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Clinical lessons learned from the first leg of the CAR T cell journey

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Chimeric antigen receptor (CAR) T cell therapy for B cell malignancies has surpassed expectations, driving an ever-expanding number of clinical trials and the first US Food and Drug Administration approvals of cell therapies for the treatment of cancer. This experience has illuminated some generalizable requirements for CAR T cell efficacy as well as the interplay between disease biology and clinical outcomes. Major CAR intrinsic variables affecting T cell behavior have been defined, and mechanisms of tumor resistance are increasingly understood. Here, we review the clinical experience with CAR T cells amassed to date, including but not limited to B cell malignancies, emphasizing factors associated with efficacy, resistance and major barriers to success. We also discuss how these insights are driving next-generation clinical trials, including those in solid tumors.

A growing understanding of the immune system's role in controlling cancer has driven an immunotherapy revolution, bringing effective and durable therapies for patients with previously incurable malignancies[1,2]. Advances in cell, gene and protein engineering have paved the way for CAR T cells to become a novel and important immunotherapy for patients with refractory, high-grade B cell malignancies[3]. These synthetic receptors combine the specificity of a monoclonal antibody with the cytolytic power and capacity for immune surveillance of a T cell, independent of the major histocompatibility complex[4] (Box 1). Thus, through genetic engineering, a T cell can be endowed with specificity for any cell-surface protein expressed by a human cancer, dramatically broadening the number of available immunotherapeutic targets. A sizable fraction of patients with relapsed and refractory large B cell lymphoma (LBCL) and B cell acute lymphoblastic leukemia (B-ALL) have achieved durable complete remissions after single infusions of these potent cellular products[5–14].

To date, the most important factors influencing the outcome and durability of the tumor response after CAR T cell therapy in the clinic appear to include the capacity for T cells to expand after administration, disease histology and probably disease-intrinsic factors that predispose tumors to resistance associated with antigen loss or downregulation. Although the results of CAR T cells in solid tumors have not been as robust, several early signs of clinical efficacy in human trials hint at oncoming success. Further understanding of the factors influencing response and resistance are driving the development of next-generation CAR therapeutics that are predicted to mediate increased efficacy in both hematologic malignancies and solid tumors. Here, we review the major factors influencing outcomes in patients treated in CAR T cell trials to date, as a means for understanding the challenges to applying these therapeutics to other cancers, including solid tumors.

Major factors affecting the clinical behavior, toxicity and efficacy of CAR T cells

The substantial clinical experience with CD19-CAR therapy for B cell malignancies accrued since 2010 spans CAR constructs using variable CD19-recognition domains; transmembrane domains and costimulatory molecules; non-selected and selected T cell subsets transduced with retroviral and lentiviral vector constructs and transposons; lymphodepleting preparative regimens incorporating different agents and doses; and manufacturing processes varying in duration, cytokine support and methods of T cell activation[5–7,9,15–17]. The trials have also included remarkable interpatient heterogeneity, with diversity in ages (ranging from infants to older people), diversity in underlying disease histology and genotypes, and genetic diversity across individuals. Despite the number of potential factors affecting variability in CAR T cell behavior, outcomes across CAR trials have been remarkably similar, and only several factors to date have been consistently identified to influence patient outcomes, namely disease histology, the lymphodepleting regimen used and the CAR architecture/costimulatory domain employed in the construct.

Disease histology. Disease histology has emerged as a major factor affecting outcomes after treatment with CAR T cells for B cell malignancies. In B-ALL, CD19-CAR therapy induces very high complete response (CR) rates, but longer follow-up has revealed high rates of relapse that limit overall success[6,9]. In LBCL, CD19-CAR therapy induces lower CR rates, but relapses are rare, thus resulting in similar rates of long-term disease control[14], whereas primary resistance remains a major barrier to the success of CD19-CAR T cells in CLL[18–20].

Disease histology of B-cell acute lymphoblastic leukemia. B-ALL, a cancer of the bone marrow, is highly susceptible to CD19-CAR therapy, and minimal residual disease (MRD)-negative CRs have been achieved in 60–93% of patients across several studies[5–10,15] (Table 1). Similarly, a CD22 CAR in pediatric B-ALL has demonstrated a 73% CR rate when administered at bioactive doses[11]. High and homogeneous expression of CD19 and CD22 target antigens is likely to contribute to the high response rates observed in B-ALL[21], but several pieces of evidence suggest that tumor cells within the bone marrow may also be more accessible and/or exist within a less suppressive tumor microenvironment (TME) than cells present within an organized tumor. Patients with leukemia who simultaneously have marrow disease and lymphomatous masses show rapid clearance of bone marrow disease (often within 28 days) but slower and sometimes decreased clearance of lymphomatous masses[11,18].

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Box 1 | CAR T cell nomenclature and structure

Basic structure. CAR T cells combine the specificity of a monoclonal antibody with the cytolytic capacity of a CAR T cell. This is achieved by fusing the signaling elements from CD3-ζ, the initiator of T cell signaling, to a transmembrane domain and an extracellular antigen-binding domain (described below). CAR T cells target surface antigens in a genetically unrestricted manner, independent of expression of the major histocompatibility complex[4].

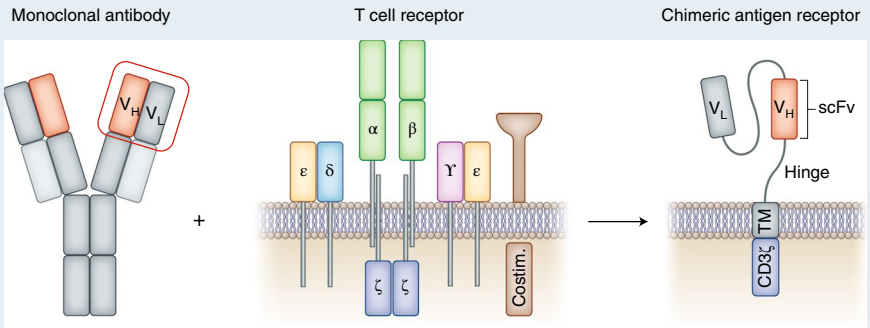
Costimulatory domain. Initial human trials of CAR T cells containing only the CD3-ζ domain (first generation) revealed limited T cell expansion and persistence[22,131,132]. Effective T cell response requires both signal one (TCR/CD3-ζ) and signal two (costimulatory signal, such as CD28 or 4-1BB). Next-generation CAR T cell constructs were engineered to contain costimulatory domains such as CD28 (refs. [133,134]) and 4-1BB[135]. Clinical trials of these constructs have resulted in high response rates in patients with B cell malignancies.

Antigen-binding domain. The antigen-binding domain imparts specificity to CAR T cells. This region is most commonly derived from an scFv of an antibody, although ligands, cytokines and other domains have also been used[3]. Importantly, CARs can be designed to recognize any cell-surface antigen.

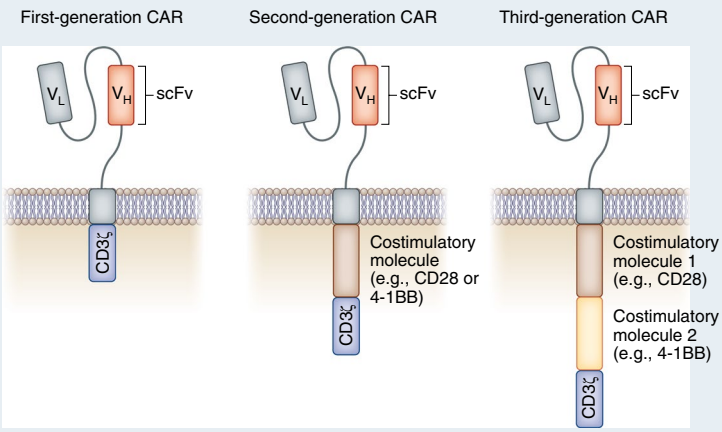
CD19 and other CAR T cell targets. To date, most clinical experience and success has been amassed with CAR T cells targeting CD19, a surface protein involved in B cell signaling that is expressed on B cell malignancies (including B-ALL, LBCL and CLL) and normal B cells. Given that its expression is restricted to the B cell lineage, and patients can live without healthy B cells, CD19 has emerged as a promising target for CAR T cell immunotherapy[16]. Other targets that have demonstrated clinical success in B cell malignancies include CD22 for B-ALL[11] and BCMA for multiple myeloma[35,37]. CAR T cell targets for solid tumors are rarely restricted to tumor and non-vital tissues, and the prospect of on-target, off-tumor toxicity must be carefully considered.

CAR T cell production and infusion. To date, most CAR T cell trials have used autologous T cells for transduction. A cancer patient's T cells are collected, activated with antibodies or antibody-coated beads, and then transduced, most commonly with a lentivirus or retrovirus, to express the CAR molecule. CAR T cells are then expanded in vitro to sufficient numbers to infuse back into the patient. Patients often receive lymphodepleting chemotherapy before T cell infusion.

CAR T cell engraftment, proliferation and persistence. Successful CAR T cell treatment relies on (1) engraftment, which requires lymphodepletion most commonly induced via chemotherapy[10,12] and (2) antigen recognition, which induces



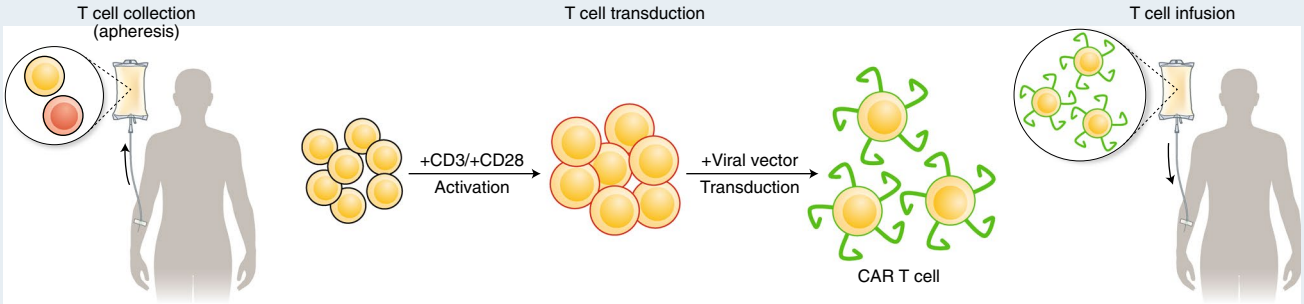
Structure of CAR T cells. CAR T cells are engineered by fusing the scFv of a monoclonal antibody (or another antigen-recognition domain) to a transmembrane domain and intracellular signaling domains capable of eliciting a T cell response. V_H, heavy chain; V_L, light chain; costim, costimulation; TM, transmembrane. Credit: Debbie Maizels/Springer Nature



CARs that contain only the CD3-ζ endodomain are known as first-generation CARs; those that contain one costimulatory domain (such as CD28 or 4-1BB) are known as second-generation CARs; and those that contain two or more costimulatory domains are known as third-generation CARs. Credit: Debbie Maizels/Springer Nature

Box 1 | CAR T cell nomenclature and structure (continued)

antigen-induced proliferation. (3) In some cases, long-term immune surveillance, in which patients retain low numbers of circulating CAR T cells for months to years after the infusion, is needed for durable tumor control[5,7,8].



T cells are collected from a cancer patient by apheresis. Those T cells are then activated with antibodies and exposed to a viral or other vector encoding the CAR molecule. CAR T cells are allowed to expand before reinfusion into the patient. Credit: Debbie Maizels/Springer Nature

Even in the limited experience for CAR T cells in solid tumors, signs that marrow disease may be particularly susceptible to these therapeutics have been observed. A first-generation GD2.z CAR has mediated a CR in a patient with neuroblastoma involving the bone marrow[22,23], a GD2.28.z CAR has mediated a CR in the bone marrow in a patient with neuroblastoma[24], and the only patient in a trial of Her2.28.z CAR T cells for sarcomas who achieved a CR had rhabdomyosarcoma limited to the bone marrow[25]. Because solid tumors that disseminate to the marrow are typically incurable with standard cancer therapies, focused efforts aimed at defining whether non-hematologic cancers involving the bone marrow might be uniquely susceptible to CAR T cell therapeutics are warranted.

Disease histology of chronic lymphocytic leukemia. At the other end of the response spectrum among B cell malignancies is chronic lymphocytic leukemia (CLL), which also demonstrates high and homogenous CD19 expression, but CD19-CAR T cells have been found to mediate CRs in only 15–30% of patients across several trials[18–20] (Table 1). Patients with advanced CLL who are eligible for CAR T cell trials typically have disease both in the bone marrow and at extramedullary sites. As observed in treatment of B-ALL and LBCL, greater CAR T cell expansion is associated with a greater likelihood of response in CLL. However, in a study comparing the relationship between the kinetics of T cell expansion and disease response in B-ALL and CLL, researchers have found that, despite identical manufacturing processes to express the same CD19.BB.z-CAR, 100% of B-ALL patients whose CAR T cells underwent high expansion experienced a CR, whereas a sizable fraction of patients with CLL whose CAR T cells underwent similarly high expansion did not experience a CR, thus implicating additional requirements for CR in CLL, which remain poorly understood[26].

A recent study has provided evidence that T cells from some CLL non-responders have impaired fitness before therapy, because T cells contained in pre-manufacturing apheresis products and manufactured CAR T cell products from non-responders showed phenotypic, transcriptomic and metabolic profiles associated with T cell exhaustion, in contrast to those from responders, which showed profiles associated with T cell memory[27]. These results align with clinical evidence indicating that immunosuppression is a characteristic feature of advanced CLL and further illustrate how underlying disease biology affects outcomes of CAR T cell use. Importantly even in B-ALL, in which response rates are much higher than those in CLL, a retrospective comparison of apheresis products from patients who achieved durable responses versus those that did not respond or experienced early relapse has revealed that increased expression of exhaustion markers on the apheresis product can predict outcome[28]. Overall, baseline T cell dysfunction appears to be an important cause of primary resistance to CD19 CAR therapy and is a major feature predicting response in CLL, in which immunosuppression is a hallmark of the disease.

Disease histology of large B cell lymphoma. CR rates after CD19-CAR therapy in LBCL are intermediate between those observed in B-ALL and CLL, and 40–50% of patients have achieved a CR in numerous trials[12–14,29–34] (Table 1). The basis for the consistently lower response rates than those in B-ALL has not been defined, but hypotheses include more variable CD19 expression, limited T cell trafficking into the lymphomatous masses and/or an inhibitory TME. The field greatly needs a more precise understanding of the basis for non-response to CD19-CAR therapy in LBCL, both to enable selection of patients more likely to respond and to develop next-generation CAR T therapeutics or regimens that can mediate improved outcomes.

Disease histology of other hematologic malignancies. Encouraging advances have been made in the treatment of multiple myeloma with CAR T cells targeting B cell maturation antigen (BCMA)[35,36,37]. In the first reported trial of a BCMA.CD28.z CAR from the US National Cancer Institute, a steep dose–response curve was observed, and 81% of patients receiving a bioactive dose experienced a CR or partial remission[35]. More recently, the results of a larger multicenter trial of a BCMA.BB.z-CAR demonstrated an overall response rate of 85% and a complete response rate of 45%. Of interest, partial remissions were typically associated with MRD-negative bone marrow, results again consistent with greater sensitivity and or accessibility of myeloma within the marrow than that in extramedullary disease[37]. Registration trials are ongoing, and studies of CAR T cells deployed in multiple myeloma at earlier points in therapy are likely in the near future.

Given the success of CAR T cells in treating bone marrow disease in general, and leukemia in particular, there is hope that CAR T cells will be successfully deployed in acute myeloid leukemia (AML). Preliminary data from a trial of CD123 CAR T cells for AML have demonstrated early clinical responses without the development of long-term cytopenias in some patients, although most patients quickly proceeded to allogeneic hematopoietic stem-cell

Table 1 | Outcomes after use of CD19-CAR T cells for B cell malignancies

Disease	Site	Population studied; phase	Construct	Vector	Reported CR (MRD^- at 28 days for B-ALL; CR at 3 months for NHL)	ITT CR (MRD^- for B-ALL)	Median follow-up	T cell persistence	Relapse rate	$CD19^-$ relapses	High-grade/ severe CRS[a]	High-grade/ severe neurotoxicity[a]	Notes and references
B-ALL	Children's Hospital of Philadelphia and University of Pennsylvania	Pediatric and adult; phase I	19.BB.z	Lentiviral	79% (22/28)[b,e]	NR	7 months	68% (PCR) at 6 months	26% (7/27)	43% (3/7)	27% (8/30)	NR[d]	5
B-ALL	Memorial Sloan Kettering Cancer Center	Adult (age 18+); phase I	19.28.z	Retroviral	67% (32/48)[c,e]	39% (32/83)[c,e]	29 months	0% (PCR) at 6 months	61% (25/41)	16% (4/25)	26% (14/53)	42% (22/53)	9
B-ALL	Novartis Global (ELIANA)	Pediatric, AYA; phase II	19.BB.z	Lentiviral	81% (61/75)	66% (61/92)	13 months	83% (BCA) at 6 months	33% (20/61)	94% (15/16)[e]	47% (35/75)	13% (10/75)	6
B-ALL	NCI	Pediatric, AYA; phase I	19.28.z	Retroviral	60% (12/20)	57% (12/21)	10 months	0% (PCR) at 6 months	17% (2/12)	100% (2/2)	29% (6/21)	5% (1/21)	10/12 responders underwent allo-HSCT[7]
B-ALL	Fred Hutchinson Cancer Center	Adult (age 18+); phase I	19.BB.z	Lentiviral	93% (27/29)	84% (27/32)	NR	NR	33% (9/27)	22% (2/9)	23% (7/30)	50% (15/30)	10
B-ALL	Seattle Children's Hospital	Pediatric, AYA; phase I	19.BB.z	Lentiviral	93% (40/43)[e]	89% (40/45)[e]	9.6 months	~30% at 6 months (BCA)[f]	45% (18/40)	39% (7/18)	23% (10/43)	21% (9/43)	8
NHL	Multiple (ZUMA-1)	Adult (age 18+); phase II	19.28.z	Retroviral	54% (55/101)	50% (55/111)	15.4 months	NR	NR	27% (3/11)[g]	13% (13/101)	28% (28/101)	14
NHL	Multiple (JULIET)	Adult (age 18+); phase II	19.BB.z	Lentiviral	40% (37/93)	22–27% (37–45/165)[i]	14 months	NR	NR	NR	22% (24/111)	12% (13/111)	13
NHL	University of Pennsylvania	Adult (age 18+); phase I	19.BB.z	Lentiviral	57% (16/28)	42% (16/38)	28.6 months	50% (BCA) at 12 months[f]	0%	NA	18% (5/28)	11% (3/28)	29
NHL	Fred Hutchinson Cancer Center	Adult (age 18+); phase I	19.BB.z	Lentiviral	33% (10/30)	27% (10/37)	NR	NR	11% (1/9)[h]	NR	13% (4/32)	28% (9/32)	Results dramatically improved after adoption of flu/cy preconditioning[12]
NHL	National Cancer Institute	Adult (age 18+); phase I	19.28.z	Retroviral	55% (12/22)	55% (12/22)	NR	NR	8% (1/12)	NR	18% (4/22)	55% (12/22)	31
CLL	Fred Hutchinson Cancer Center	Adult (age 18+); phase I	19.BB.z	Lentiviral	17% (4/24)	13% (4/30)	NR	100% (PCR) at 6 months	NR	NR	8% (2/24)	25% (6/24)	18
CLL	University of Pennsylvania	Adult (age 18+); phase I	19.BB.z	Lentiviral	29% (4/14)	22% (4/18)	19 months	100% (PCR and BCA) in responders at 6 months	0%	0%	43% (6/14)	7% (1/14)	19

NR, not reported; NA, not applicable; MRD, minimal residual disease, defined as <0.01% by flow cytometry; ITT, intent-to-treat response rate; AYA, adolescent and young adult; BCA, B cell aplasia;PCR:, polymerase chain reaction detection of CAR transgene. [a]As defined by the study authors or grade 3 or above. [b]Three patients not assessed for MRD. [c]Five patients not assessed for MRD. [d]High-grade neurotoxicity not reported; overall neurotoxicity 43%. [e]Some patients had no detectable disease at the time of infusion and were considered to be in CR, but whether treatment mediated clinical benefit was unclear. [e]Five patients were not assessed for CD19 expression at relapse. [f]Among responders only. [g]Includes patients with progressive disease after initial infusion; only a limited number of patients were analyzed. Strict cutoff for $CD19^-$ <1% by immunohistochemistry. [h]Only reported data for patients receiving lymphodepletion with fludarabine and cyclophosphamide (flu/cy). [i]Inferred from the text; the ITT overall response rate was reported as 34%, but no ITT CR rate was reported.

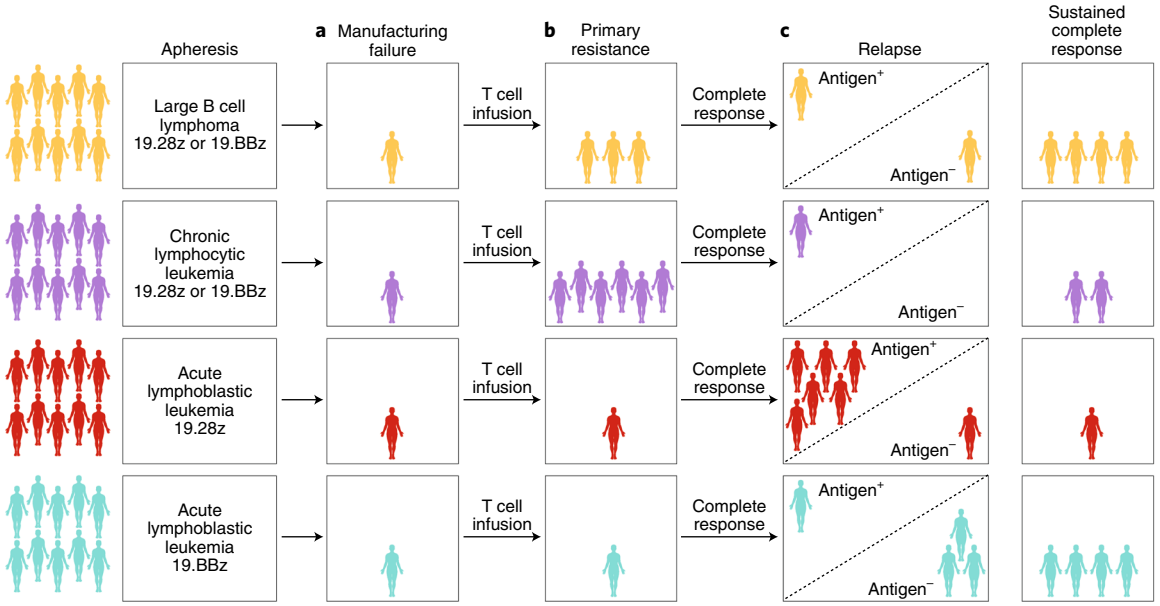


Fig. 1 | Patterns of failure after CD19-CAR T cell therapy and potential causes. Each row depicts mechanisms of failure and relapse for a different disease histology and/or CAR T cell construct. There are ten figures per row; each figure represents approximately 10% of patients, and each figure within a box represents patients in that category of treatment failure or resistance. **a**, In some series, manufacturing failures are an important cause of treatment failure. The rate of manufacturing failure has not been associated with underlying disease or the costimulatory domain. In general, with improved manufacturing processes this failure can be limited to <10% of cases[6–8,13,14]. **b**, Primary resistance is highly associated with underlying disease, with CLL[18–20,27] > LBCL[13,14,29,31] > B-ALL[5–9]. **c**, Among patients with B-ALL, CD19^{+} relapse tends to occur more commonly after treatment with CD19.28.z-CARs, which manifest short persistence[79], whereas CD19$^{-/lo}$ relapse tends to occur after treatment with CD19.BB.z-CARs[6], which often induce prolonged immune pressure. The incidence of CD19^{+} versus CD19$^{-/lo}$ relapse in LBCL occurs but remains incompletely characterized[14,95,96]; approximately 50% of relapses have been reported to be due to loss of CD19[136]. Whether CD19 expression at relapse in LBCL correlates with the costimulatory endodomain remains unknown. Credit: Debbie Maizels/Springer Nature

transplantation (allo-HSCT), which presumably eradicated the CAR T cells[38]. CARs targeting several other antigens, including CD33, CLL1, NKG2D and FLT3, are also in clinical trials, and more clinical data are expected in the next few years. Overall however, there have been fewer trials of CAR T cells in AML than in B cell malignancies, probably because of the paucity of antigenic targets that are not simultaneously expressed broadly in the hematopoietic compartment. Thus, owing to the risk of myelosuppression, CAR T cells for AML are likely to emerge, at least initially, as a bridge to allo-HSCT[39] (Box 2).

Lymphopreparative regimen. Adoptive transfer of T cells rarely results in substantial engraftment in the absence of lymphodepletion, and clinical experience has clearly demonstrated that robust expansion of CAR T cells after adoptive transfer is essential for clinical efficacy[5–7,26,35,40]. Thus, an essential component of effective CAR T cell therapy is pre-treatment with a lymphodepleting preparative regimen[10,12,16,41].

Lymphodepletion elevates levels of homeostatic cytokines, such as IL-7 and IL-15, which enhance T cell expansion[42]; transiently diminishes regulatory T cells[43] and potentially myeloid-derived suppressor cells; alters the TME and consequently enhances T cell trafficking; and inhibits anti-CAR immune responses, which could affect persistence[44,45]. Through the analysis of results from single arm non-randomized trials, regimens containing cyclophosphamide and fludarabine have been found to be associated with higher CAR T cell expansion and greater long-term persistence[10,12,46]. This phenomenon is well illustrated in relapsed and refractory LBCL treated with CD19.4-1BB.z CAR T cells, wherein CR rates after cyclophosphamide preconditioning alone are less than 10% but increase to 50% after the addition of fludarabine[12]. This finding has been attributed to fludarabine's potent effect on lymphocyte depletion, which drives higher IL-15 levels[31] and diminishes anti-CAR immune responses[10,12]. Because profound T cell depletion in humans results in incomplete and protracted recovery of T cell numbers and repertoire diversity[42], and because profound T cell depletion can diminish epitope spreading induced by adoptive T cell transfer[47], next-generation therapeutics under development include approaches to selectively expand engineered populations in the absence of a lymphodepleting regimen[48].

Costimulatory domains and T cell persistence. Incorporation of a CD28 versus 4-1BB costimulatory domain has emerged as the factor most consistently affecting CAR T cell behavior. T cells expressing CARs incorporating a CD28 endodomain demonstrate more rapid and higher peak expansion but rarely persist beyond 1 to 2 months[7,9]; in comparison, those incorporating a 4-1BB endodomain show slower and lower peak expansion, and often persist for months or even years[5,6,8].

Several studies have demonstrated that CD19.28.z-CAR T cells, compared with CD19.BB.z-CAR T cells, expand more rapidly after infusion and, on average, to higher levels[5,7,26]. The biology responsible for this finding remains incompletely understood, but greater signal strength downstream of CD28- versus 4-1BB-containing CAR T cells has been reported[49], as have mitochondrial changes in 4-1BB CAR T cells that are not found in CD28-containing CAR T cells[50]. To date, there is no clear evidence that the more rapid and profound expansion of CD28-containing CD19-CARs results in greater anti-tumor efficacy, but differential expansion could affect outcomes in solid tumors. The optimal costimulatory domain for use in targeting solid tumors with CAR T cells is underexplored and remains an area of active study.

Box 2 | Allogeneic hematopoietic stem-cell transplant

What is it? The transplantation of hematopoietic stem cells from another individual into a patient.

When is it used for cancer? Patients with hematologic malignancies often receive allo-HSCT to act as a form of long-term immune surveillance against chemotherapy-resistant cancer cells.

What are the downsides? Allo-HSCT is associated with high levels of morbidity and mortality, often related to infection and graft-versus-host-disease.

How does it relate to CAR T cells? CAR T cells may be able to replace allo-HSCT as a form of long-term disease surveillance, but the evidence is not yet clear. Patients who undergo allo-HSCT after CAR T cell therapy lose their CAR because of lymphoablative preparatory regimens.

In addition to differential levels of peak expansion, CD19.CD28.z and CD19.BB.z-CARs also demonstrate striking differences in persistence. CAR T cell persistence results in long-term immunosurveillance as CAR T cells continue to eliminate all CD19$^+$ cells, including both tumor cells and normal B cells (which also express CD19). Therefore, CD19 CAR T cell persistence can be monitored by measuring the recovery of non-malignant B cells after their initial destruction by CAR T cells. Patients receiving CD19.28.z-CAR T cells typically show B cell recovery within 60 days of infusion[7,9,15,30], whereas 83% of children and young adults treated with tisagenlecleucel, a CD19.BB.z-CAR, have been found to show B cell aplasia (BCA) at 6 months after infusion and a median time to B cell recovery of approximately 11 months[6,40]. The basis for diminished persistence of CD28 versus 4-1BB CAR T cells probably relates to a greater tendency of CD28-containing CAR T cells to develop exhaustion[51]. In addition, 4-1BB-containing CAR T cells have higher levels of the anti-apoptotic proteins BCL-2 and BCL-XL, and a metabolic profile that may enhance memory formation[50,52].

Although long-term follow-up data are limited, there is no evidence to date that the distinction in CAR behavior induced by the costimulatory endodomain affects the response in treatment of LBCL because CR rates after CD19.BB.z and CD19.CD28.z CAR treatment are similar, and most complete responders experience long-term disease control after a single infusion of CAR T cells[8,12–14,29,31,32]. Indeed, CRs induced by CD19-CAR T cells in LBCL are remarkably durable because nearly all patients who were in remission at 6 months remained disease free throughout the period of follow-up, even if they had B cell recovery (a proxy for loss of functional CAR)[14,32] (Fig. 1). As a result, clinical decisions regarding the optimal CAR construct for treatment of LBCL are not currently focused on the potential for long-term persistence but instead are increasingly relegated to distinguishing features between CD28- versus 4-1BB-containing CARs as they relate to product availability and/or toxicity.

In children and young adults with B-ALL treated with CD19-CARs, in contrast to results in individuals with LBCL, the persistence of CAR T cells appears to be an important requirement for cure[5–8,53] (Fig. 1). CD19.28.z-CAR T cells in pediatric B-ALL are associated with rapid B cell recovery and disease relapse in complete responders who do not proceed to allo-HSCT[7,9,53,54] (Box 2). In a trial of patients with B-ALL treated with a CD19.BB.z CAR, all children who achieved an MRD-negative remission but had short BCA (<3 months) and did not undergo allo-HSCT relapsed[28]. The distinctions between LBCL and pediatric and young-adult B-ALL with regard to the need for CAR persistence probably relates to the underlying disease biology; effective chemotherapeutic treatment of pediatric and young-adult B-ALL requires multiple years of therapy, whereas effective chemoimmunotherapy for LBCL is typically administered over approximately 6 months. Because limited CAR T cell persistence is associated with a diminished likelihood of durable responses in pediatric and young-adult B-ALL, there appears to be a preference for 4-1BB CARs for treatment of this disease when post-CAR allo-HSCT is contraindicated or undesired[6,8,55].

Delineating which children and young adults with B-ALL in remission after CD19-CAR T cell treatment should proceed to allo-HSCT is an area of evolving clinical practice. There is a general consensus that patients who have never previously received an allo-HSCT and have a suitable donor should be recommended for consolidative allogeneic allo-HSCT if they enter remission with a CD19.28.z-CAR or if they receive a CD19.BB.z-CAR but experience short CAR persistence, as evidenced by B cell recovery. For patients who demonstrate persistent B cell aplasia after CD19.BB.z-CAR T cells, some researchers have also recommended allogeneic allo-HSCT, because the risk for relapse with CD19$^-$ leukemia is high[21] (described below). In an abstract describing long-term follow-up of children with B-ALL who achieved remission after CD19.28.z-CAR T treatment, more than 50% who underwent post-CAR allo-HSCT experienced event-free survival[53].

The requirement for CAR T cell persistence for cure of B-ALL in older adults has not been demonstrated, and important differences in the biology of this disease across the age spectrum might possibly lessen the effects of the costimulatory domain on long-term disease control after CD19-CAR therapy. Indeed, in a study of CD19.28.z-CAR for B-ALL in older adults[9], the long-term outcomes were poor regardless of whether post-CAR allo-HSCT was administered; these findings may reflect inferior outcomes after allo-HSCT in older individuals. Thus, the question of whether CD19.BB.z-CARs are superior to CD19.28.z-CARs for long-term disease control in older adults with B-ALL remains unanswered. Currently, no CD19 CAR product is approved by the US Food and Drug Administration for this population.

Costimulatory domains and toxicity. The major toxicities observed to date in clinical trials of CARs for B cell malignancies have been cytokine-release syndrome (CRS) and CAR-associated neurotoxicity (reviewed in refs. [56–58]). CRS, a syndrome marked by high levels of inflammatory cytokines that results in a sepsis-like picture in patients, is thought to be largely a result of IL-6 and IL-1 (refs. [59,60]). Neurotoxicity is not as well understood but is thought to occur as a result of endothelial dysfunction in the central nervous system (CNS), owing to a highly inflamed state[61]. The different rates and magnitudes of CAR T cell expansion induced by CD28 versus 4-1BB-containing CARs also appear to affect acute toxicity, because CRS- and CAR-associated neurotoxicity are associated with higher CAR T cell expansion[5–10,12,14,15]. Randomized controlled trials of CARs incorporating CD28 versus 4-1BB costimulatory domains are not available, but reviews of non-randomized trials in B-ALL have shown no apparent difference in the rate or severity of CRS or neurotoxicity with CARs incorporating CD28 versus 4-1BB costimulatory domains[5–8,10] (Table 1). In all trials of CD19 CAR for B-ALL, toxicity has generally correlated closely with disease burden and T cell expansion, and not the CAR costimulatory domain[5–10], possibly because B-ALL is permissive for CAR T cell expansion, and therefore the magnitude of the effect of the costimulatory domain is lessened.

In contrast, in LBCL, preliminary results from non-randomized trials of CARs incorporating CD28 versus 4-1BB costimulatory domains suggest that rates of high-grade CRS and neurotoxicity may be lower with 4-1BB-containing CD19-CAR T cells. In the published phase II ZUMA-1 trial of a CD19.28.z-CAR for LBCL, 94% of patients developed CRS, with 13% of patients having grade 3 or higher, and 64% of patients developed neurologic events, with 28% grade 3 or higher[14]. In the LBCL cohort of the TRANSCEND NHL trial of a

Table 2 | Clinical trials of CAR T cells for solid tumors

Construct	Disease	Site	No. patients treated	Response rate	Notable toxicity	Notes and references
GD2.z	Neuroblastoma	Baylor College of Medicine	11	CR 27% (3/11) (2 sustained)	Transient pain (≤ grade 3) only at site of disease in 3/19 patients	Epstein–Barr virus–specific CTL; T cell persistence correlated with long-term survival; lymphodepletion only in some patients with anti-CD45 antibody[22,23]
GD2.28.Ox40.z	Neuroblastoma	Baylor College of Medicine	11	No objective responses; 3/11 showed decreased MIBG avidity	None	Evidence for robust T cell expansion when lymphodepletion was added, but transient persistence; no benefit to addition of anti-PD-1 therapy[41]
L1CAM.z	Neuroblastoma	City of Hope	6	No objective responses	None	No lymphodepletion; minimal T cell persistence[136]
Her2.28.BB.z	Colon cancer metastatic to the lung and liver	National Cancer Institute	1	NA	Patient died of respiratory failure; had very high levels of circulating cytokines consistent with CRS	Patient administered 10^{10} cells and exogenous IL-2; no dose escalation performed[75]
Her2.28.z	Sarcomas	Baylor College of Medicine	23	Without lymphodepletion: PR 6% (1/17) Tumor necrosis in some patients with lymphodepletion: CR 17% (1/6)	None	Evidence for robust T cell expansion and clinical efficacy when lymphodepletion was added, with no evidence for toxicity[25,76]
Her2.28.z	Glioblastoma	Baylor College of Medicine	16	PR 6% (1/16)	None	Intravenous administration without lymphodepletion; virus-specific T cells used[77]
IL13Rα2.z	Glioblastoma	City of Hope	3	NA (patients treated post-resection, but all patients relapsed post-infusion)	None	Intracavitary administration; antigen downregulation observed in one patient, with no lymphodepletion[127]
IL13Rα2.BB.z	Glioblastoma	City of Hope	1	CR, case report	None	Patient had PD with intratumoral injection but then had multifocal response to intracavitary injection into cerebrospinal fluid; no lymphodepletion[81]
EGFRvIII.BB.z	Glioblastoma	University of Pennsylvania	10	None noted	None	IV administration without lymphodepletion; evidence of T cell expansion, trafficking to the CNS, antigen-negative selection, and upregulation of inhibitory receptors[82]
CD133.BB.z	Hepatocellular, pancreatic and colorectal carcinoma	Chinese People's Liberation Army General Hospital	23	PR 13% (3/23)	None	Intravenous administration; some patients without lymphodepletion; biopsies demonstrated selection of $CD133^-$ tumor cells in some patients[137]
Mesothelin.BB.z	Pancreatic cancer	University of Pennsylvania	6	No objective responses; 1/6 showed decrease in PET avidity	Anaphylaxis in one patient	No lymphodepletion; RNA-based CAR; one PR also reported in a case report of a patient with mesothelioma[80,129,130]
CAIX.z	Metastatic renal cell carcinoma	Erasmus University Medical Center	12	No objective responses	Transient liver enzyme elevations probably caused by on-target, off tumor toxicity	No lymphodepletion; toxicity prevented by administration of anti-CAIX monoclonal antibody before T cell infusion[73]

Continued

Table 2 | Clinical trials of CAR T cells for solid tumors (continued)

Construct	Disease	Site	No. patients treated	Response rate	Notable toxicity	Notes and references
CEACAM5.z	Metastatic gastrointestinal cancer	Christie Hospital (UK)	14	No objective responses	Transient respiratory symptoms without a need for intubation in the setting of increased systemic cytokines; possible on-target, off-tumor toxicity or CRS	Increased T cell engraftment with flu/cy conditioning; biopsies demonstrated T cell trafficking to tumor sites[74,138]
CEA.28.z	Metastatic colon cancer	Southwest Hospital (China)	10	No objective responses; most patients showed decreases in tumor markers	None	Evidence of robust T cell expansion and clinical efficacy when lymphodepletion was added, with no evidence for toxicity[87,138]
CEA.28.z	Gastrointestinal cancer metastatic to liver	Roger Williams Medical Center	6	No objective responses; some patients showed decreases in tumor markers and tumor necrosis	None	Regional delivery through hepatic-artery infusions without lymphodepletion[139]
PSMA.z	Prostate cancer	Roger Williams Medical Center	5	No objective responses; some patients showed decreases in tumor markers	None	Lymphodepletion and exogenous IL-2 administered[140]
TAG-72.z	Colorectal cancer metastatic to liver	University of California, San Francisco; Stanford; and Mary Crowley Cancer Center	16	No objective responses	Symptoms consistent with CRS in two patients	No lymphodepletion; ten patients administered CAR intravenously, others regionally delivered through hepatic-artery infusions; exogenous interferon-α administered; many patients developed anti-CAR antibody response[141]
c-MET.BB.z	Breast cancer	University of Pennsylvania	6	No objective responses	None	No lymphodepletion; RNA-based CAR; intratumoral injections[142]

MIBG, metaiodobenzylguanidine; PET, positron emission tomography; PR, partial response; PD, progressive disease; NA, not applicable.

CD19.BB.z-CAR T cell for non-Hodgkin's lymphoma (NHL), preliminary data presented in abstract form indicate that only 30% of patients overall developed CRS; only one patient (1%) developed CRS of grade 3 or higher; and 20% of patients developed neurotoxicity, only 14% of whom had CRS of grade 3 or higher[34,62,63]. An earlier phase 1 trial of the same CD19.BB.z-CAR showed higher rates of CRS and neurotoxicity; however these findings may have been related to dose, because treatment at higher doses consistently elicits high levels of CRS and neurotoxicity[12]. Similarly, the JULIET trial using tisagenlecleucel (CD19.BB.z) in DLBCL demonstrated lower rates of severe neurotoxicity (12%) than were observed in the ZUMA-1 trial (28%) of axicabtagene ciloleucel (CD19.28.z). Together, emerging data from non-randomized trials suggest that in LBCL, CD19.BB.z-CAR T cells may manifest a more favorable safety profile.

Recent data from preclinical and clinical studies indicate that decreasing the signal strength of CAR T cells could result in diminished toxicity and enhanced T cell persistence. For instance, one group has engineered a CAR with a CD28 costimulatory domain and a truncated CD3-zeta domain containing only one of three ITAM domains that results in enhanced persistence and efficacy in animal models[64]. Other groups have engineered alternative regions of the CAR molecule to decrease CAR potency to decrease cytokine production and, by extension, severe CRS and neurotoxicity in patients[65–67]. CAR T cells engineered to decrease toxicity have already shown some efficacy in early-phase clinical trials[66–68], but whether such maneuvers to reduce potency might also dampen clinical outcomes remains unclear.

With regard to long-term toxicity, children treated with CD19.BB.z-CAR and CD22.BB.z-CAR T cells who show CD19-CAR or CD22 CAR persistence for 3 months typically develop hypogammaglobulinemia, and many have received replacement with pooled immunoglobulin[5,6,11], with very few infectious complications. However long-lived CD19$^-$ plasma cells are retained in some patients despite long-lasting CD19-CAR-induced B cell aplasia, and such cells can provide pathogen- and vaccine-specific immunoglobulin[69].

Lessons learned from clinical results by using CAR T cells for solid tumors

Despite evidence in preclinical models that CAR T cells can mediate impressive effects against solid tumors, definitive results in clinical trials are lacking (Table 2). Understanding the striking contrast between consistent CAR T efficacy in relapsed or refractory B cell malignancies and the limited signals of CAR T cell efficacy in solid tumors is a primary focus of investigation in the field.

Solid-tumor CARs and the potential for toxicity. A major concern facing CAR T cell therapies for solid tumors is the risk of on-target, off-tumor toxicity, because tumor-specific cell-surface antigens are

rare, and CARs targeting tumor-associated cell-surface antigens could target vital tissues expressing the same antigen. This concern has limited the number of trials launched and has led to substantial preclinical investment in creating systems to enhance specificity, such as CARs that kill only target cells that express both of two tumor-specific antigens, although these systems have not yet entered the clinic[70–72].

Of interest however, only limited on-target, off-tumor toxicity has been observed to date, and most trials have observed an absence of both toxicity and efficacy, often with limited expansion of the CAR T cells after adoptive transfer (Table 2). Some of these trials have used RNA-based CARs as an additional safety mechanism; these CARs are transiently expressed in T cells, but responses (and also potentially toxicity) are blunted as CAR expression is diluted out with each T cell division. This system may allow for the vetting of a new antigen with potential off-tumor toxicity, but the temporary expression may limit the usefulness of this approach. Overall, whereas the safety profiles from trials using CAR T cells for tumor-associated antigens in solid tumors are reassuring, many studies have not provided definitive evidence that the target is safe, because it remains possible that off-target toxicity might occur in the presence of greater CAR T cell expansion.

The most definitive evidence to date seen for on-target, off-tumor toxicity is in patients with renal cell carcinoma treated with a first-generation CAR targeting carbonic anhydrase IX (CAIX) administered with recombinant human IL-2, who experienced reversible liver-enzyme elevation[73]. The toxicity was ameliorated in subsequent cohorts by pre-treatment with an anti-CAIX monoclonal antibody. Low levels of CAIX-CARs persisted for a maximum of 74 days, but clinical responses were not observed[73]. Additionally, a study administering a first-generation CEACAM5 CAR with recombinant human IL-2 was halted because of transient respiratory toxicity associated with CAR T cell expansion and cytokine release; whether this finding was due to on-target, off-tumor toxicity remains unclear[74].

A therapeutic window for CAR T cells to target antigens shared with normal tissues. A third-generation Her2.28.BB.z-CAR, containing the single-chain variable fragment (scFv) derived from trastuzumab (a monoclonal antibody that recognizes Her2 and is clinically used in breast cancer patients) was tested in 2011, before the field's emerging understanding of the risks of CRS and the strong relationship between CAR T cell dose and CRS[75]. The first patient treated on this protocol received 10×10^9 CAR T cells, a number orders of magnitude higher than what has since been deemed a safe dose of CD19 CAR T cells, developed cardiorespiratory toxicity within 1 hour of infusion and ultimately died of multiorgan system failure[75]. The death was initially attributed to on-target off-tumor toxicity due to low-level expression of Her2 in lung tissue, but further analysis suggests that this death is more likely to be attributable to CRS. This patient was administered a dose well beyond what was eventually established as the maximum tolerated dose for CD19-CAR T cells and demonstrated very high levels of circulating cytokines, which are characteristic of CRS[75]. Furthermore, additional data now available demonstrate that Her2-CAR T cells administered in appropriate doses are safe, and show signs of clinical activity in sarcomas and gliomas, but no evidence of on-target, off-tumor toxicity[25,76,77].

The lack of on-target, off-tumor toxicity with Her2 CAR T cells is likely to be explainable by data demonstrating that CARs require high antigen expression for effective targeting, as has been shown for the CD22 CAR[11], a CAR targeting anaplastic lymphoma kinase[78] and a CAR targeting B7H3 (ref. [79]) (reviewed in ref. [21]). This property provides a potential therapeutic window for targeting tumor-associated cell-surface molecules with high differential expression between tumors and vital tissues.

Clinical results from using CAR T cells to target the diaganglioside GD2 are consistent with such a therapeutic window, because GD2 is highly expressed in several cancers but weakly expressed in the CNS[21] and peripheral nerves. A first-generation GD2-CAR has been reported to mediate CRs in 3 of 11 patients with neuroblastoma and to show no evidence of on-target, off-tumor toxicity[22,23]. A subsequent study using a third-generation GD2.28.OX40.z CAR (incorporating the same scFv) with or without anti-PD1 yielded no objective responses[41]. Despite these disappointing results, transient but substantial expansion did occur in some patients treated with the GD2.28.OX40.z-CAR, and the treatment was not associated with toxicity. Recently, preliminary data presented in abstract form have reported a CR in the bone marrow of a patient with neuroblastoma after GD2.28.z-CAR therapy incorporating a different scFv[24]. Finally, a GD2.BB.z-CAR targeting mouse and human GD2 administered to mice with diffuse intrinsic pontine glioma has been found to mediate impressive antitumor activity without evidence of on-target, off-tumor neurotoxicity, thus providing further evidence supporting a therapeutic window in which CAR T cells can preferentially target tumors with high levels of antigen while leaving tissues that express lower levels of the same antigen largely intact[21].

CARs targeting mesothelin, an antigen overexpressed in many carcinomas but also expressed on some normal endothelium, are also undergoing clinical testing in human studies in an attempt to exploit a therapeutic window between high expression in pancreatic, gastric, ovarian and lung cancers and low expression on serosal surfaces. Using RNA electroporation to transiently express the CAR, researchers have demonstrated T cell trafficking to the tumor site and transient partial responses in patients with mesothelioma and pancreatic ductal adenocarcinoma[80], without evidence of on-target, off-tumor toxicity. Further clinical studies using mesothelin-based CARs are ongoing.

CAR T cells for brain tumors. Substantial preclinical and clinical efforts are underway to develop effective CAR T cell–based treatments for brain tumors[77,81,82]. EGFRvIII, a mutated version of the epidermal growth factor receptor, is a compelling CAR target because it is absent in non-malignant tissues. EGFRvIII-CAR T cells have been administered intravenously to patients with relapsed glioblastoma multiforme (GBM), and post-infusion biopsies demonstrated CAR T cell trafficking to the site of disease, emergence of antigen-negative tumor cells and upregulation of PD-L1 (ref. [82]). This study demonstrated that CAR T cells can traffic across the blood–brain barrier but also revealed that clinical responses after treatment with monospecific CARs in GBM are likely to be limited both by tumor heterogeneity (discussed below)[82] and by adaptive resistance. Numerous groups are developing next-generation CAR T cell therapies for GBM, including multispecific CAR T cells and incorporation of next-generation CAR engineering approaches to overcome the inhibitory tumor microenvironment (discussed below)[83,84].

Emerging evidence suggests that the efficiency of CAR T cell trafficking to the CNS can be enhanced by administration of CAR T cells directly into the tumor bed and/or into the cerebrospinal-fluid space. IL13Rα2 CAR T cells administered into the cerebral ventricle mediated a CR in a patient with multifocal disease[81]; this report is notable for both complete disease eradication (even though IL13Rα2 expression was limited to only 70% of tumor cells, according to immunohistochemistry) and the ultimate emergence of $IL13^-$ disease. These results emphasize the importance of multispecific targeting and/or the induction of natural endogenous immune responses in eradicating heterogeneous tumors such as GBM[83,85]. As more clinical trials of CAR T cells for CNS tumors are undertaken, care will be required to assess safety from multiple perspectives, because patients with immune responses in the CNS may be susceptible to traditional CAR-mediated toxicities, such as

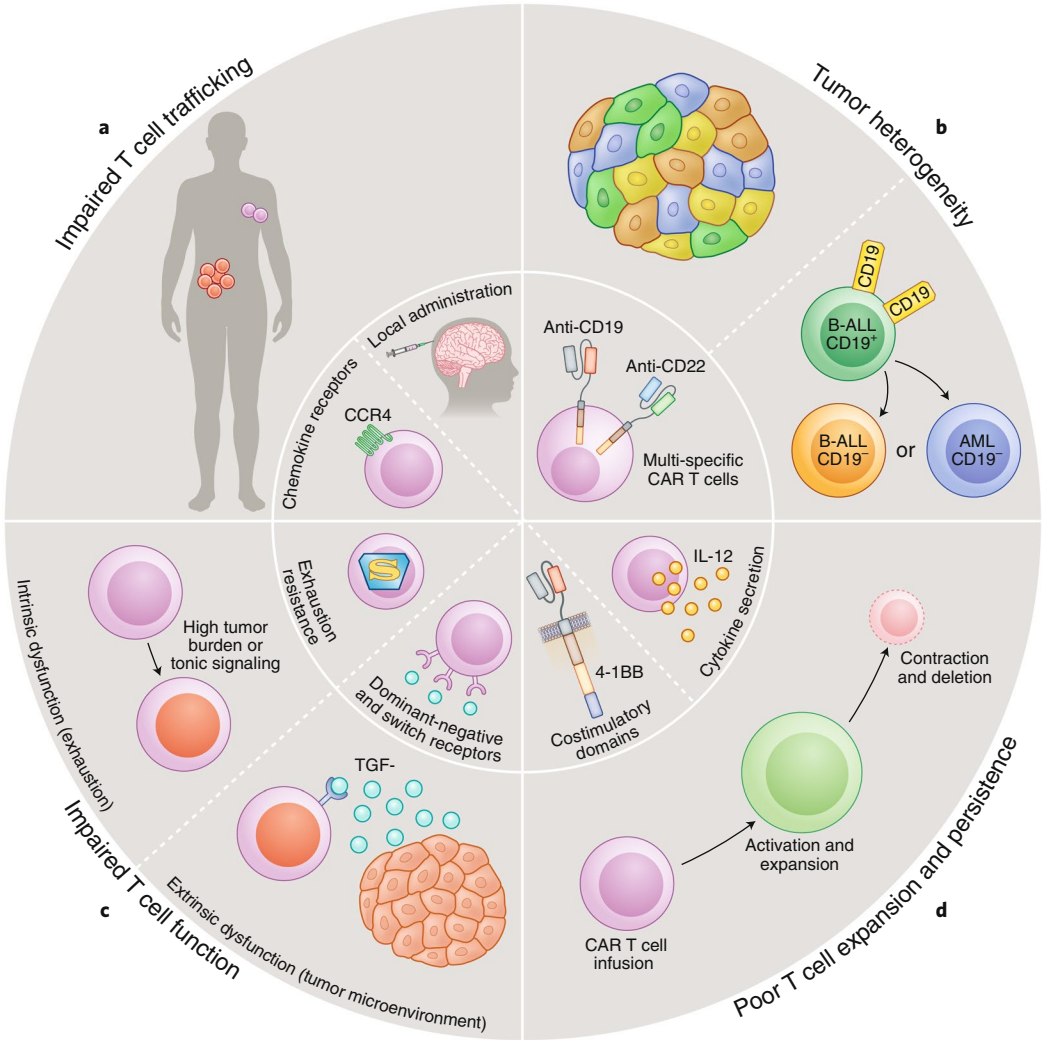


Fig. 2 | Overcoming obstacles to enhance CAR T cell efficacy. a, Intravenously administered CAR cells must traffic throughout the body to find tumor sites. T cell trafficking is limited in solid tumors, and also in the case of brain tumors, by the blood–brain barrier. T cell trafficking can be enhanced through coexpression of chemokine receptors on CAR T cells, which drive the cells to the sites of disease. Alternatively, CAR T cells can be administered regionally or directly into the site of the tumor. **b**, Tumor heterogeneity is a fundamental property of cancer, and clinical trials have already demonstrated relapse associated with CD19 loss induced by CAR T cell therapy. Next-generation CAR T cells can be endowed with specificity for multiple targets or could be engineered to induce natural antitumor immune responses to address this problem. **c**, T cells can be rendered dysfunctional by both intrinsic and extrinsic factors. Intrinsic dysfunction primarily results from T cell exhaustion driven by high antigen loads and/or tonic signaling. Next-generation exhaustion-resistant T cells are currently being designed. CAR T cell function can also be suppressed by extrinsic factors, such as cytokines (for example, TGF-β), ligands that signal via inhibitory receptors (for example, PD-L1) or competition for nutrients within the TME. So-called dominant-negative and switch receptors can convert or transform these signals. **d**, CAR T cell clinical success is dependent on engraftment and sufficient expansion to eliminate tumors. CAR T cell populations eventually either disappear or are maintained as a memory-like population protecting against future malignancy. Costimulatory domains such as 4-1BB contribute to CAR T cell persistence. So-called armored CARS constitutively secrete cytokines that can aid in T cell expansion and persistence. Credit: Debbie Maizels/Springer Nature

CRS and neurotoxicity; on-target, off-tumor toxicity; and toxicity due to tumor-associated inflammation, which could be associated with neurologic compromise caused by tumor swelling in precarious anatomic locations[21].

Although preclinical data have demonstrated the potential to target numerous proteins expressed in solid tumors, including B7-H3 (ref. [79]), EGFR[86], PSCA[87], CSPG4 (ref. [88]) and TEM8 (ref. [89]), fewer clinical trials of CAR T cell immunotherapies for solid tumors and CNS malignancies, compared with B cell malignancies, have been completed or even initiated. As principles regarding the safety of targeting antigens shared by normal tissues continue to evolve, and more sophisticated CAR T cell constructs become available to enable regulation[90] and logic gating[71], we anticipate increasing clinical activity in this area.

Enhancing CAR T cell efficacy

In addition to defining safe and promising antigen targets for solid tumors, researchers have focused on development of next-generation CAR therapeutics capable of overcoming therapeutic resistance (Fig. 2).

Overcoming antigenic heterogeneity. Heterogeneity is a hallmark of cancer, and therapeutics targeting one molecule rarely mediate complete tumor eradication. Unsurprisingly therefore, CD19

loss has emerged as the major cause of relapse after treatment with CD19-CAR T cells. In the global registration trial of tisagenlecleucel for B-ALL, wherein sustained immune pressure was induced by prolonged persistence of the CD19.BB.z-CAR T cells, 94% of relapses analyzed were attributed to CD19$^-$ disease[6] (Table 1). High rates of CD19$^-$ B-ALL were also observed after an alternative CD19.BB.z-CAR[8]. Although CD19$^-$ relapses have been reported after treatment with CD19.28.z-CAR T cells in B-ALL, CD19$^+$ relapse has been found to occur more commonly[9], probably because of an absence of sustained immune pressure (Fig. 1). Antigen escape has occurred as a result of CD19 splice variants that lack the exon recognized by the CAR or the transmembrane domain[91], mutations leading to truncation or absence of the CD19 transmembrane region[91,92], intracellular retention of CD19 (ref. [91]) and lineage switching from a lymphoid to a myeloid phenotype[8,93]. According to current thinking, cells bearing these genetic variants are present before therapy and are enriched by selection[94]; however, technologies are not yet available to identify patients at risk for immune escape before therapy.

The breadth of mutations reported in CD19 and the propensity for intracellular retention of the protein has lessened enthusiasm for targeting other epitopes on the CD19 molecule and fueled interest in targeting alternative pan–B cell antigens, such as CD22. CAR T cells targeting CD22 have been found to render most B-ALL patients into MRD-negative remission. These patients included CAR-naive patients and those who had previously developed CD19$^-$ leukemia after treatment with CD19-CAR T cells, thus providing evidence that intrinsic resistance to CAR-mediated killing is not likely to be an important cause of acquired resistance to CAR T cells[11].

CD19 loss has been observed in LBCL after CD19-CAR therapy[95–97]; however, the frequency and whether the biological mechanisms responsible for CD19 loss are the same as those in B-ALL remain unknown. BCMA$^-$ multiple myeloma has also emerged as a cause of relapse after response to treatment with a BCMA CAR[35], and relapse associated with downregulation of CD22 below a threshold necessary for CAR activation, rather than outright antigen loss[11], has been observed after CD22.BB.z-CAR T cell therapy.

Resistance due to antigen remodeling is expected to present an even greater obstacle to success when CAR T cells are used to treat acute myelogenous leukemia and solid tumors, because they demonstrate greater antigenic heterogeneity than B cell malignancies[98,99]. Consequently, several groups have developed bispecific CAR T cells, which can be accomplished by using one receptor incorporating two scFvs linked in a single molecule (tandem CAR); a bicistronic CAR in which two monospecific CARs are expressed from the same vector; or a mixture of vectors for expression of two different CARs during the transduction process, thus resulting in a mixed product[21] (Fig. 2b). Bispecific CD19/CD22 and CD19/CD20 CARs have recently entered clinical trials, and early signs of clinical activity associated with a favorable safety profile have been reported with all three approaches[100–104]. Preclinical studies using multispecific CARs to target solid tumors and CNS malignancies have been reported, including a tandem Her2/IL13Rα2 CAR that eliminated single-antigen-positive tumor cells and demonstrated enhanced activity against double-antigen-positive cells, owing to the formation of a stronger immune synapse[83]. Trispecific CARs have also been developed for both glioblastoma and B cell malignancies[99,105].

An alternative and potentially complementary approach to overcome tumor heterogeneity is to enhance the capacity for CAR T cells to induce a native T cell response. In so-called epitope spreading, the inflammation induced by CAR T cells enhances the presentation of neo-antigens recognized by the host immune system, thus sparking an anti-tumor immune response by the native immune system. This response could lead to elimination of CAR-targeted antigen-negative tumor cells, because the unleased native T cells recognize other tumor antigens through their T cell receptors (TCRs). The potential of this approach has been demonstrated in a mouse model in which EGFRvIII CAR T cells led to elimination of both antigen-positive and antigen-negative tumor cells[85]. This effect can be potentiated in mice by combining CAR T cells with radiotherapy and increasing the susceptibility of tumor cells to death mediated by TNF-related apoptosis-inducing ligand[106]. There is no evidence to date in clinical trials that CAR T cells induce native T cell responses, but this possibility has not been extensively studied, and the absence of such reported phenomena may relate to the low inherent immunogenicity of the diseases, such as B-ALL, that have been targeted to date[107].

Improving CAR potency and persistence. T cell dysfunction can be driven by T cell intrinsic factors, known as T cell exhaustion, as well as extrinsic immunosuppression mediated by the TME (Fig. 2c). T cell exhaustion is emerging as a major feature limiting the efficacy of CAR T cells, and it can be induced by excessive stimulation due to high tumor burdens, as well as antigen-independent signaling induced by aggregation of CAR receptors[51,108,109]. In a trial of CD19.BB.z-CAR T cells for LBCL, non-responders demonstrated higher expression of canonical exhaustion markers on CAR T cells found at tumor sites and the bone marrow than did patients who had a CR[29]. Moreover, in a trial of the same agent in CLL, features of exhaustion in T cells contained in both the apheresis products used to engineer the CAR T cells and the manufactured CAR T cell products themselves have been found to be predictive of non-response[27].

A recent case report has demonstrated impressive potency of a single T cell clone in which disruption of the Tet methylcytosine dioxygenase 2 (*TET2*) gene occurred because of CAR integration into that locus and consequently resulted in loss of function, thus raising the prospect that genetic engineering might render CAR T cells exhaustion-resistant[110]. Alternatively, placing the CAR under control of the native TCR promoter by using CRISPR–Cas9 and AAV6-mediated insertion has been reported to diminish exhaustion and enhance potency[111]. A plethora of approaches to engineer CAR T cells that are more resistant to suppressive factors within the TME are also under development (reviewed in ref. [3]), including dominant-negative TGF-β receptors coexpressed with a CAR[112], switch receptors that activate CD28 in response to PD1 ligation[84] and CARs that specifically target tumor-associated macrophages[113]. Clinical trials of such next-generation therapeutics are not yet mature, and therefore whether one particular approach will emerge as the most effective remains unknown.

In addition to engineering T cells for enhanced potency, a related challenge is enhancing CAR T cell persistence. Whether persistence beyond several days or weeks will be required for effective CAR T cell therapy of solid tumors remains unclear, because the experience in B cell malignancies suggests that the need for long-term persistence may vary with disease. In a trial of a first-generation anti-GD2 CAR in neuroblastoma, prolonged T cell persistence was associated with longer survival[23], and a recent study of adoptively transferred T cells transduced with an NY-ESO-specific TCR in synovial sarcoma has reported that clinical responses occurred over several months and were associated with prolonged T cell persistence[114]. As discussed above, CARs containing the 4-1BB costimulatory domain typically persist longer than those incorporating a CD28 costimulatory domain, although they do not expand to such high numbers in patients. Recent work has demonstrated that expression of 4-1BB ligand alongside a CAR containing the CD28 costimulatory domain increases both anti-tumor efficacy and T cell persistence[115]. Other preclinical studies have focused on integrating cytokine signaling (such as IL-7 signaling) into CAR T cells, which has resulted in enhanced potency and persistence of CARs in preclinical models of solid tumors[116,117]. Finally, so-called 'armored CARS' that constitutively secrete cytokines such as IL-12, IL-15 and IL-18 appear capable of enhancing T cell persistence and host anti-tumor immunity in animal models and are now being translated to the clinic[118–121] (Fig. 2d).

An alternative approach to improving CAR T cell persistence is to decrease host anti-CAR T cell responses by using an effective lymphodepletion regimen (discussed above)[12] or by administering CARs incorporating humanized or fully human binders[122,123]. Early clinical data from a trial in B-ALL have demonstrated that complete responses can be induced by a humanized CAR in some patients who experienced loss of persistence of a CD19-CAR containing a murine scFv[122], presumably because of host T cell–mediated rejection.

Enhancing T cell trafficking. Trafficking of CAR T cells to tumor sites has been demonstrated in non-CNS solid tumors[80,124] and brain tumors[82]; however, the limited overall efficacy might possibly relate, at least in part, to too few cells penetrating the tumor parenchyma[76]. Preclinical studies have shown that endowing T cells with chemokine receptors such as CXCR2, CXCR4 and CCR2 can enhance T cell trafficking to the site of disease in both lymphomas and solid tumors[125–128]. Clinical trials are testing this approach for $CD30^+$ NHL with CAR T cells cotransduced with CCR4, but more sustained efforts to measure and enhance T cell trafficking in clinical trials are warranted (Fig. 2a).

An alternative approach to enhance trafficking is to deliver CAR T cells either directly or regionally at the site of the tumor. Regional delivery appears particularly appealing for CAR therapy of CNS tumors, because CAR T cells can be delivered safely into the cerebrospinal fluid[81], and preclinical models demonstrate that lower doses are necessary for complete tumor response in using this approach[129,130]. Administering CAR T cells directly into the CNS could also potentially diminish the risk of systemic toxicity and thus allow for targeting of antigens whose expression on vital normal tissues is restricted to the periphery (Fig. 2a).

Conclusions and future directions

CAR T cell therapy has induced impressive responses and significant clinical benefit in patients with several B cell malignancies, and early results indicate some signals of clinical activity in solid tumors. As advances in bioengineering enable a plethora of approaches to improve the efficacy of CAR T cells in preclinical models[3], physician scientists must carefully select which of these to test in human trials. The most effective approaches will draw on clinical observations and careful correlative studies from human trials to understand the major drivers of resistance biology for this new class of therapeutics. To date, human trials of CAR T cells for B cell malignancies have highlighted the dual challenges of antigen escape due to tumor heterogeneity and T cell dysfunction as major barriers to progress. Next-generation therapeutics designed to overcome these barriers are poised to improve upon already impressive outcomes in B cell malignancies and to enable the reach of these therapeutics to other hematologic malignancies and solid tumors.

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