells targeting multiple antigens Secretion of bispecific T cell engagers to engage non-targeted tumour antigens with endogenous $CD3^+$ T cells Secretion of agonists that will enhance cross-presentation, such as FLT3L, IL-12, type 1 IFNs or STING agonists Condition patients with cyclophosphamide to reduce T_{reg} levels and activate DCs	CAR T ligand vaccination 'Universal' CARs	Could use multiplexed mRNA to introduce multiple changes that will be present while the CAR is expressed

CAR, chimeric antigen receptor; DCs, dendritic cells; ROS, reactive oxygen species; STING, stimulator of interferon genes; TME, tumour microenvironment; T_{reg}, $CD4^+$ T regulatory cells.

on selectin-binding ligands ex vivo. This modification increases the number of selectin-binding sites, thus enabling greater tumour infiltration owing to upregulated selectin expression in the inflamed peritumoural regions[150,151]. Activation of the TME could also upregulate the expression of appropriate selectins and ICAM1 (ref. 59).

Thirdly, many tumours are surrounded by a dense network of fibroblasts capable of generating large amounts of extracellular matrix consisting of collagens, heparan sulfate proteoglycans, hyaluronic acid and other matrix proteins[152]. This desmoplastic stroma presents a formidable physical barrier that retains T cells within the stroma and prevents them from entering regions containing greater densities of tumour cells[153]. Furthermore, the structure and stiffness of the matrix are important as these can provide biomechanical signalling cues to T cells. For example, T cells have a number of collagen receptors, some of which, such as LAIR1, can inhibit T cell function[154], as well as certain biochemical signals (for example, by mediating and regulating the expression of TGFβ and other cytokines or chemokines). One approach that might overcome this barrier is to develop CAR T cells expressing matrix-digesting enzymes such as heparinase[155] or hyaluronidase[156,157] on the cell surface. An alternative approach taken by my group[158,159] and others[160–162] is to deplete cancer-associated fibroblasts (CAFs) with a CAR directed against fibroblast activation protein (FAP). In a variety of preclinical models, FAP-targeted CAR T cells have been shown to reduce the amount of matrix within the tumour and reduce the extent of CAF-induced immunosuppression. In a study published in August 2023 using two-photon microscopy, reductions in the density of the tumour matrix induced by FAP-targeted CAR T cells led to markedly increased trafficking of endogenous T cells and enhanced the antitumour activity of the subsequently administered CAR T cells targeting mesothelin in a mouse model of pancreatic cancer[48]. Data from one initial preclinical study suggest that FAP-targeted CAR T cells are highly toxic[163]; however, many subsequent studies (including one small-cohort clinical trial) have shown that these cells have antitumour activity and are well tolerated[48].

Finally, as discussed previously (Fig. 1), misdirection of the T cells towards lymphoid tissues and away from tumours remains an important and under-studied issue. If strategy 2 is adopted (in which persistence is not a priority), the ex vivo T cell expansion process should be changed to favour more-effector-like cells (with limited expression of CD62L or CCR7) that would be expected to enhance tumour trafficking. This change in phenotype could be accomplished by using IL-2, rather than IL-7 or IL-15, during CAR T cell expansion[164,165]. Alternatively, providing TGFβ during expansion could augment the differentiation of CAR T cells towards tissue-resident memory cells, which are more likely to be retained within tumours[166,167]. The use of a CD28 versus a 4-1BB cytoplasmic domain within the CAR construct would also

favour effector-like CAR T cells[12,15,168]. CD28-expressing CAR T cells generally are less persistent; therefore, this approach would probably complement strategy 2.

Local cavitary installation of the CAR T cells provides another possible way of overcoming systemic trafficking issues. This approach has been used with some success in patients with brain tumours[28,29] and in those with mesothelioma[169,170]; however, this route of administration might not be successful in patients with other tumour types and/or in patients with distant metastases owing to limited systemic spread of the CAR T cells after intratumoural injection[171].

Persistence

In strategy 2, CAR T cell persistence is not a major concern owing to the use of repeat administrations of shorter-acting CAR T cells. However, a major goal of strategy 1 (the haematological cancer paradigm) is to generate CAR T cells with the longest possible persistence. As described in detail in a number of excellent reviews[19,106], strategies include altering ex vivo cell culture conditions, blunting host responses through preconditioning (using fludarabine and cyclophosphamide), reducing the immunogenicity of the CAR construct, T cell subset selection, manipulation of signalling molecules (such as AKT), pharmacological inhibition, manipulation of cytokines or their receptors, ectopic expression of cellular immortalization genes and reducing the extent of oxidative stress. Inhibiting apoptosis is another strategy, which involves expression of anti-apoptotic molecules such as BCL-2 family members or knocking down pro-apoptotic molecules such as BIM, BID, DR5 and Fas[106,172,173]. Data from a non-peer-reviewed publication suggest that the presence of the CD47 'don't eat me' signal on CAR T cells is important to avoid clearance by innate immune cells and that overexpression of CD47 on CAR T cells could lead to increased persistence[174].

Increased persistence (and often resistance to hypofunction) can also be achieved by CAR construct engineering approaches, such as changing the size of the extracellular spacer (hinge) region and the affinity of the scFv[15,175]. Expression of a transcription factor such as FOXO1 has been shown to promote T cell expansion, especially that of effector memory cells[176].

The most widely studied CAR engineering approaches designed to optimize persistence involve modifications of the co-stimulatory domains. Changes that favour persistence include mutations in the CD3ζ immunoreceptor tyrosine-based activation motif (ITAM) domains or CD28 cytoplasmic domain, using a 4-1BB versus CD28 cytoplasmic domain, use of ICOS or OX40 cytoplasmic domains, or inclusion of 4-1BB ligand. An approach of particular interest is the use of multiple co-stimulatory domains, usually the combination of sequences from 4-1BB and CD28. This is a somewhat controversial topic, in that some studies have seen enhanced CAR activity with so-called third-generation CARs (in which the CD28 and 41BB domains are linked together as a fusion protein)[177], whereas this effect has not uniformly been observed in other studies. Of note, the previously described successful trial testing GD2-targeted CAR T cells in patients with neuroblastoma[31] used such a CAR design. Interestingly, a set of studies published over the past few years suggest that the locations of the co-stimulatory domains in the cell membrane are important and that enhanced efficacy can be achieved only when the co-stimulatory domains are expressed separately, albeit in close proximity on the cell membranes. In one study[178], the investigators saw enhanced activity of paired CARs (with different cytoplasmic domains, permitting co-expression in parallel rather than in series) relative to standard third-generation CARs. In a separate study[179], the authors reported enhanced activity using a GD2–CD3ζ–CD28 CAR paired with a B7-H3–4-1BB CAR and a mesothelin–CD3ζ–CD28 CAR paired with a chondroitin sulfate proteoglycan–4-1BB CAR. However, the enhanced activity required that the CAR T cells share a single CD3ζ domain. In a third study[180], enhanced efficacy was observed with a BCMA–CD3ζ–CD28 CAR paired with a CD38–4-1BB receptor. The paired receptor concept has been tested clinically in a phase I trial using a CD19–CD3ζ–4-1BB CAR paired with a switch receptor encoding the extracellular domain of PD-1 fused to the transmembrane and cytoplasmic domains of CD28. This study showed acceptable safety and signs of efficacy (objective response rate 58%) in patients with B cell lymphoma[181].

An evolving and encouraging alternative approach is the development of CAR-like constructs that engage or mimic the multichain endogenous TCR[175]. Representative designs in this area[175] include: (1) a T cell antigen coupler (TAC), a fusion protein comprising three domains (an antigen-binding scFv domain, an anti-CD3 scFv that recruits the endogenous TCR and a transmembrane and cytoplasmic domain that anchors the construct near to the TCR); (2) TCR fusion constructs, in which antigen-targeting scFvs are fused to the amino terminus of various TCR subunits (such as the CD3ε chain); and (3) synthetic TCR antigen receptors (STARs), which are double-chain TCR-based receptors with variable VH and VL constant region antibodies fused to the TCR constant α/β regions. Based on promising data from preclinical studies[182,183], clinical trials designed to determine safety and efficacy are underway (such as NCT04727151, testing TAC T cells in patients with HER2-positive solid tumours, and NCT05344976, testing mesothelin-targeted STAR T cells in patients with advanced-stage solid tumours). A phase I trial testing a mesothelin-targeted CD3ε TCR fusion construct showed some evidence of efficacy with a response rate of 30% and longer persistence in many patients (30–90 days) than has been typically seen in studies testing CAR T cells in patients with solid tumours, although some pulmonary toxicity was observed at higher doses[184]. Based on these findings, a phase II trial combining this CAR with immune checkpoint inhibitors is underway.

An important component of strategy 1 is to use CAR T cells with the most undifferentiated cellular phenotype possible. In addition to some of the CAR engineering approaches described previously, this can involve optimizing cell culture conditions for this purpose during production. Most research in this area has focused on the use of common γ-chain cytokines (such as IL-2, IL-7, IL-15 and IL-21). Although high doses of IL-2 were originally used during CAR T cell expansion, this tends to produce more effector-like cells (and would thus be of value in strategy 2). Low doses of IL-2 (ref. 165) or the use of IL-7, IL-15 and/or IL-21 would be likely to result in CAR T cells of a less-differentiated phenotype[164]. Other mediators added during expansion that have been reported to enhance persistence include *N*-acetyl cysteine, IL-12, IL-18, or inhibitors of AKT, GSK3i, MEK or PI3K[106,185,186]. As mentioned previously, adding TGFβ during expansion appears to augment T cell differentiation towards the tissue-resident memory phenotype that favours intratumour accumulation of CAR T cells[167].

As discussed previously, CAR T cells initially traffic to lymph nodes where their ligands are not usually expressed, thus limiting activation and expansion. This liability can be converted into an advantage by artificially expressing the CAR ligand on DCs present in lymph nodes. Such an approach allows the CAR T cells to encounter their cognate antigen and/or ligand in the optimal context of a lymph node where co-stimulation is maximal and immunosuppression minimal compared to the TME. This idea was originally developed using mouse models in which transgenic T cells targeting the melanoma antigen, pmel, or the

xenoantigen, chicken ovalbumin, were injected into tumour-bearing mice; their efficacy was dramatically increased by vaccinating the mice with pmel or chicken ovalbumin protein antigens[187,188]. A vaccine against gp100 has been shown to markedly augment the effects of adoptively transferred T cells expressing both a HER2-targeted CAR and a TCR targeting gp100 in immunocompetent mouse models[189].

This approach has been tested in clinical trials. The Baylor group pioneered the modification of cytomegalovirus (CMV)-specific or Epstein–Barr virus (EBV)-specific T cells to express TCRs and CAR T cell transgenes while retaining the ability of these cells to expand and differentiate within lymph nodes and tumours following exposure to constitutive viral antigens (such as CMV and EBV)[190]. An EBV-directed vaccine augmented the persistence of CD19-targeted CAR T cells manufactured from EBV-specific T cells in a phase I/II trial involving paediatric patients with acute lymphocytic leukaemia[191]. This idea was extended by transducing antitumour T cells with a TCR capable of recognizing a bacterial pathogen[192].

This lymph node 'vaccine' strategy has been innovatively adapted to priming CAR T cells (reviewed in detail elsewhere[193]). Investigators designed protein amphiphile CAR ligands (amph-ligands) that, upon injection, trafficked to lymph nodes and decorated the surfaces of antigen-presenting cells with a CAR ligand, thereby enabling the priming and/or stimulation of CAR T cells in the native lymph node microenvironment[194,195]. Amph-ligand boosting triggered massive CAR T cell expansion, increased donor cell polyfunctionality, induced a bystander effect (see below), and enhanced antitumour activity in multiple immunocompetent tumour-bearing mouse models.

A similar observation was made following the successful in vivo expansion of CAR T cells targeting the developmentally regulated tight junction protein claudin 6 (CLDN6)[196]. In this study, the authors employed a lipid nanoparticle (LNP)-based RNA vaccine to deliver the CLDN6 CAR antigen into lymphoid compartments. Presentation of the natively folded target on resident antigen-presenting cells promoted the cognate and selective expansion of CAR T cells. After injection of CLDN6 CAR T cells into syngeneic mice, subsequent vaccines (two to five in total, administered 1 week apart) with a LNP encoding CLDN6 mRNA led to improved CAR T cell engraftment and regression of large tumours in difficult-to-treat mouse models. In a separate study from the same group, enhancement of a TCR-like CAR was augmented by an mRNA vaccine[197]. A clinical trial testing the use of a CLDN6-targeted CAR followed by multiple doses of a LNP–CLDN6 mRNA vaccine is underway (NCT04503278). Initial results presented in abstract form[198,199] are highly encouraging with response rates of >30% (with one complete response), disease control rates of >67% and acceptable toxicities.

Hypofunction

The conventional approach to this challenge using the 'haematological cancer paradigm' has been to develop methods of modifying either the T cells or the TME to render the CAR T cells less susceptible to hypofunction. Many potential strategies focusing on different pathways have been proposed and have been discussed in detail in a number of excellent reviews[15–18,82]. A partial list of the most successful preclinical approaches involving CAR T cell engineering includes: (1) immune checkpoint inhibition by secretion of anti-PD-1 scFv-only antibodies, expression of dominant-negative or 'switch' receptors, or *PD1* knockdown using CRISPR; (2) deletion or knockdown of intracellular inhibitory molecules such as SHP1, PTEN, cbl-b, diacylglycerol kinase (DGK), PTNPN2 phosphatase, DNMT3A[81], NR4A, BLIMP, or regnase-1/roquin-1 (ref. 200); (3) reduction of Fas expression; (4) engineering CAR T cells to be more resistant to the effects of inhibitory factors present in the TME such as TGFβ (by expression of a dominant-negative *TGFBR2* allele[201]), adenosine and PGE2 (using an intracellular cAMP blocking peptide[140]), and ROS (by expression of catalase[202]); (5) secretion of various activating mediators such as bispecific antibodies, activating RNA molecules[144], cytokines such as IL-12 (ref. 176), and TLR5 agonists[149]; (6) expression of an orthotopic IL-2 receptor that can be stimulated by injection of modified IL-2 (ref. 203); and (7) engineering cytoplasmic domains that resist hypofunction. In this regard, CAR T cells containing the CD28 co-stimulatory domain are more resistant to suppression by TGFβ owing to increased IL-2 secretion[204]. A report published in June 2023 indicates that addition of a constitutively active intracellular domain of c-kit to CAR T cells can activate STAT3 and STAT5 signalling, leading to enhanced IFNγ secretion, cytotoxicity, and CAR T cell persistence, resulting in increased antitumour activity[205]. A CAR design featuring the transmembrane and cytoplasmic domains of KIR2DS2 (a stimulatory killer immunoglobulin-like receptor normally found on natural killer cells) combined with DAP12 (an ITAM-containing adaptor) seems particularly promising[206].

An interesting approach relevant to strategy 1 is based on the idea that temporary 'resting' of CAR T cells could break the cycle of chronic stimulatory hypofunction, especially in those with CARs that are tonically active. This has been accomplished in preclinical studies by using 'switchable' CAR T cells (in which the CARs are activated by injection of an antibody)[207], temporary pharmacological inhibition of CAR signalling using the tyrosine kinase inhibitor dasatinib[208], or the introduction of an inducible degradation domain[209,210]. These approaches differ from the use of inducible suicide switches incorporated to eliminate CAR T cells if toxicity is encountered because they do not kill the CAR T cells, but instead temporarily disable them, theoretically enabling them to recover once the switch signal is withdrawn.

Approaches focused on altering the TME (instead of the CAR design) to reduce the extent of immunosuppression and/or enhance CAR T cell activity have also shown promise and would be applicable in both strategies. A selective list of these methods includes the use of immune checkpoint inhibitors or adenosine receptor inhibitors, altering the microbiota[211], intratumoural injection of enterotoxins or poly-IC, increasing tumour pH using systemically administered bicarbonate[212], administration of oncolytic viruses[143,213–216], and reducing the activity of myeloid suppressor cells (through agents capable of cell-mediated myeloid reduction, such as anti-granulocyte–macrophage colony-stimulating factor or anti-PD-(L)1 antibodies or all-*trans* retinoic acid)[217] or $CD4^+$ T regulatory cells (through agents such as Fas ligand (FasL), CCR4 or CCR8 inhibitors, monoclonal antibodies targetting specific overexpressed proteins, anti-sense oligonucleotides or bispecific antibodies[218,219]).

Heterogeneity

Tumour heterogeneity is a major issue that must be addressed in both strategy 1 and strategy 2. Three main approaches have been used to overcome tumour heterogeneity in the context of CAR T cell administration: targeting multiple tumour antigens, 'arming' CAR T cells with the ability to kill antigen-negative tumour cells, and stimulating the TME to enable activation of the endogenous immune system, thus enabling epitope spreading. Again, these mechanisms are described in detail elsewhere, in several excellent reviews[15,83,86].

T cell engineering has been used to develop various CAR configurations that enable the targeting of multiple antigens[15]. These include transducing T cells with more than one CAR construct or designing

a CAR with two (or more) binding sites, such as CD19 and CD20 or CD19 and CD22. Another promising approach is the use of 'universal' CARs[220–222] that function through the binding of an extracellular adapter domain that acts as a bridge between the CAR and a soluble tumour antigen-targeting ligand. This ligand consists of a tumour-binding domain (such as an scFv antibody) coupled to a molecule that binds with the universal CAR. The dissociation of tumour antigen targeting and T cell signalling seen with this approach confers certain advantages over standard CAR designs, such as dose control of T cell effector function, and importantly, the ability to simultaneously or sequentially target multiple tumour antigens.

The ability to multiplex mRNA potentially makes the expression of multiple CAR T cells relatively straightforward in strategy 2. T cells can also be modified to secrete factors that will kill tumour cells indirectly, such as bispecific T cell engagers[223,224].

A potential tool to expand the cytotoxicity of CAR T cells beyond those expressing the target antigen involves interactions between FasL on T cells and the death receptor Fas, which is expressed on many tumour cells[225]. However, these interactions are complex as the CAR T cells also express Fas on the cell surface and can undergo fratricide if exposed to FasL on other CAR T cells.

Current CAR T cell designs stimulate only very weak cross-presentation and activation of endogenous T cells[34]; therefore, enhancing this effect is an attractive option. CAR T cells have been engineered to express DC activators such as surface-expressed CD40L[226,227] or to secrete DC activators and/or chemoattractants, such as IL-12 or FLT3-ligand (in combination with an activating 4-1BB antibody)[85]. Intratumoural injections of a STING agonist in combination with systemic PSMA-targeted CAR T cells are effective in mouse models[228]. Systemic approaches that might address this issue include the use of low-dose cyclophosphamide (which might promote DC activation and suppress $CD4^+$ T cell levels)[34] and modulation of the gut microbiota using the non-absorbable antibiotic vancomycin or faecal microbiota transplantation[211]. Interestingly, the amph-ligand-boosted CAR T cell approach described previously[194] has been reported to induce endogenous T cell responses[195,229], as well as to promote increased CAR T cell persistence.

Lessons from successful CAR T cell trials in patients with solid tumours

As mentioned previously, two reports published over the past few years (involving strategy 1, the central memory paradigm) have described levels of efficacy similar to those seen in some trials testing CD19-targeted CAR T cells in patients with lymphoma. In a phase I trial, 37 patients with heavily pretreated gastrointestinal malignancies received a second-generation claudin18.2-specific CAR T cell and had an overall response rate of 48.6% and a disease control rate of 73.0%, with 44.8% of responses lasting ≥6 months[30]. These results are impressive given that these patients were heavily pretreated and previously reported third-line therapies were associated with overall response rates of 1.7–13% with median PFS durations of 1.6–2.6 months. In the second study, 27 children with neuroblastoma received a third-generation GD2-targeted CAR T cell, resulting in a complete response rate of 33% with partial responses in a further 30% (objective response rate 63%). Among the patients who received the highest dose of CAR T cells, 3-year overall survival and event-free survival were an impressive 60% and 36%, respectively.

The reasons for the notably higher success rates in these two trials are not clear, although worthy of further consideration. In the trial involving patients with gastrointestinal cancers[30], patients were selected for high levels of target expression (≥40% claudin18.2-positive tumour cells and staining intensity of 2+), and this trial also excluded patients with one or more target lesions with diameters of ≥4 cm and/or with lung or liver metastases. Most patients received more than one CAR T cell infusion and, in addition to standard fludarabine plus cytarabine lymphodepletion, they also received nab-paclitaxel or gemcitabine. From the CAR perspective, high doses ($2.5–5 \times 10^8$ CAR T cells per infusion) of a short-lived lentivirally expressed CD3z–CD28 CAR were administered, resulting in moderate circulating CAR T cell levels (the median number of copies per microgram DNA was 6,713 after first infusion); however, the duration of persistence was short (~28 days). Investigators noted that responders had higher peak CAR numbers than non-responders (10,553 versus 4,980 copies per microgram DNA).

In the second trial[31], features of note include that the patients had neuroblastoma, a tumour that grows in bone and bone marrow (similar to many haematological cancers) and that patients with a lower tumour burden had better outcomes (with bone marrow being the site of best response). All patients received lymphodepletion. From the CAR perspective, a third-generation CAR including both CD28 and 41BB co-stimulatory domains was used. T cells were transduced at very high levels (>70%) using a retroviral vector and the cells were expanded using both IL-7 and IL-15. High CAR T cell doses were administered (in the phase II part of the trial patients received a dose of 10^7 cells/kg, which would be 3×10^8 cells in a 30-kg patient) and this resulted in very high circulating CAR T cell levels (200,000 copies per microgram DNA), similar to those seen in successful trials testing CD19-targeted products in patients with leukaemia or lymphoma. CAR T cell levels peaked at 14 days, albeit with a low level of persistence at later time points. No notable differences in persistence between responders and non-responders were observed. Multiple doses were administered to 11 of the 27 patients (up to four infusions per patient).

These trials were quite different in many ways, making firm conclusions difficult; nonetheless, commonalities include the use of high CAR T cell doses with multiple infusions permitted and that the tumours in both trials expressed moderate or high levels of the target antigen on the majority of tumour cells. The observation of high circulating CAR T cell levels and detectable CARs for longer time periods (~2 months versus 2 weeks) relative to most previous trials involving patients with solid tumours might be important. Interestingly, trends towards better responses in patients with longer CAR T cell persistence emerged from both trials. However, these levels of persistence are still limited relative to those seen in previous, successful trials of CD19-targeted CAR T cells, suggesting a need for repeat infusions. The success seen with the third-generation CAR T cell[31] is intriguing given the mixed conclusions of data on the value of CAR constructs incorporating multiple co-stimulatory cytoplasmic domains. In summary, these data support the idea that strategy 1 could potentially be successful in patients with solid tumours, provided high levels of functionally persistent CAR T cells can be generated. As additional promising clinical data are reported, which will hopefully include information on CAR T cell trafficking and function, the 'rules for success' (such as the need for high doses and/or multiple CAR T cell injections) will hopefully become more clearly established.

Conclusions

Addressing the numerous challenges facing the application of CAR T cell therapy to patients with solid tumours is a formidable task. Some investigators may lose hope, although the successes achieved with CAR

T cells in B cell malignancies, adoptive T cell transfer in patients with melanoma, and the previously described trials testing claudin18.2-targeted and GD2-targeted CAR T cells in patients with solid tumours indicate that continued efforts in this area will be valuable and have the potential to lead to similar breakthroughs. Based on the considerable work done so far, my conclusions are as follows:

1. Our incomplete understanding of why current therapies are largely ineffective in patients with solid tumours remains a major issue. Initial data suggest very inefficient tumour homing. Obtaining more, and better information on CAR T cell trafficking, persistence and function in treated patients will be critical to addressing these aspects. Better T cell imaging approaches could be especially helpful in providing information on trafficking and persistence. Based on data from preclinical studies, CAR T cells are likely to rapidly lose functional capacity after entering the tumour. Developing non-invasive methods of measuring T cell function might be possible, although biopsy samples are likely to continue to be needed to study this issue in detail. Future trials should thus strive to consistently obtain such samples whenever possible.
2. The strengths and weaknesses of current preclinical models need to be recognized and these models should be used appropriately. Studies testing human-derived CAR T cells in immunodeficient mice have dominated the literature, although models using mouse-derived CAR T cells in mice with intact immune systems might be more predictive of what we see in clinical trials and should be used more often. Advances in humanized mouse technologies might be helpful.
3. The large number of barriers to therapeutic success will probably require co-targeting of multiple mechanisms of immune evasion. These strategies will include optimization using CAR engineering (including using multiple parallel cytoplasmic co-stimulatory domains or greater levels of engagement with the endogenous TCR), optimizing conditions during ex vivo T cell expansion to promote the emergence of specific T cell subtypes, and manipulation of the host to alter the TME and/or stimulate the native T cells. All of these approaches might need to be used to achieve optimal outcomes.
4. Owing to these multiple challenges, as we pursue the conventional memory cell paradigm, I believe the two areas of greatest promise will be perfecting the use of iPSCs to enable the introduction of multiple genetic alterations into allogeneic CAR T cells and the use of 'vaccines' that express the target antigen on DCs located in lymph nodes in order to maximize proliferation, enhance persistence and augment bystander effects.
5. The current clinical trials testing CAR T cells in patients with solid tumours are closely modelled on approaches that were successful in patients with haematological cancer and are based on producing T cells with the least effector-like and most memory-like or stem cell-like phenotypes, resulting in CAR T cells that will initially traffic to bone marrow and lymph nodes and therefore have the highest probability of long-term persistence. This approach might not be optimal for patients with solid tumours. An alternative to this approach involves administering repeat doses of highly active effector-like cells that can traffic more efficiently to tumours and thus kill tumour cells more effectively, and be 'replaced' by repeat administration as they lose function. Here, altering conditions during ex vivo T cell expansion, activating the TME and multiplexing could all optimize both trafficking and short-term cytotoxic potential. CARs produced by mRNA transduction could be used. Approaches designed to generate CAR T cells in situ are especially attractive as these could reduce both costs and waiting times, enhance safety (because no lymphodepletion would be needed), increase accessibility and possibly improve efficacy.

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