nature biomedical engineering

Review article

https://doi.org/10.1038/s41551-025-01492-8

Fine tuning towards the next generation of engineered T cells

Received: 27 August 2024

Accepted: 1 August 2025

Published online: 10 October 2025

Tham T. Nguyen[1], Patrick Ho[2], Sarah Staudt[2], Celine Gregoire[3,4], Kai Ziegler-Martin[2], Megane Jassin[1], Alix Block[1], Michael Hudecek[2,5,6,7], J. Joseph Melenhorst[3], Jo Caers[1,4] & Maik Luu[2,5,6,7]

Chimeric antigen receptor (CAR) T cell therapy has achieved remarkable success in treating haematologic malignancies. However, the rise in clinical use has highlighted substantial challenges related to T cell- and tumour-intrinsic mechanisms. Additionally, the tumour microenvironment can render these treatments dysfunctional. Extensive attempts in the field are optimizing the key elements of CAR T cell products for therapy, including antigen specificity and affinity, metabolic fitness, phenotypic stability and manufacturing. Recent efforts in transcriptomic and epigenetic profiling, as well as high-throughput functional screening methods, have identified new classes of targets, binders and mechanisms to be exploited. Advances in gene editing and delivery offer opportunities to translate those strategies into clinical trials. Here we discuss the multifaceted exploration of CAR T cell engineering approaches and emerging directions, highlighting the available strategies that can be built on to create the next generation of cellular therapies.

Chimeric antigen receptor (CAR) T cell therapy involves the genetic engineering of a patient's T cells to express CARs that specifically target cancer cells in a major histocompatibility complex (MHC)-independent manner. This allows the treatment of patients with malignancies carrying a common target antigen with the same CAR construct—which can be introduced into their polyclonal T cell pool—despite the diversity in haplotypes. Originating from work using retroviral vectors for gene introduction in T cells, this technique enabled the development of a first-generation 'T-body' CAR, and subsequently five further generations (so far)[1–3]. Over the past decade, CAR T cell therapy has shown success in treating haematological malignancies, with a substantial boost when the United States Food and Drug Administration (FDA) approved the first two CAR T cell products, Tisa-cel and Axi-cel, developed at the University of Pennsylvania and the National Cancer Institute, respectively. Ongoing preclinical research and clinical trials are expanding CAR T cell therapies to combat solid tumours by improving the homing, infiltration, expansion and survival of CAR T cells within the hostile tumour microenvironment (TME). This has been accompanied by advances in smart CAR design and engineering strategies. In this Review we summarize the current landscape of developments in the CAR T cell field, providing an overview of the currently available concepts and tools as a foundation for the next generation of tailored T cell therapies.

Key elements of the CAR design

CARs combine the MHC-independent ability of antibodies to bind either membrane-bound or soluble antigens with the signal-transducing properties of the T cell receptor (TCR) machinery. The binding domain specifies antigen reactivity, and the precise combination of signalling and non-signalling (that is, structural) domains determines antigen sensitivity.

[1]Laboratory of Hematology, GIGA, University of Liege, Liege, Belgium. [2]Lehrstuhl für Zelluläre Immuntherapie, Medizinische Klinik und Poliklinik II, Universitätsklinikum Würzburg, Würzburg, Germany. [3]Cell Therapy & Immuno-Engineering Program, Center for ImmunoTherapy and Precision Immuno-Oncology, Lerner College of Medicine, Cleveland Clinic, Cleveland, OH, USA. [4]Department of Hematology, University Hospital of Liege, Liege, Belgium. [5]National Center for Tumor Therapy (NCT WERA), Würzburg, Germany. [6]Bavarian Cancer Research Center (BZKF), Würzburg, Germany. [7]Außenstelle Zelluläre Immuntherapie, Fraunhofer Institut für Zelltherapie und Immunologie (IZI), Würzburg, Germany. e-mail: Luu_m@ukw.de

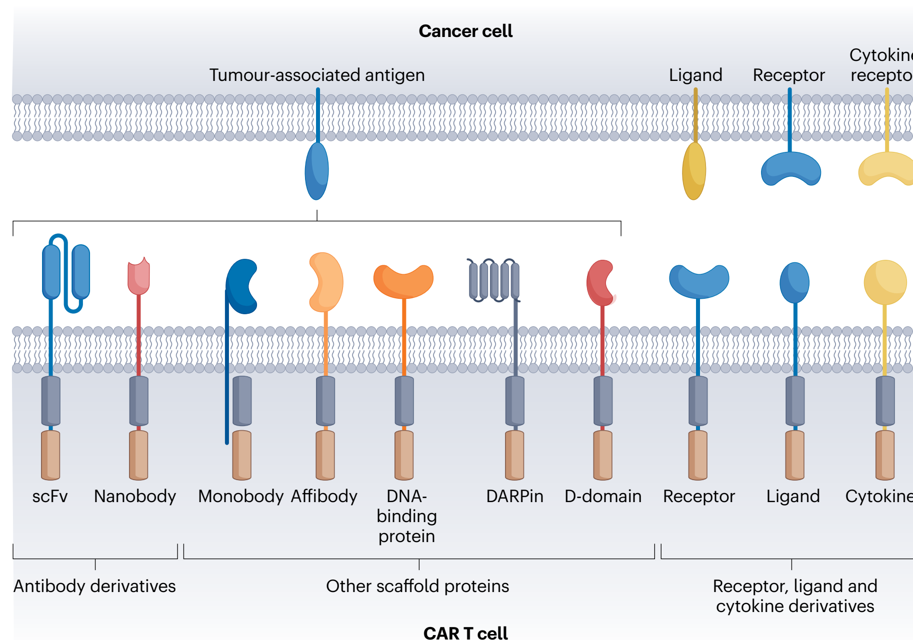

Fig. 1 | Alternative binding domains in CAR engineering. The binding domain of conventional CARs can be replaced by alternative antibody derivatives and other scaffold proteins such as monobodies, affibodies, DNA-binding proteins, DARPins or D-domains. Physiological binding can be exploited for CARs by use of the ectodomain of natural receptors, ligands and cytokines.

Binding domains

Antibody binding domain. The earliest CAR architecture substituted the TCR Vα and Vβ domains with a single-chain variable fragment (scFv) derived from the variable heavy (VH) and light (VL) chains of a conventional antibody. The resulting T-bodies triggered TCR-mediated signalling upon scFv-mediated antigen engagement, a strategy that has been refined through subsequent generations of CAR design[3,4]. As antibody-derived moieties, scFvs can be generated rapidly against defined target antigens through well-established methods of antibody development, including in vivo immunization, hybridoma technology, in vitro phage display, and recombinant protein production. Coupled with their high degree of antigen specificity, their relative ease of development makes scFvs a flexible platform for CAR engineering[5]. However, the size (30 kDa) and multidomain structure of scFvs can lead to improper protein folding or protein aggregation, which manifest in tonic signalling, T cell exhaustion and loss of therapeutic efficacy[6,7]. Moreover, scFvs carry a risk of immunogenicity unless fully humanized[8]. Therefore, despite their widespread use in CAR design, various alternatives have been developed (Fig. 1).

Single-variable heavy domain of heavy-chain (VHH) antibody (nanobody). Nanobodies are the smallest antigen binding unit (~15 kDa) of a heavy chain-only antibody that contain a long complementarity determining region 3 (CDR3) essential for mediating specific epitope binding with high affinity[9]. Their compact and small structure simplifies production and integration into multiple target constructs. VHHs can replace scFvs in CAR constructs, giving rise to the so-called nanoCAR, which may induce less tonic signalling[10,11]. Notably, ciltacabtagene autoleucel became the first and only B cell maturation antigen (BCMA) nanoCAR T cell product approved by the FDA in 2022, using two VHHs that bind to two distinct epitopes of BCMA[12]. More recently developed nanoCARs tested in preclinical settings are summarized in Table 1.

Alternative scaffold proteins. Further attempts have been made to generate CAR T cells with even smaller protein binders that aim to reduce immunogenicity, simplify folding and enhance binding avidity. Unlike natural protein-binding scaffolds, designed ankyrin repeat proteins (DARPins) are de novo synthetic binders characterized by their compact size, typically around 14–17 kDa, with two α-helices and one β-turn. They are usually composed of four or five repeats with ~33 amino acids per unit. Preclinical studies have shown that HER2-targeting DARPin-based CAR T cells exhibit comparable cytotoxicity, cytokine secretion and antitumour responses relative to conventional scFv-based controls[13]. Moreover, multispecific DARPin CAR T cells against epidermal growth factor (EGFR), epithelial cell adhesion molecule and human epidermal growth factor receptor 2 (HER2) have shown synergistic antitumour effects in heterogeneous tumours[14].

The D-domain is another synthetic binding domain recently implemented in CAR designs. It represents a compact 8-kDa de novo-designed mini protein complex comprising three helices, devoid of disulfide bonds and N-linked glycosylation[15]. D-domain-based CAR T cells (ddCAR) have shown comparable activity to scFV-based CARs against $CD123^+$ and $BCMA^+$ tumour cells in preclinical models[16,17]. Based on this observation, a phase 1 clinical trial study has reported the safety of CART-ddBCMA, with high response rates, in relapsed/refractory multiple myeloma (MM)[18]. Of note, the potential immunogenicity of non-traditional binding domains remains a key concern. As of today, several studies have mentioned this aspect but have not investigated the persistence of the constructs.

Natural receptor-, ligand- and cytokine-based CARs. Physiological interaction partners can also be exploited to generate receptor- or ligand-based CARs. However, the incorporation of native receptor–ligand interactions carries a heightened risk of off-target effects, requiring careful context-specific consideration. Examples of receptor-based approaches include CD27-based CAR T cells targeting CD70, CD16-based CAR T cells targeting fragment crystallizable (Fc) and NKG2D-based CAR T cells being tested in various cancer models[19–26] (Table 1).

Similarly, certain cytokines have been used to replace the scFv as binding domain in CARs, creating cytokine-based CAR T cells that redirect their cytotoxicity towards cancer cells expressing the

https://doi.org/10.1038/s41551-025-01492-8

Table 1 | Alternative binding domains for CAR designs

Binding domain of CAR	Target for CAR T	Cancer models	Reference
Antibody-derived fragment			
Nanobody	BCMA	MM	12
	TAG-72	CRC, ES, T-ALL	213
	GPC2	NB	214
	CD38	MM	215
	CD20, HER2	T-ALL	216
	CD20	B-NHL	217
	PD-L1, EIIIB	Melanoma, CRC	218
	EGFR	BC, OC	219
	CD7	ALL	220 221
	CD105	HCC	222
	B7H3	NB, HCC, ES	223
Other protein scaffold			
Monobody (adnectin)	EGFR	Solid tumours	32
Affibody	HER2, EGFR	ALL	33
Thermostable DNA-binding protein	RBP4	ALL	34
D-domain	CD123	AML	17
	BCMA	MM	16
		RRMM	NCT04155749
DARPin	HER2	BC, melanoma	224
		BC, OC	13
		Solid tumours	225
	EGFR, EPCAM, HER2	BC, NSCLC	14
Ligand-based CAR			
FLT3L	FLT3	AML	226
EPHRIN B2	EPHB4	RMS	227
CTLX	CTLXR	GBM	228
T1E	ERBB2	HNSCC	NCT01818323
		BC	229
APRIL	BCMA, TACI	MM	NCT03287804 NCT04657861
TriPRIL	BCMA, TACI	MM	230
BAFF	BAFF-R, BCMA, TACI	MCL, MM, ALL	231
LFA 1	ICAM-I	TC	232 NCT04420754
FSH	FSHR	OC	233
TPO	MPL	AML	234
GM-CSF	GMR	AML, JMML	235
EGFRL	EGFR	NSCLC	236
Receptor-based CAR			
CD27	CD70	Various	19 NCT02830724
CD16	Fc	Various	22,237 NCT03266692 NCT02776813
NKG2D	NKG2DL (MICA, MICB, ULBP1-6)	Various	NCT04107142 NCT03415100 NCT02203825
B7H6	NKp30	TLBL, OC	238
BAFT, B7H6	NKp30	Various	239,240
Cytokine-based CAR			
IL-10	IL-10R	AML	27
IL-11	IL-11R	OS	28
IL-13	IL-13R	GBM	30 NCT05540873 NCT04661384

Disease models: CRC, colorectal cancer; ES, epithelioid sarcoma; T-ALL, T cell acute lymphoblastic leukaemia; NB, neuroblastoma; MM, multiple myeloma; B-NHL, B cell non-Hodgkin lymphoma; ALL, acute lymphoblastic leukaemia; HCC, hepatocellular carcinoma; AML, acute myelogenous leukaemia; RRMM, relapsed/refractory multiple myeloma; RMS, rhabdomyosarcoma; GBM, glioblastoma; HNSCC, head and neck squamous cell carcinoma; JMML, juvenile myelomonocytic leukaemia; NSLCC, non-small lung cancer cells; OS, osteosarcoma; OC, ovarian cancer; BC, breast cancer; T-LBL, T cell–lymphoblastic lymphoma; TC, thyroid cancer. Other abbreviations: TAG-72, tumour-associated glycoprotein 72; GPC2, glypican 2; HER2, human epidermal growth factor receptor 2; PD-L1, programmed death-ligand 1; EIIIB, extra domain B fibronectin; EGFR, epidermal growth factor receptor; B7H6, B7-homologue 6; RBP4, retinol binding protein 4; EpCAM, epithelial cell adhesion molecule; FLT3, FMS-like tyrosine kinase-3; CTLXR, chlorotoxin receptor; ErbB, receptor tyrosine kinases including HER1, HER2, HER3, HER4; TACI, transmembrane activator and CAML interactor; ICAM-I, intercellular adhesion molecule; FSHR, follicle-stimulating hormone receptor; MLP, myeloproliferative leukaemia protein; GMR, granulocyte–macrophage colony-stimulating factor GM-CSF receptor; NKG2D, natural killer group 2D; MICA and B, MHC class I polypeptide-related sequence A and B; NKp30, NK cell-activating receptor.

corresponding cytokine receptors, such as interleukin-10 (IL-10), IL-11 and IL-13[27–30]. In a different engineering approach, IL-12 has been incorporated in tandem between the scFv and transmembrane domain to trigger endogenous IL-12 signalling in T cells, rather than acting as binding domain. The engineered cells showed increased reactivity against cancer cells with antigen loss through an antigen-independent NK-like killing mechanism[31]. Other examples of small binding modules currently in the preclinical investigation phase include monobodies (10 kDa), affibodies (5 kDa) and thermostable DNA-binding proteins[32–34].

Signalling domains

The activation of T cells relies primarily on antigen-driven TCR stimulation and a co-stimulation signal. Mimicking both signals using synthetic CARs was achieved by fusion of binding domains to co-stimulatory domains such as CD28 or 4-1BB (CD28- or 4-1BB-based), followed by the CD3ζ signalling domain (Fig. 2). The incorporation of a single co-stimulatory domain marks the second generation of CAR design, and has enhanced CAR-mediated T cell activation and antitumour responses, enabling translation into the clinic.

Upon TCR stimulation by antigen–MHC binding, the three immunoreceptor tyrosine-based activation motifs (ITAMs) in CD3ζ are phosphorylated at their tyrosine residues by Lck, initiating downstream activation cascades[35,36].

In the effort to enhance persistence, a recent study introduced point mutations at ITAM1, 2 or 3 within CD3ζ in CD28-based (28ζ) CAR T cells. The study found that single-ITAM-based CARs, particularly ITAM1 (1xx), favour a memory T cells and anticancer response[37], a finding later confirmed by others[38]. In a phase I clinical study for relapsed or refractory diffuse large B cell lymphoma, CD19-28ζ1xx CAR T cells were demonstrated to be safe and able to induce complete remissions in 63% of patients[39]. Alternatives to the CD3ζ signalling domain have been proposed over the years, from FcεRIγ to TREM1/DAP12, and most recently CD3δ, CD3ε or CD3γ[40–42]. Although these candidates require further validation, CD3ζ has been established as a reliable but tunable signalling backbone that can potentially direct T cell fate during the antitumour response.

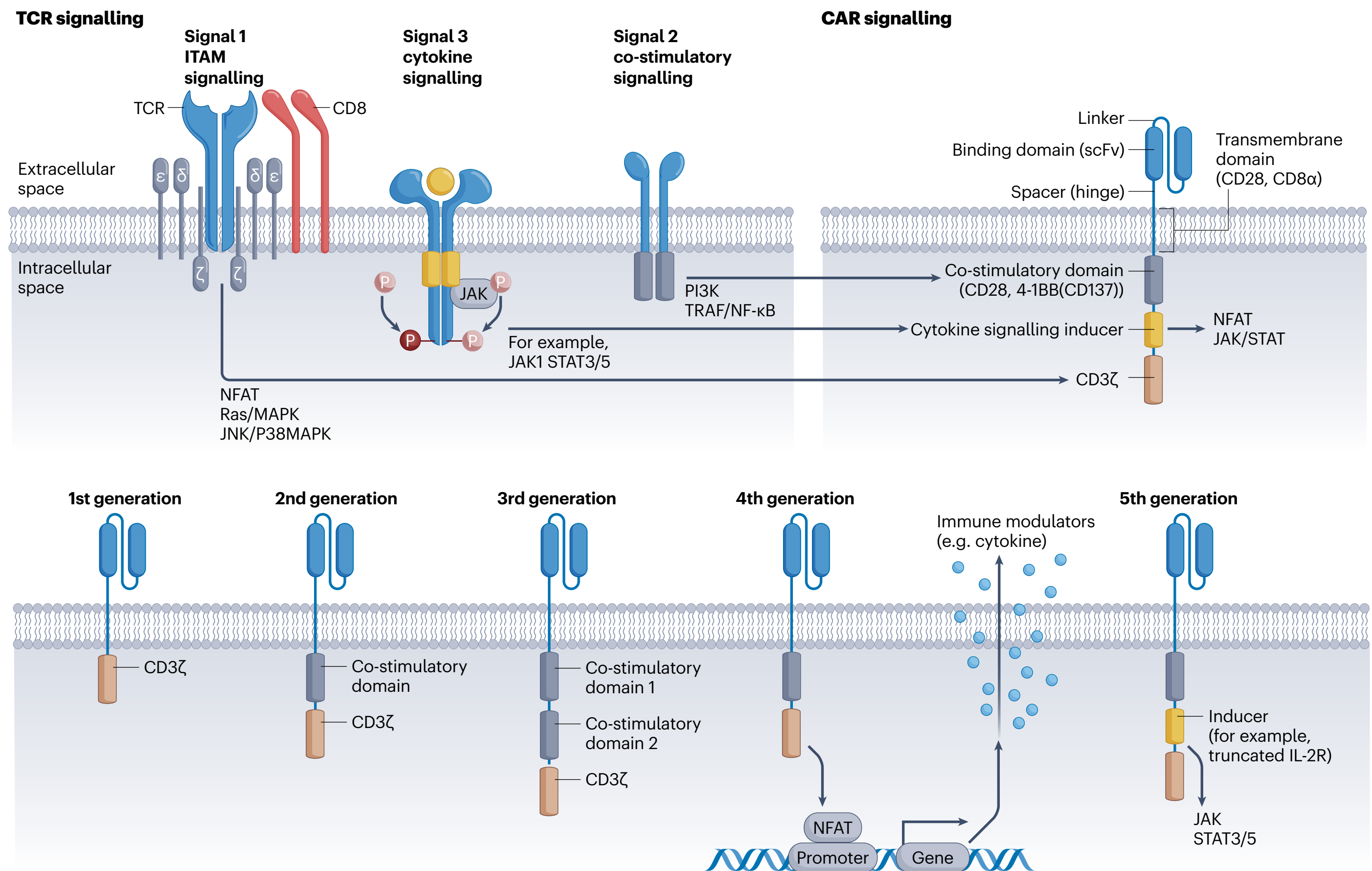

Fig. 2 | Evolution of the CAR structure. The CAR concept originated from exploitation of TCR signalling elements including CD3ζ (ITAM), co-stimulatory and cytokine signalling. The CAR structure has evolved from the first generation (1G) to the fifth generation (5G). Initially, the CAR intracellular domain featured solely the CD3ζ signalling domain (1G). Subsequent iterations involved the addition of one co-stimulatory domain (2G) or two co-stimulatory domains (3G). The 4G CAR incorporated nuclear factor of activated T cells (NFAT) signalling to enable the secretion of immunomodulators such as cytokines. The 5G CAR contains an inducer element derived from the IL2RB inserted between CD3ζ and the co-stimulatory domain (5G). The IL2RB fragment contains a STAT3 binding motif to facilitate antigen-dependent activation of the JAK-STAT pathway.

Co-stimulatory domain

Introduction of the co-stimulatory domain CD28 into the CAR structure allowed CAR T cells to survive longer in vivo, and plays a crucial role in determining CAR T cell efficacy, differentiation, safety and kinetics[3,37–40,43–46]. Screens for additional effective co-stimulatory domains have identified several candidates.

For example, the CD28 co-stimulatory domain, derived from the natural CD28 receptor found on T cells, serves as an accelerator of CAR T cell activation. Incorporation of this domain into CAR constructs enhances the recognition and elimination of cancer cells, promoting cell proliferation and expansion while ensuring a sustained antitumour response[40,45]. CD28 signalling triggers PI3 kinase/AKT/Bcl-XL-mediated activation in CD8$^+$ T cells[45]. However, although CD28-based CAR T cells demonstrate superior cytotoxicity, they are associated with higher toxicity and reduced persistence compared to their 4-1BB counterparts[46–49].

CD28-based CAR T cells tend to favour a glycolytic metabolism, which provides rapid bursts of energy enabling immediate cell activation upon antigen encounter and enables an effective and rapid cytotoxic response of CAR T cells towards target cells[50,51]. However, unchecked CD28-mediated signalling and metabolic rewiring can also result in limited persistence. Mutations in the CD28 signalling motif have been shown to calibrate 28ζ-CAR T cells to retain high responsiveness without compromising durability, and long-lasting antitumour responses both in vitro and in vivo[52].

The 4-1BB (CD137) co-stimulatory domain, derived from the natural 4-1BB receptor expressed on T cells, is a key driver of T cell activation signalling and significantly enhances CAR T cell persistence. CAR T cells bearing 4-1BB (4-1BB-based) fused to CD3ζ (4-1BBζ) showed improved survival and efficacy in mice[53]. In line with this, it was found that 4-1BB specifically promotes CAR T cells memory formation, cytokine secretion, and especially persistence in vitro and in vivo[53], CD19 4-1BB-based CAR T cells could induce tumour control in patients with chronic lymphocytic leukaemia that lasted up to ten years[54]. Mechanistically, CAR T cells bearing 4-1BB are less prone to exhaustion induced by tonic signalling[7]. Relative to 28ζ CAR T cells, 4-1BBζ CAR T cells show enhanced expression of activation gene signatures, including human leukocyte antigen (HLA) class II genes, ENPP2 and IL-21 axis genes, and decreased PD-1 expression[55]. Moreover, 4-1BB-based CAR T cells exhibit a preference for oxidative metabolism and tend to promote prolonged and sustained activity, potentially leading to more durable therapeutic responses. 4-1BB promotes the expression of peroxisome proliferator-activated receptor-γ co-activator-1α (PGC-1α) through the p38 MAPK pathway, thereby initiating mitochondrial biogenesis and oxidative metabolism[56]. Of note, the combination of CD28-4-1BB fused to an scFv binder, referred to as third-generation CAR (Fig. 2), has been demonstrated to elicit superior function in certain tumour models, such as B cell acute lymphoblastic leukaemia (B-ALL), mesothelioma or neuroblastoma, as compared to the second-generation CAR design[50,53,57,58].

https://doi.org/10.1038/s41551-025-01492-8

Table 2 | Alternative co-stimulatory domains for CAR T cells

Co-stimulatory domains	Target for CAR T cells	Cancer models	Preclinical–clinical studies
OX40	GD2	NB	241
	CEA	CRC	242
	GD2	NB	243
	CD30	HL, NHL	244
	CD20	BCL	245
OX40+ICOS	ROR1	MCL	62
ICOS	MSLN	MESO	60
	CD19	Various	246
CD27	CD19	BC, OC	63
	NKG2D	BC	247
	NKG2D	BC	246
	GD2	Solid tumours	248
MyD88/CD40	EPHA2	GBM, OS, BCL	64
	CD19, CD123	GBM	65
CD2	HER2, EGFR, CD19	Various	249
TLRs (TLR2)	CD19	RR-ALL	250
	CD19, MSLN	ALL, NSCLC	251
IL-2Rβ	CD19	ALL	59
IL-15R	CD19	ALL, CNSL	252

Disease models: RR DLBCL, relapsed/refractory diffuse large B cell lymphoma; MCL, mantle cell lymphoma; MESO, mesothelioma; LCC, large cell carcinoma; B-ALL, B cell acute lymphoblastic leukaemia; HL, NHL, Hodgkin or non-Hodgkin lymphomas; BCL, B cell lymphoma; PCa, prostate cancer; RR-ALL, relapsed/refractory acute lymphoblastic leukaemia; CNSL, central nervous system leukaemia. Other abbreviations: MSLN, mesothelin; GD2, disialoganglioside; CEA, carcino-embryonic antigen; ROR1, receptor tyrosine kinase-like orphan receptor 1; EPHA2, erythropoietin-producing hepatocellular carcinoma A2; PSMA, prostate-specific membrane antigen.

Efforts are under way to explore alternatives to CD28 and 4-1BB, resulting in an expanding body of reports (Table 2). One example is the incorporation of a truncated IL-2Rβ between the co-stimulatory domain, 4-1BB or CD28, and CD3ζ, which facilitates the recruitment of STAT3 and STAT5, initiating signalling cascades for T cell activation (Fig. 2), and can achieve superior efficacy, proliferation and persistence compared to 4-1BB or CD28 second-generation configurations in a context-dependent manner[59]. Commonly activated pathways such as MAPK, PI3K–AKT–mTOR and TRAF–NF-κB operate at moderate levels to promote cell survival and immune responses while inhibiting exhaustion and differentiation. ICOS (CD278) acts as an analogue of CD28, featuring a distinct binding motif (YMFM) for activation of the PI3K–AKT pathway[60]. In line with that concept, the combination of 4-1BB with membrane-proximal ICOS (ICOS-41BB-CD3ζ), rather than membrane-distal ICOS (41BB-ICOS-CD3ζ), induces a higher antitumour response and cytokine secretion in solid tumours[61]. Furthermore, the tandem of ICOS and OX40 replacing the CD28 co-stimulatory domain showed a durable tumour response in a lymphoma xenograft model[62]. A CD27 (TNFRSF7)-CD3ζ tandem CAR was designed capable of upregulating Bcl-xL in CAR T cells, promoting persistence comparable to 4-1BB[63]. Furthermore, CAR T cells bearing MyD88/CD40 as co-stimulatory domains outperformed 4-1BB in T cell persistence in preclinical models[64,65]. Notably, MyD88/CD40 CARs induce a high level of key cell-cycle regulators during receptor-mediated activation. The modified T cells are characterized by enhanced proliferative capacity and antitumour activity in chronic activation assays, as well as more robust tumour control in vivo[64].

Non-signalling domains

Although the signalling domains of CARs are well recognized for their role in initiating CAR T cell responses against cancer cells, non-signalling domains—including the linker, spacer, hinge and transmembrane regions—have an important yet often overlooked impact on CAR accessibility and affinity for their targets.

The spacer, located between the binding domain and transmembrane domain, can be derived from CD8α, IgG3-Fc, FcγRII or IgG4 Fc, CD28 or TNFSFR19, and impacts both structural flexibility and orientation towards the antigen. We have reported that a longer spacer improves the binding interaction between a ROR1 CAR and ROR1 antigen, therefore enabling engineered T cells to kill ROR1$^+$ cells more efficiently than alternative designs with a shorter spacer[66,67]. The use of spacers derived from the IgG4 Fc was found to potentially bind to FCγRs impeding CAR T cell function. Introducing mutations within the IgG4 Fc spacer that abolish Fc receptor binding results in improved T cell engraftment and effector function in vivo[68]. Furthermore, a CD8α-derived transmembrane domain elicited decreased secretion of inflammatory cytokines and activation-induced cell death (AICD) compared to the CD28-derived counterpart, while showing similar efficacy in a CD19-dependent in vivo model[69].

The transmembrane domain anchors the CAR construct in the T cell membrane, connecting the extracellular antigen-recognition domain and the intracellular signalling components. One of the most established domain anchors is derived from CD28 and can form homodimers upon T cell activation. This dimerization process is thought to bring the cytoplasmic signalling domains into close proximity, promoting downstream signalling through molecules such as PI3K, AKT and mTOR. These pathways are important for cell survival, proliferation and cytokine production. Besides exploitation of the TCR and its co-stimulatory elements for conventional CARs, ongoing research efforts are considering domains beyond the established designs, such as by using DNAX-activating protein 10 (DAP10) as a transmembrane domain to improve 4-1BBζ CAR T cells targeting anti-glypican 3 (GPC3)[70]. DAP10 is a downstream interaction partner of the natural killer group 2D (NKG2D) natively expressed on the T cell surface to target NKG2D ligand-positive cancer cells. Thus, CAR constructs using a fusion of scFvs to DAP10 transmembrane domains can benefit from both the CAR antigen and the endogenous NKG2D-DAP10 interaction synergistically[70]. A GPC3-DAP10-CAR is under investigation in clinical trials (NCT03198546, NCT03198052). In summary, the importance of the transmembrane domain is growing as alternative options to the conventional transmembrane domain are being proposed.

The impact of the linker on CAR T cell efficacy was unclear until a recent study showing modulation in anti-CD22 CAR T cells[71]. Singh and colleagues found that short linkers with only one repeat of GGGGS connecting the V_L and V_H of an anti-CD22-scFv promoted homodimerization, leading to antigen-independent tonic signalling. Interestingly, in this specific instance, the CD22-directed 4-1BB-based CAR is characterized by a low natural degree of tonic signalling, and the resulting increase in tonic signalling enhanced immune synapse formation, expression of inflammatory gene signatures and effector function. Meanwhile, the same beneficial effect was not observed in CD28-based CARs, which already exhibit higher basal levels of tonic signalling[71].

Controlling CAR expression

The expression level of the CAR plays a pivotal role in terms of safety and efficacy of CAR-cell products. In contrast to viral transduction using constitutive promoters, genome-editing tools including CRISPR–Cas9, zinc finger proteins (ZFPs) or transcription activator-like effector nucleases (TALENs) enable site-specific CAR transgene integration into defined loci[72–74] and more reliable site-specific integration approaches enable endogenous promotors, such as the TRAC and TET2 promotors, to more uniformly regulate CAR expression at physiological levels[74–76]. In a prominent example, T cells engineered to express the CAR under

control of the endogenous T cell receptor-α constant (TRAC) locus promoter exhibited more uniform CAR expression, reduced exhaustion and superior tumour control, although their superiority has not yet been proven in clinical studies[77]. Other approaches aim to restrict CAR expression, thereby minimizing off-target effects as well as exhaustion. Toward this end, CAR expression can be placed under the control of inducible promoters that respond to small-molecule reagents such as doxycycline or contain hypoxia-response elements (HREs)[78–80]. Finally, ongoing efforts are exploring synthetic T cell-specific promoters for in vivo manufacturing[81]. However, the application and safety of delivering the CAR transgene in vivo and specifically restricting expression to T cells remain to be further validated.

TCR-inspired CAR designs

Not only the physiological expression level of the TCR but also its unique architecture make T cells highly sensitive to detecting their cognate antigen. Lipid-bilayers and single-molecule live-cell imaging have been used to determine the antigen sensitivity of TCRs and CARs. Thereby, the proximal signalling of CARs was shown to be ~1,000 times less efficient compared to TCR signalling due to inefficient recruitment of ZAP-70[43]. This lack of sensitivity is a major challenge in CAR T cell engineering with regard to the treatment of antigen heterogeneity in tumours. Selective pressure caused by the antigen-directed targeting of tumours has been reported to induce antigen downregulation or even loss[82,83].

To improve the sensitivity of CAR T cells, several groups have proposed CAR designs that are inspired by the TCR architecture, such as replacement of the variable regions of the TCRα and TCRβ chains by light and heavy chains of an antibody. The synthetic TCR and antigen receptor (STAR) construct was further modified to contain mutated murine TCRα and TCRβ chains to avoid mispairing with the endogenous human ones (muSTAR)[84]. In a similar approach, HLA-independent TCRs (HIT) were established, reducing the mispairing with endogenous constant regions by applying a targeted knock-in of the modified TCRα and TCRβ chains into the TCRα locus[44]. The muSTAR and HIT elicited better proliferation, cytokine secretion and higher antigen sensitivity compared to the conventional CAR format, both in vitro and in vivo[44,84]. Another approach extended this concept to address antigen loss by developing bispecific chimeric TCRs (Bi-ChTCRs). Fusion of a CD19-specific scFv to the TCRα and of CD22-scFv to the TCRβ constant region conferred a potent antitumour response against a mixed target antigen population[85].

The increased antigen sensitivity of these designs may enable robust elimination of cancer characterized by low and heterogeneous expression of the target antigen. However, evaluation of the therapeutic potential of the muSTAR, HIT and Bi-ChTCR formats is currently restricted to the limitations of preclinical models. Standard models rely on immortalized cell lines, often engineered to overexpress the target antigen and differing in the physiological distribution and endogenous regulation of the antigen. Moreover, the transplantation of xenogeneic cell lines into immunodeficient systems does not replicate the heterogeneity and evolutionary dynamics of genetic models on a syngeneic background[86–88]. To evaluate the muSTAR, HIT and Bi-ChTCR concepts in immunocompetent host animals, a partial or fully murinized design would be required.

Administering TCR-inspired CAR T cells as primary engineered T cell therapy might not provide sufficient insight into their performance in comparison to second-generation CAR T cells, as both would exert similar selective pressure driving tumour evolution. In this context, it would be unclear whether the antigen levels present were insufficient for the CAR T cell product to control the tumour. Moreover, antigen heterogeneity is probably not the sole determinant of therapy outcome. Dynamic changes in the TME, along with the potential advantages in T cell fitness conferred by TRAC locus-targeted insertion of TCR-inspired CARs, may also impact efficacy. Future studies should aim to compare these constructs to the commercially available CAR formats in models that closely mimic the selection pressure-driven antigen escape observed in patients.

Advanced genetic engineering facilitates tailored T cell generation

Although the second generation of CAR T cells show remarkable response rates in clinics, tonic signalling, exhaustion, graft-versus-host disease (GvHD), antigen escape and immunosuppressive TME factors all present challenges to therapeutic persistence and potential, especially in solid malignancies. The use of genome editing, gene transfer technologies and synthetic biology tools have enabled the design of various strategies to reduce toxicities and enhance the functionality of engineered immune cells, including the deprivation of inhibitory signals, overexpression of TME-modulatory factors, and metabolic rewiring.

Metabolic engineering of CAR T cells

T cells undergo metabolic changes during their expansion and differentiation process. Memory T cells primarily rely on oxidative phosphorylation (OXPHOS) and catabolism, whereas effector T cells shift towards aerobic glycolysis, the pentose phosphate pathway (PPP) and the anabolic pathway[89]. These pathways are not very energy-efficient, but their intermediate products serve as building blocks for cellular compartments, preparing the cells for rapid division, proliferation and expansion. The previously discussed differences between CD28- and 4-1BB-based CARs have inspired modulation of T cell metabolism to achieve favourable phenotypes in vivo (Fig. 3).

Nutrient transporters. Improving the capacity of CAR T cells to take up essential nutrients in a hostile TME could optimize their function. Recently, CAR T cells expressing amino-acid transporters such as SLC7A5 or SLC7A11 have been shown to survive in nutrient-deficient conditions such as low tryptophan and cysteine, while maintaining their function[90]. Modulation of the tryptophan metabolism and activation of the aryl hydrocarbon receptor (AHR) can also affect the differentiation of regulatory T cells (T_{reg} cells)[91]. Overexpression of the glucose transporter GLUT1 promotes glycolysis and the proliferation of effector CD4 and CD8 T cells (Fig. 3)[92]. GLUT overexpression in CAR T cells could enhance their response in the TME and help them compete with tumour cells, although this hypothesis requires clinical validation.

Accumulation of unwanted glycolysis by-products, such as lactate, in the TME creates an acidic environment, which can result in suppressed T cell functions. Consequently, knocking down the acid loader chloride/bicarbonate anion exchanger 2 (Ae2) gene or overexpressing the proton channel HvCN1 may restore the favourable pH, and promote the survival and functionality of T cells in the TME[93].

Metabolic enzymes and regulators. Metabolic enzymes are crucial to convert nutrients into useful sources for the metabolic cycles that fuel T cell activities. For example, glutamic-oxaloacetic transaminase 2 (GOT2) facilitates the conversion of aspartate into oxaloacetate, ensuring a consistent source for the tricarboxylic acid cycle (TCA) in mitochondria. Enzymes such as argininosuccinate synthase (ASS) and ornithine transcarbamylase (OTC) are integral components of the urea cycle, facilitating arginine biosynthesis and ammonia detoxification[94]. Overexpression of GOT2 or ASS/OTC in CAR T cells bolsters their expansion, antitumour efficacy and persistence, especially in arginine-low microenvironments[94,95]. Furthermore, depletion of isocitrate dehydrogenase (IDH) in CAR T cells leads to memory formation[96]. Interestingly, it was shown in cancer cells that IDH mutants can favour accumulation of 2-hydroxyglutarate (D2-HG), which is a weak competitive inhibitor of α-KG dioxygenases, including the Tet family[97].

 https://doi.org/10.1038/s41551-025-01492-8

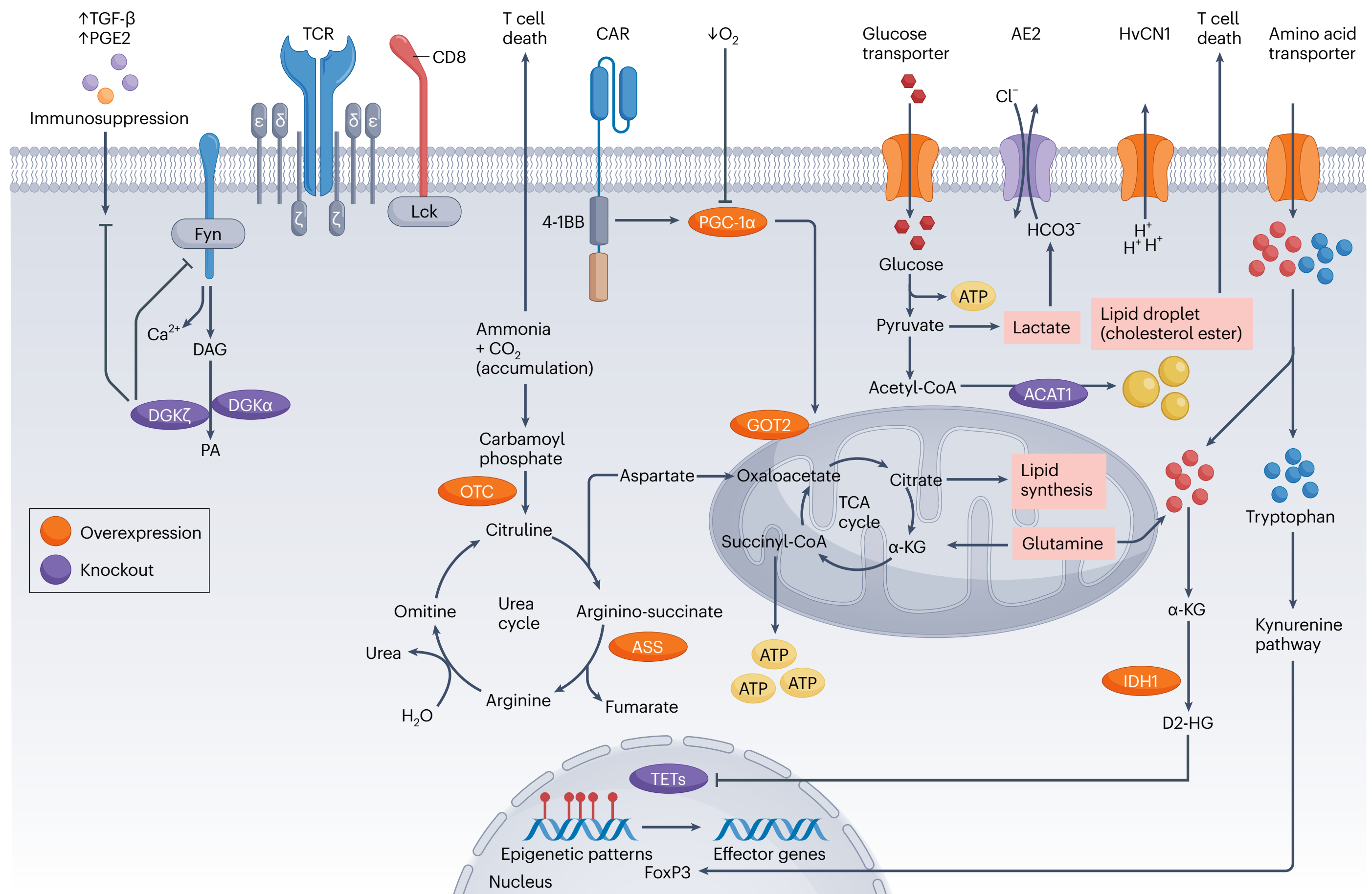

Fig. 3 | Engineering strategies for favourable CAR T cell metabolism. Targets to be knocked out and knocked in are shown in red and green, respectively. Diacylglycerol kinase (DGK) phosphorylates diacylglycerol (DAG) to phosphatidic acid (PA), thereby inhibiting T cell signalling. Functional DGK disruption induces resistance to TGF-β and prostaglandin E2 (PGE2) signalling. The co-stimulatory domain of CAR and overexpression of PGC-1α promote mitochondrial biogenesis. Overexpression of glucose, amino-acid transporters (GLUT1, SLC7A5 or SLC7A11) and enzymes in metabolic cycles (OTC, ASS, GOT2) enable cells to survive in nutrient-deprived conditions as well as to avoid toxic substance accumulation. Knockout of DGK prevents excessive activation of the T cell response and enables them to resist TGF-β and PGE2 in the TME. Knockout of ACAT1 prevents cholesteryl ester accumulation, thereby avoiding cell death. Knockout of AE2 and overexpression of HvCN1 enable the cell to resist high lactate and low pH intracellularly.

Unlike the above-mentioned enzymes, whose activities favour the T cell response, other enzymes work as metabolic checkpoints[98]. For instance, disruption of diacylglycerol kinase (DGK) induces resistance to immunosuppressive signals and empowers killing activity in the hostile milieu of a glioblastoma model[99]. As another example, acyl-CoA:cholesterol acyltransferase 1 (ACAT1) mediates the conversion of free cholesterol and fatty acids into cholesteryl esters, whose accumulation leads to T cell death (Fig. 3). Disruption of ACAT1 in MSLN CAR T cells enhances cytotoxicity and cytokine production, ultimately resulting in enhanced tumour control[100].

Peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α) is a master regulator of oxidative phosphorylation. In particular, PGC-1α acts as a co-activator for several transcription factors that control a network of genes related to mitochondrial biogenesis and function, including transcription factors NRF-1 and NRF-2. Accordingly, it can finetune the energy-production machinery within T cells[101]. EGFR CAR T cells overexpressing a full-length PGC-1α exhibit substantially enhanced efficacy in vivo compared to a truncated version (Fig. 3)[101].

Engineering strategies to overcome T cell dysfunctionality

The main challenges for CAR T cell therapy are resistance and toxicity. Resistance can be caused by intrinsic T cell mechanisms, including checkpoint pathways, cell death programs and exhaustion-related dysfunction, immune rejection, suppression or antigen escape in tumour cells (Fig. 4).

Inhibition of T cell signalling. Upon antigen exposure, T cell signalling cascades are activated, some of which serve to inhibit T cell activation, referred to as intracellular immune checkpoints[102]. Mechanisms include recruiting certain proteins such as Grb2 and Crk-L that destabilize TCR activation engagement such as by E3 ubiquitin-protein ligase CBL-B, phosphorylating tyrosine residues on specific signalling molecules, such as CD3, ZAP-70 or the lymphocyte-specific protein tyrosine kinase (Lck), or phosphorylating the adapter protein SLP76, thereby disrupting the stability of the TCR signalling complex[103–108]. Unlike the above negative regulators of T cell signalling, Lck acts as a key enzyme that promotes T cell signalling. However, its functional disruption by gene editing or pharmacological inhibition can also lead to enhanced CAR T cell function[109–111]. Mechanistically, Lck depletion shifts the activation signalling cascades toward FYN dependence, which eventually induces a stronger and more durable CAR T cell response by enhancing expansion, proliferation and memory formation[109].

Immune checkpoints and inhibitory receptors. Following persistent antigen exposure, immune checkpoints are expressed as crucial indicators of T cell exhaustion and dysfunction, restraining T effector functions. To some extent, these checkpoints are beneficial in controlling

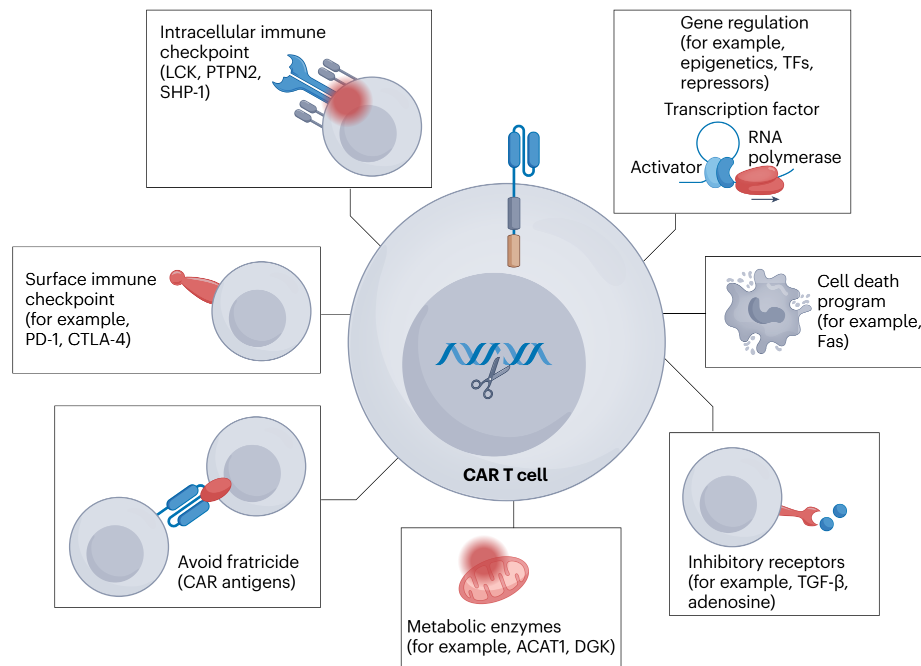

Fig. 4 | Engineering strategies and targets to overcome T cell dysfunction. Strategies include empowering CAR T cell functionality by knocking out the negative-feedback mechanism influencing T cell signalling (for example, LCK, PTPN2 and SHP-1), immune checkpoints (for example, PD-1 and CTLA-4), cell death programs (for example, FAS) and gene regulation (including transcription factors (TFs), repressors and epigenetic enzymes). Increasing T cell survival by knocking down fratricide-inducing antigens or downregulation of inhibitory receptors (for example, for TGF-β and adenosine) might serve as suitable approaches. By engineering metabolically relevant enzymes and nutrient transporters, CAR T cells can be adapted to the hostile TME.

excessive immune responses in T cells, and are important for T cell survival and functional persistence. Indeed, it is argued that these immune checkpoints should not be downregulated as it makes T cells rapidly progress to terminal differentiation and cell death[110]. However, given the limited amount of infused CAR T cell product, exhaustion hinders its potency and long-term persistence. Genetic disruption of these immune checkpoints, such as PD-1, LAG-3 and CTLA-4, has been demonstrated to rejuvenate the antitumour response in multiple CAR T cell products and different cancer models, and is advancing to clinical trials (Table 3)[112–116].

Apart from immune checkpoint molecules, inhibitory molecules present in the TME, such as TGF-β or adenosine, can render T cells dysfunctional. Knocking out TGF-βRII by CRISPR–Cas9 in mesothelin CAR T cells restored effector function, reduced T_{reg} cell transformation and resulted in a sustained memory phenotype among circulating CAR T cells. This strategy demonstrated enhanced tumour control in a squamous cell carcinoma model[117]. In line with the idea of blocking TGF-β signalling, other approaches such as protein inhibitors or the overexpression of dominant-negative TGF-βRII have been investigated[118,119]. A phase I clinical trial assessing TGF-β-insensitive PSMA CAR T cells has reported a decline in prostate-specific antigen (PSA) in approximately 30% of treated patients, but responses were of limited durability[120]. Adenosine is secreted by cancer cells and binds to the A2 adenosine receptor (A2AR) to suppress T cell responses. Knocking down this receptor by shRNA enhances cytokine production, activation and efficacy in MSLN CAR T cells encountering their targets[116,121]. In line with these findings, A2AR knockout using CRISPR–Cas9 improves antitumour response and avoids exhaustion of CAR T cells in vitro, eventually leading to better tumour control in solid tumour models[122].

Programmed death regulator. It is reported that CAR T cells are prone to cell death due to a high level of cell death program-mediating proteins including Fas, as well as FasL, DR5 and TRAIL, hindering their efficacy[123,124]. Therefore, disruption of these signalling pathways in T cells may reinvigorate T cell activity. In particular, the Fas receptor (also named CD95 or APO-1) ablation reduces apoptosis in CD19 CAR T cells after repeated exposure to $CD19^+$ cancer cells, and boosts T cell expansion[125]. Attempts to design universal CAR T cells by triple knock-out of TCR/HLA-I/Fas in allogeneic CAR T cells resulted in enhanced degranulation, killing capacity, cytokine production and prolonged survival in vitro and in vivo[125].

Transcriptional and epigenetic regulators. Epigenetic regulators, transcription factors and co-repressors work in concert to govern the plasticity and function of T cells, operating at various levels across histone, DNA modification, and transcriptional and post-transcriptional regulation (Fig. 4). For example, epigenetic modifiers such as histone-3, lysine-9 methyltransferase (SUV39H1), DNA methyltransferase 3α (DNMT3A) and DNA modifier methylcytosine dioxygenase encoded by the ten-eleven translocation 2 (TET2) gene inhibit BATF, cJUN and TCF7 activity, thereby promoting the terminal differentiation of T cells[126–130]. At the DNA level, transcription factors regulate key T cell differentiation and exhaustion genes, and disruption of these transcription factors has been associated with condensed chromatin at their target gene locus[131–133]. Additionally, factors favouring gene programs opposing T cell effector function might be a suitable target for editing. Among those are regnase 1 and roquin 1, which disrupt the transcription of inflammatory genes[134–137]. Other examples include transducin-like enhancers of split 4 (TLE4), which inhibits the interferon-γ (IFN-γ) response through epigenetic silencing, and Ikaros family zinc finger protein 2 (IKZF2), associated with mediating T_{reg} cell gene signatures. Notably, double knockout of TLE4 and IKZF2 has resulted in enhanced CAR T cell activation signalling and memory phenotype, while reducing exhaustion[138,139].

Off-tumour CAR targets. Targeting antigens that are widely expressed could avoid issues of mutational burden leading to potential antigen

 https://doi.org/10.1038/s41551-025-01492-8

Table 3 | Engineering strategies to overcome T cell dysfunction

Target genes	Knockout tools	CAR target	Cancer models/entities	Preclinical–clinical studies
Immune checkpoint inhibitors				
PD-1	shRNA	CD38	RR AML	253
	CRISPR–Cas9	CD33	GBM	113
	shRNA	CD19	PCa, CML	254
	shRNA	MSLN	MESO	255
PD-1, CTLA-4	siRNA	CSPG4	Melanoma	256
TIGIT	shRNA	MSLN	MESO	257
TIM-3	shRNA	MSLN	MESO	258
LAG-3	CRISPR–Cas9	CD19	CML	112
PD-1, TIM-3, LAG-3, CTLA-4, TIGIT	shRNA	CRC01	RR BCL	NCT04836507
Inhibitory receptor				
TGF-βRII	CRISPR–Cas9	EGFR	Solid tumours	NCT0497621
	CRISPR–Cas9	MSLN	MESO	117,118
Adenosine receptor: A2AR	shRNA	HER2	BC	259
	CRISPR–Cas9	MSLN (P4)	MESO	122
	shRNA	MSLN	MESO	116,121
Apoptosis				
FAS	CRISPR–Cas9	CD19	ALL, CML	125
Transcription factors				
TOX or TOX2	shRNA	CD19	Melanoma	131
BLIMP1 and NR4A3	CRISPR–Cas9	CD19	PCa	133
NR4As	Cre system	CD19	Melanoma, COAD	132
Epigenetic enzymes				
TET2	E1879Q mutant	CD19	CML	73
	CRISPR–Cas9	CD19	B-ALL, PCa	260
SUV39H1	CRISPR–Cas9	PSMA	PCa	130,261
DNMT3A	CRISPR–Cas9	EphA2, CD19, HER2, IL-13Rα	ALL, MESO, GBM	126
Transcription repressors				
Regnase-1 and roquin-1	CRISPR–Cas9	MSLN, CD19, NYESO TCR	CML, MESO	262
TLE2/ IKZF2	CRISPR–Cas9	IL-13Rα2	GBM	138
Avoid fratricide				
CD38	CRISPR–Cas9	CD38	ALL	75
CD7	CRISPR–Cas9	CD7	ALL	142
CD70	TALEN	CD7	T-ALL	143
Metabolic enzymes				
ACAT1	siRNA	MSLN	PCa	100
DGK	CRISPR–Cas9	EGFRvIII	GBM	99

Disease models: RR AML, relapsed/refractory acute myeloid leukaemia; CML, chronic myeloid leukaemia; RR BCL, relapsed/refractory B cell lymphoma; COAD, the invasion and metastasis of colon adenocarcinoma. Other abbreviations: EGFRvIII, epidermal growth factor receptor variant III; shRNA, short hairpin RNA; CRISPR–Cas9, clustered regularly interspaced short palindromic repeats; Cre system, enzyme recombinase Cre; TALEN, transcription activator-like effector nuclease; siRNA, small interfering RNA.

downregulation or loss; however, this often results in more on-target/off-tumour toxicities, and in some case even fratricidal killing. A SLAMF7-targeting CAR T cell product has shown efficacy against MM, but also depleted SLAMF7$^{+/high}$ NK cells, CD4^{+} and CD8^{+} T cells, and B cells. However, SLAMF7-low expressing cells among those subsets remained spared from fratricide, and were thus functional[140]. The CARAMBA trial has built on these preclinical data and demonstrated the efficient production, safety and feasibility of autologous SLAMF7 CAR T cells for the treatment of patients with MM[141]. For CARs that do not spare antigen-low cells, functional disruption or engineered epitope masking on the CAR T cell surface could offer a potential strategy to mitigate fratricide, as reported for targets such as CD38, CD70, CD7 and NKG2D[75,142–144]. Although antigens with essential functions for T cell signalling are not amenable to total genetic ablation, precision genome-editing tools can modify the target epitope to protect genetically modified CAR T cells. Furthermore, the application of adenine base editing to introduce a mutation in CD45 preserves its function while not being recognized by CD45-directed CARs. Epitope-edited CAR T cells were resistant to CD45-mediated fratricide but effective against haematologic malignancies. Moreover,

 https://doi.org/10.1038/s41551-025-01492-8

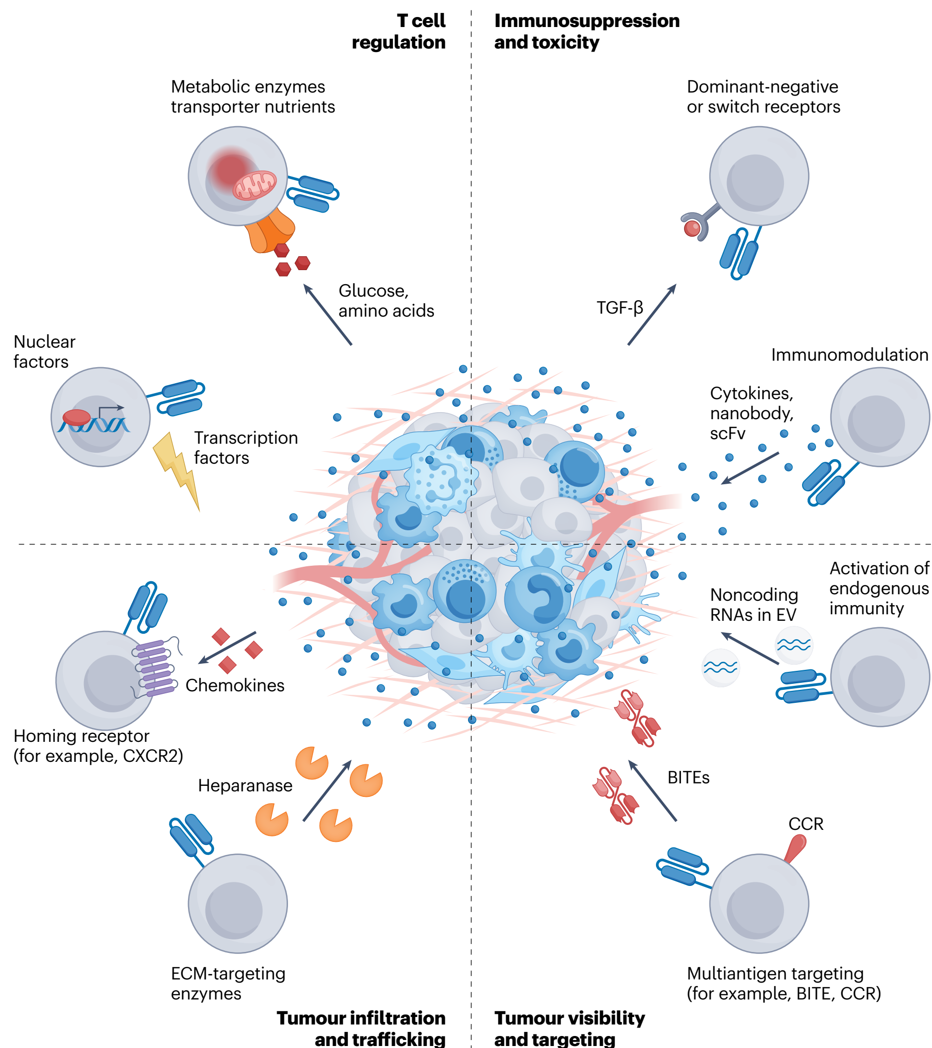

Fig. 5 | Engineering strategies to overcome challenges in the TME. (1) Regulation of CAR T cells by upregulating nuclear factors, metabolic enzymes and nutrient or ion transporters to support the T cell response. (2) Modulation of T cells expressing dominant-negative receptor or switch receptors, or secreting immunomodulators against immunosuppressive or toxicity-mediated cytokines. (3) Improving tumour infiltration and trafficking by upregulating homing receptor or ECM-targeting enzymes. (4) Multiantigen targeting and antigen presentation by noncoding RNA upregulation secreted within extracellular vesicles to improve tumour visibility and targeting.

the same edit in haematopoietic stem cells allowed engraftment and differentiation in mice as a potential for sufficient immune reconstitution combined with CAR T therapy[145].

Armouring T cells to reach and modulate the TME

The efficacy of CAR T cells in patients can be limited by the hostile, immunosuppressive environment characteristics of solid tumours or the bone marrow niche. Current strategies to improve migration through the TME involve boosting intrinsic T cell activity, improving infiltration and trafficking to and into the tumour sites, strengthening survival and countering immune suppression (Fig. 5).

Homing and infiltration capacities. Tumour cells often express several chemokines that attract immunosuppressive cells such as neutrophils (CXCL1 and CXCL2), T_{reg} cells (CCR10, CCR4 and CCR5) or MSDCs (CCL2, CXCL5 and CXCL12). CAR T cells can be engineered to express chemokine receptors to mediate homing to tumour sites[146–149]. Several clinical trials are ongoing to assess the potential of homing receptors in CAR T cells, including CCR5 (NCT05060796) and CCR4[150]. Although homing towards the tumour is an essential feature, simply equipping CAR T cells with chemokine receptors may not be sufficient to support tumour invasion. In particular, cancer-associated fibroblasts produce dense collagen networks that can inhibit CAR T cell interactions with tumour cells and broadly exclude immune cells. Recently, it was demonstrated that inhibition of collagen crosslinking facilitates T cell migration into the islets in solid tumour slices[151]. In line with these findings, engineering CAR T cells to secrete heparanase increased their capability to infiltrate and reject tumours, an approach that may synergize with homing receptor expression[152].

Switch and cytokine fusion receptors. Chimeric switch receptors (CSRs) are designed to promote T cell functions in response to suppressive signals present in the external TME. Fusion of an extracellular domain derived from an inhibitory receptor to the intracellular signalling domain of an immunostimulatory receptor can convert a suppressive signal from the TME into an activating one[153]. There is a growing body of data supporting the real potential of the switch receptor to boost CAR T cell function including TIM-3/CD28, TIGIT/CD28 and PD-L1.4-1BB[154,155] (Table 4).

The PD-1/CD28 CSR has been studied both in preclinical and clinical settings, showing enhanced CAR T cell efficacy, proliferation and

https://doi.org/10.1038/s41551-025-01492-8

Table 4 | Engineering strategies to overcome challenges in the TME

Modification	CAR target	Cancer model/entity	Preclinical/clinical studies
Homing receptors			
CCR2	GD2	NB	263
CXCR5	EGFR	NSLCC	149 NCT05060796
CXCR4	CD30	HL	147
CXCR2	MSLN	MESO	146
CXCR1, CXCR2	CD70	GBM	148
Natural or chimeric co-stimulatory receptor			
CD40L	CD19	ALL	264
	MSLN	Solid tumours	NCT05693844
ICOS-L/4-1-BB	GPC3	HCC	265,266
C7R (CD34/IL-7R)	GD2	GBM	NCT04099797
		Solid tumours	NCT03635632
IL-2/IL-9R	MSLN	PDAC	266
Chimeric switch receptor			
TIGIT/CD28	CD19	Melanoma	154
	CD19	RR DLBCL	NCT04850560
PD-1/CD28	C-Met	GC	156 267
	MUC-1	CCA	268
T3/CD28 (TIM-3)	CD19	B-ALL	269
IL-4R/IL-2Rβ	T1E	HNC	NCT01818323
TGF-βR-4-1BB	STEAP1	PCa	270
IL-4R-IL-7R	EBV	LCL	271
GM-CSF/IL-18R	EPHA2, HER2	B-ALL, melanoma	272
Dominant-negative receptors			
TGF-β DNR	PSMA	PCa	NCT04227275, NCT03089203
FAS DNR	CD19	B-ALL	160
PD-1 DNR	MSLN	MESO	NCT04577326
Enzymes			
Heparanase	GD2	NB	152
CpNA	CD19, EGFR	Melanoma, GBM	273
Adenosine deaminase 1	CD19	Colon	274
hTERT	CD19	B-ALL	275
Noncoding RNAs			
RN7SL1	CD19, MSLN	Melanoma	179
miR155 or LSD1 shRNA	CD19	B-ALL	180
miR-17-92	EGFRvIII	GBM	178
Immunomodulators			
VHH			
Anti-CD47, anti-PD-L1, anti-CTLA-4	PD-L1, EIIIB	Melanoma	218 276
scFv			
Anti-PD-L1	CD22	CCS	NCT04556669
	CAIX	ccRCC	277
Anti-CD40	MSLN	MESO	278
Anti-PD-1	MSLN	MESO	NCT05373147 NCT03030001 NCT03615313
Anti-PD-/anti-CTLA-4	MUC-1	Solid tumours	NCT03179007
	EGFR	Solid tumours	NCT03182816
	MSLN	MESO	NCT03182803

Table 4 (continued) | Engineering strategies to overcome challenges in the TME

Modification	CAR target	Cancer model/entity	Preclinical/clinical studies
Anti-IL-6, -IL-1	CD19, BCMA	NHL, MM	279
BITE			
EGFR BITE	EGFRvIII	GBM	NCT05660369 NCT05024175
IL-13Rα2 BITE	EGFR	GBM	166
B7H3 BITE	GPC3	HCC	280
PD-L1 nanobody BITE	HLA-G	Solid tumours	163
FAP BITE	MSLN	MESO	281
Cytokines			
Superkine IL-2	B7H6	Solid tumours	157
IL-7/CCL-21	CLDN18.2	Solid tumours	282
IL-7/CCL-19	CD19	B-ALL	NCT04833504
	CD19	ALL	169,283
	GPC3	HCC	NCT03198546
	Integrin β7, BCMA, CS1, CD38 or CD138	MM	NCT03778346
IL-7/CCL-19 or IL-12	Nectin-4/FAP	Solid tumours	NCT03932565
IL-7/Flt3L	EGFRvIII	GBM	284
IL-12	MUC16	OC	NCT02498912
	GPC3	HCC	285
	CD19	BCL	174
	EGFR	CRC	NCT03542799
	CD19	B-ALL, HBL, BC, OC	286
IL-15	GD2	NB	NCT03294954 NCT03721068
	IL-13Rα2	GBM	173
IL-18	CD19	RRNHL, DLBCL, FL, MCL, CLL, SLL	NCT04684563
		BCL	287
IL-23	IL-23-PSMA	PCa	288
IL-23p40	GD2	NB	172
IL-33	B7H6	Melanoma, NSCLC	157
IL-36γ	CD19	B-ALL	289
Nuclear factors			
JUN	CD19, GD2, HER2, CD22	NB	175
BATF	CD19	Melanoma, GBM	177,290
TF AP4+BATF	CD19, HA, GD2	Melanoma, B-ALL	176

Disease models: HL, Hodgkin lymphoma; RR DLBCL, relapsed/refractory diffuse large B cell lymphoma; HBL, human Burkitt lymphoma; FL, follicular lymphoma; CLL, chronic lymphocytic leukaemia; SLL, small lymphocytic lymphoma; PDAC, pancreatic ductal adenocarcinoma; GC, gastric cancer; CCA, cholangiocarcinoma; HNC, head and neck cancer; LCL, lymphoblastoid cell lines; CCS, cervical carcinosarcoma; ccRCC, metastatic clear cell renal cell carcinoma. Other abbreviations: GPC3, glypican 3; c-Met, mesenchymal-epithelial transition factor; MUC-1, mucin 1; STEAP1, six transmembrane epithelial antigen of the prostate 1; EBV, Epstein-Barr virus; EphA2, erythropoietin-producing hepatocellular carcinoma A2; PD-L1, programmed death-ligand 1; CAIX, carbonic anhydrase IX; HLA6, human leukocyte antigen 6; CLDN18.2, claudine 18.2; HA, homology arms; CCR2, c-c chemokine receptor type 2; CXCR, CXC chemokine receptor; TIGIT, T cell immunoreceptor with Ig and ITIM domains; IL, interleukin; GM-CSF, granulocyte–macrophage colony-stimulating factor; TGF-β, transforming growth factor β; FAS, TPO1 or CD95; PD-1, programmed cell death 1; CpNA, *Clostridium perfringens* neuraminidase; hTERT, human telomerase reverse transcriptase; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; FAP, Flt3 (Fms-like tyrosine kinase-3); JUN, transcription factor JUN.

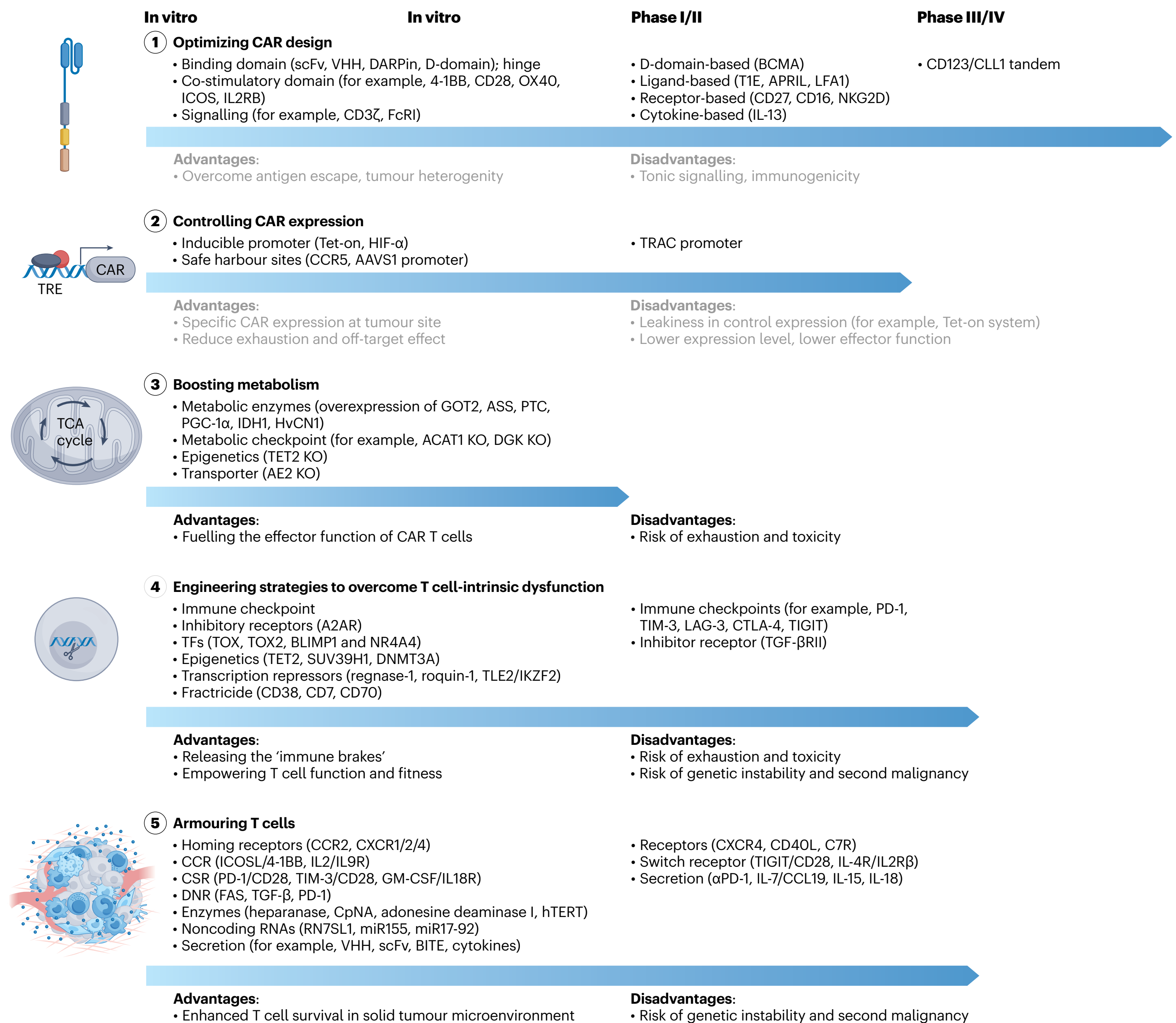

Fig. 6 | Clinical readiness level of current CAR T cell engineering approaches. Engineering strategies targeting CAR design, CAR expression, metabolism, T cell-intrinsic mechanisms and armouring are undergoing intensive investigation, moving from preclinical (in vitro and in vivo studies) to clinical research (phases I–IV).

cytokine expression, while being tolerable and safe, with encouraging responses in patients with refractory lymphoma[156,157]. Similar to switch receptors derived from immune checkpoint molecules, chimeric cytokine receptors (CCRs) or inverted cytokine receptors were generated to sustain CAR T cell function despite inhibitory signalling cytokines in the TME. For instance, the IL-4R extracellular domain can be grafted with an intracellular domain from signalling receptors providing stimulation such as common γ-chain receptors. CAR T cells expressing a variety of inverted cytokine receptors have been explored in preclinical studies, and are being assessed in clinical phase I trials (NCT01818323) (Fig. 6). In line with the concept of converting inhibitory signals, the development of a TGF-β-responsive CAR has demonstrated an alternative approach that can sustain T cell function in a suppressive TGF-β-high environment[158].

Dominant-negative receptors. Dominant-negative receptors (DNRs) are truncated or dysfunctional variants of inhibitory receptors lacking the intracellular signalling or functional binding domain. The expression of DNRs for ligands such as TGF-β, PD-1/PD-L1 or Fas diminishes the immunosuppressive effects of ligand binding. Overexpression of a TGF-βRII DNR on PSMA CAR T cells has been found to enhance T cell proliferation and tumour control in prostate cancer models[119,120]. In addition, CD19 CAR T cells with a PD-1 DNR have shown promising results in patients with refractory/relapsed B cell lymphoma (RR BCL)[159]. Based on this concept, T cells expressing Fas DNR have demonstrated enhanced survival and persistence in a murine CD19-dependent leukaemia model[160].

Secretion of antibody fragments (VHH, scFv, bispecifics). Cell engineering methods enable T cells to actively secrete modulatory mediators. In particular, CAR T cells can be engineered to express scFv, VHH, bispecific T cell engagers (BITEs) or cytokines delivered locally to the TME. Typically, these immunomodulators target immune checkpoint proteins[161,162] (Table 4).

Recently, BITEs that simultaneously bind to CD3 on T cells and antigens on cancer cells were introduced into clinical practice, exemplified

 https://doi.org/10.1038/s41551-025-01492-8

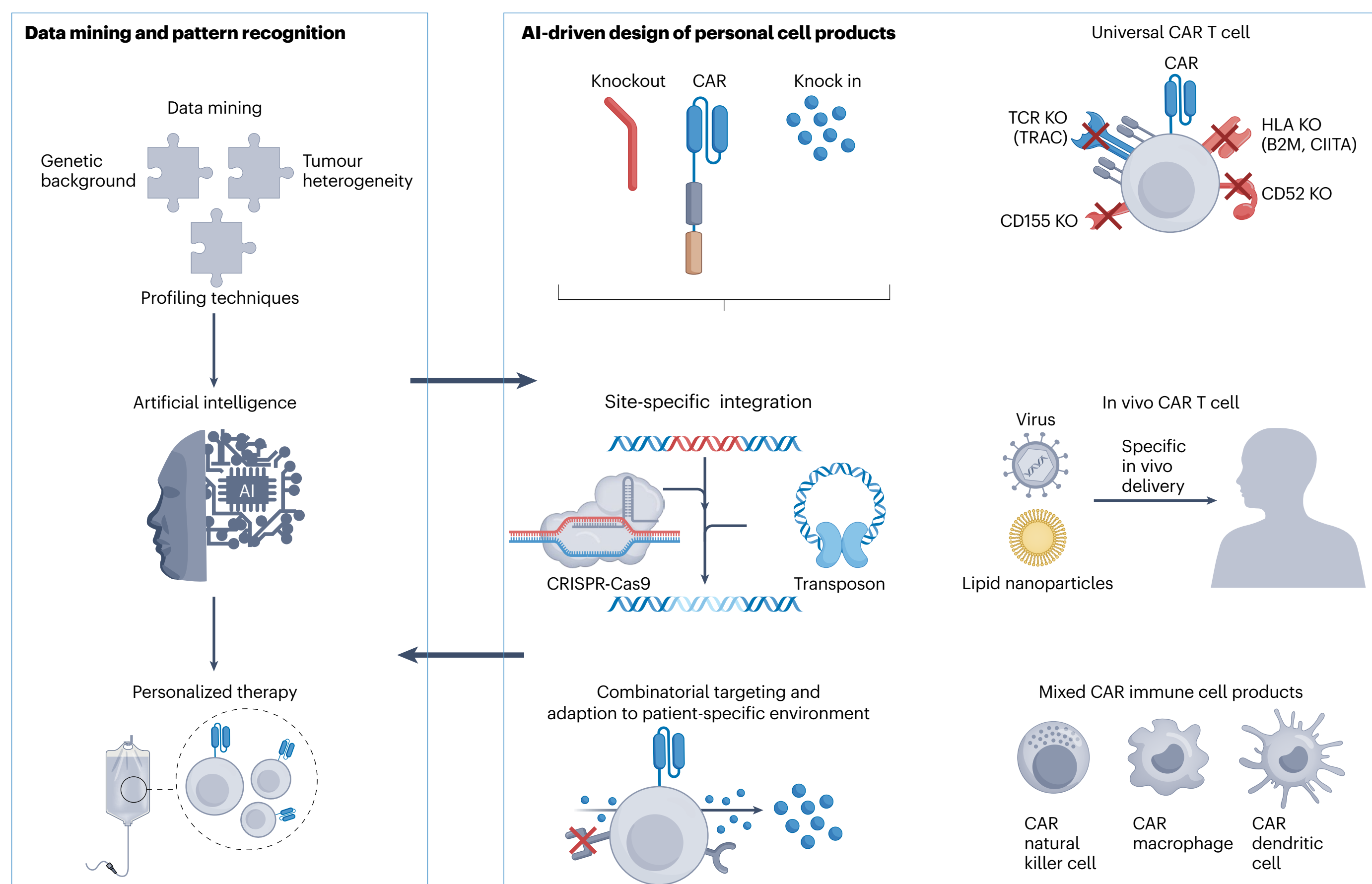

Fig. 7 | Future direction of engineering CAR T cell therapy. Treatment involves combining multiple modifications of T cells, including knock in and knockout, to obtain multifunctional CAR T cell products. Off-the-shelf therapeutics include the generation of universal CAR T cells, in vivo gene transfer and alternative CAR immune cells such as natural killer cells, macrophages and dendritic cells. Patient data exploration, mining and AI enable outcome prediction as well as personalized CAR T cell products.

by the FDA approval of blinatumomab. By targeting additional tumour antigens and activating bystander T cells, BITEs could complement CAR T cell therapy by helping to overcome limitations associated with heterogeneous solid tumours and antigen escape, while potentially reducing dosing[163–167]. For example, EGFRvIII CAR T cells secreting an EGFR-targeting BITE demonstrate tumour control of heterogeneous glioblastoma without detectable toxicity[164,168]. Additionally, CAR T cells can secrete cytokines that are important for T cell self-renewal, proliferation and stemness, or that favour sustained effector function and activation of endogenous immune cells in hostile environments[157,169–174] (Table 4).

Transcriptional regulators. Understanding and reprogramming gene networks can optimize the maintenance of T cell responsiveness through the use of transcriptional regulators (Fig. 5). Whereas depletion of the transcription factor JUN and FOS dimers was described to cause T cell exhaustion, overexpression of JUN boosts the proliferation and effector function of GD2 and CD19 CAR T cells, and confers resistance to T cell exhaustion, resulting in more robust tumour regression in mouse models[175]. Similarly, basic leucine zipper ATF-like transcription factor (BATF) interacts with Interferon regulatory factors IRF4 and IRF8 to regulate early responses to TCR activation, and drives cells towards effector function or exhaustion. BATF overexpression promotes CD19 CAR $CD8^+$ T cell proliferation and enhanced granzyme and cytokine production, resulting in prolonged cytotoxicity and long-term tumour regression in mice[176]. In contrast, BATF depletion preserves CAR T cell effector functions and improves resistance to T cell exhaustion in models of mesothelin-expressing cancers[177]. Furthermore, a double knock-in of BATF and transcription factor AP4 enhances T cell persistence[176].

Apart from transcription factors, noncoding RNAs, particularly microRNAs (miRNAs), play a pivotal role in regulating various aspects of T cell function through intricate mechanisms. For instance, miR-17-92 targets phosphatase and TENsin homologue (PTEN) and promotes the expansion and survival of EGFRvIII CAR T cells[178]. RN7SL1 is an RNA scaffold molecule for RNA-binding proteins involved in protein translation, therefore promoting CAR T cell proliferation and differentiation[179]. Under pathological conditions, RN7SL is packaged in the extracellular vesicles and transported into the TME, where it triggers enhanced antigen presentation on cancer cells. Finally, engineered CAR T cells expressing miR155 exhibit boosted effector function[180].

Transient modulation of CAR T cell activity

The genetic-engineering approaches discussed to modulate CAR T cell function represent long-lasting, usually irreversible cellular changes. CAR T cell activity has been transiently modulated by the addition of soluble drugs such as dasatinib, an inhibitor of Lck. Consequently, blocking the phosphorylation of ZAP-70 ablates CAR signalling and induces a reversible off state. Such an intervention can serve as an approach to mitigate CAR T cell mediated toxicities such as cytokine release syndrome[111].

To limit on-target, off-tumour toxicity, CARs that forms a reversible covalent bond with a 1,3-diketone hapten-conjugated adaptor molecule were developed. The adaptor molecule specifies and

https://doi.org/10.1038/s41551-025-01492-8

regulates sensitivity to the target antigen. Screening of small-molecule binders by a DNA-encoded library could facilitate the generation of various soluble adapters that regulate CAR T cell function in a concentration-dependent manner[181]. Small molecules have also been used to control CAR dimerization using an alternative on-switch mechanism. Implementation of binding domains for small molecules between the CD3ζ and co-stimulatory domain enabled the activation of CAR signalling and cytotoxic function upon small-molecule administration in vitro and in vivo[33]. In a different iteration of an on-switch system, binders were developed that specifically recognized conformational changes in the human retinol binding protein 4 induced by the small molecule A1120. A CAR platform was established that consisted of a hRPB4-anti-CD19 scFv binding to a membrane-bound 28ζ adapter. On administration of the A1120, a conformational change led to assembly of the CAR construct[34]. The control of CAR T cells using small molecules is a versatile and dynamic strategy to control CAR T cell activity over time and thereby limit potential toxicities. On–off–on schedules could further prolong their fitness. However, as these reversible approaches depend on the presence of small-molecule drugs, it remains to be evaluated how biodistribution and availability, especially in the sequestering TME, will affect the kinetics in a clinical situation.

Translational challenges

Most engineering approaches described in this Review involve gene vectors that integrate semi-randomly into the host genome, which can disrupt gene regulation and potentially lead to cancer. Gammaretroviral and lentiviral vectors are particularly risky due to their tendency to integrate near active gene regulatory regions. Although non-viral systems such as Sleeping Beauty or PiggyBAC may offer safer alternatives, cases of T cell lymphoma linked to PiggyBAC-engineered CAR T cells have raised concerns and prompted FDA reviews. Large-scale data show no T cell malignancies in over 3,000 CAR T cell infusions, but low rates of secondary cancers and the increasing use of CRISPR have intensified safety discussions[182–186]. The lack of robust genotoxicity models and clear product release criteria underscores the need for better assays and consensus on acceptable risk levels[187].

Predicting the behaviour of next-generation cell therapies remains difficult due to limited and non-standardized test models. In vitro and immunodeficient mouse models often overestimate CAR T cell efficacy and fail to capture the complexity of the TME and immune interactions. Syngeneic mouse models, using immunocompetent hosts, better replicate clinical conditions by including native immune components and allowing the assessment of persistence, biodistribution and lymphodepletion strategies[188]. These models have successfully reflected patient responses to immunotherapies and highlight the role of the microbiome in treatment outcomes. However, differences in antigen expression and limited cross-reactivity of CAR constructs with murine targets restrict their clinical relevance.

When murine targets are lacking, organotypic human tissue slices offer a complementary approach to assess toxicities in healthy human or efficacy in tumour tissues, but they are limited by tissue availability and potential allogenicity[188–190]. Despite the advances in ex vivo models and recent changes allowing human trials without animal testing, in vivo models still offer critical insights into systemic effects that in vitro systems currently cannot fully replicate[191]. Future efforts should focus on developing models that accurately predict efficacy and toxicity, preserve or mimic the TME, as well as the host tissue architecture, while using samples derived from clinical studies that allow meaningful correlative assessments.

Besides efforts in benchmarking genetic and non-genetic modifications, another step forward will be to investigate the impact of their combined use in a single cell product, or the co-administration of multiple engineered products to benefit from synergistic effects. At the same time, it is essential to evaluate whether such combinations may negatively impact cell performance. An earlier study using TALEN technology reported on a dual gene-editing approach (TRAC and CD52) to generate an allogeneic CAR T cell product, observing minimal toxicity despite translocations[192]. In one of our studies, we described the feasibility of a triple KO (TRAC, TRBC and PDCD1) for a phase I TCR T cell trial, highlighting minimal off-target effects and a low frequency of translocations[193]. These clinical studies support the feasibility of multiplexed gene editing with low genotoxicity, increasing the confidence in the use of more sophisticated engineering strategies tailored to specific therapeutic needs. However, as gene editing will become more prominent in the future, approaches such as base editing might be a safer and more scalable alternative to classic CRISPR editing[194]. Although multiplexed gene editing has been performed in preclinical studies, its long-term impact in patients remains to be determined and will warrant extended clinical follow-up.

Future directions and perspectives

Initially, CAR T cell therapy was designed to be a versatile single-shot treatment that can be adapted to a variety of malignant entities. Although CAR T cells have indeed revolutionized cancer immunotherapy and are even offering a chance to cure many B cell malignancies, the growing body of clinical experience emphasizes that a lack of sustainable response and persistence, especially in solid tumour and cold TMEs, remain roadblocks (Fig. 6)[157,195].

Advances in the field of gene delivery and editing using CRISPR-based methods have become handy tools to swiftly knock in or knock out elements of interest with high precision at distinct loci[77,106,196]. Although these technologies have demonstrated the ability to generate new cell products with various edits under research conditions, it remains to be determined whether patient immune cells—after several lines of treatment and pre-conditioning—retain sufficient fitness to withstand extensive genetic engineering. Furthermore, recent cases of rare malignant transformation by administered CAR T cell products have raised an awareness for potential genotoxic events asking for safety profiling and measures in the future, especially in light of multiplex-edited strategies[182,197]. Thus, future approaches should also consider non-genetic modification options to enhance T cell fitness. Epigenetic and metabolic small-molecule modulators have been shown to improve CAR T cell manufacturing and antitumour activity, offering alternative engineering strategies, or a synergy with genetic engineering[187,188] (Fig. 4). Moreover, discussion with regulatory authorities will be key to finding an optimal solution balancing the safety of multi-gene-edited cell products and the ability to flexibly adapt them to a patient's TME.

Another option to obtain fitter T cells is the use of allogeneic sources. To implement allogeneic CAR T cells in the clinic, key immunological and technical challenges must be addressed. Immune rejection and GvHD are major concerns, requiring substantial gene editing[198,199]. Enhancing persistence is critical and may involve engineering strategies exploiting virus-like immune escape mechanisms[200]. Scalable, off-the-shelf manufacturing must ensure consistent quality, potency and safety across batches. Regulatory frameworks need to evolve to define standards for gene edits, safety monitoring and product approval. Ethical and logistical considerations, including informed consent and long-term follow-up, should also be addressed.

Despite ongoing efforts to tune efficacy, reliable safety measures need to be implemented to prevent severe cytokine release syndrome or mitigate risks from insertional mutagenesis. Besides equipping CAR T cells with a controllable 'safety switch', small-molecule administration can precisely control CAR T cell activity. However, future approaches could consider designing advanced circuits that integrate the expression or repression of cellular functions depending on the environmental circumstances. For instance, the expression of gene programs or TME-modulating factors could be controlled by the metabolic cell state or the nutrients available in the tumour milieu, thereby

increasing efficacy and target specificity. Another iteration of this concept could be linking effector function to the differentiation state or lineage commitment of the engineered immune cell.

An additional layer of safety can be provided by local delivery technologies such as hydrogels or scaffolds. Biomaterial-supported systems might help to reduce systemic exposure, enhance infiltration in the target tissue and adjust the required dose[201]. In contrast to such local delivery concepts, in vivo generation of engineered immune cells requires thorough investigations of its systemic effects. Lipid nano and adeno-associated virus particles have shown that gene transfer of a CAR construct in vivo is feasible, and could substantially decrease the required infrastructure and costs for autologous manufacturing as part of a bedside manufacturing set-up[202–204]. Increasing target cell specificity of the vector and exploring the feasibility of multiplexed gene editing will be among the next steps forward.

The modular use of CAR technology has mainly been demonstrated in T cells. Engineering in different immune cell subsets that will be combined with each other to assess synergistic effects can be a strategy to optimize the features of a heterogeneous cell product. The versatility of cell therapy may be exploited in disease settings even beyond cancer. Recent examples have demonstrated the use of CD19 CAR T cells in B cell-mediated autoimmune diseases such as lupus erythematosus and myasthenia gravis[205,206]. By redirecting the CAR T cell response towards autoreactive plasma cells, the pool of autoantibody-producing cells has been successfully depleted, leading to sustained drug-free remissions.

Finally, the interplay between the tumour, immune environment and therapy outcome remains a crucial area for exploration. In particular, host-derived factors such as the microbiome composition have been shown to have predictive value regarding response and resistance to immunotherapy approaches[207]. Thus, the future of CAR T cell therapy involves predicting efficacy and toxicity by integrating multidimensional profiling information on biomarkers, clinical parameters and individual patient history[208,209]. Machine learning and artificial intelligence could play a crucial role in CAR T cell therapy by assisting in patient selection through the analysis of genetic and clinical data, aiding in the design of CAR constructs by predicting optimal receptor configurations and streamlining manufacturing processes through automated workflows[210,211]. Dual-targeting concepts such as Boolean-logic AND-gated platforms might support the translation of targeting AI-identified antigen profiles into cell products with reduced on-target, off-tumour toxicity[212]. The use of these advanced tools could optimize bench-to-bedside translation and provide help to create accessible therapy tailored to individual patient needs, thereby improving outcomes and reducing healthcare costs (Fig. 7).

References

1. Sadelain, M. & Mulligan, R. C. Efficient-retroviral-mediated gene transfer into murine primary lymphocytes. In *Proc. 8th International Congress of Immunology* (ed. International Union of Immunological Societies) Abstract no. 34 (Springer, 1992).
2. Miller, A. D. Retrovirus packaging cells. *Hum. Gene Ther.* **1**, 5–14 (1990).
3. Eshhar, Z., Waks, T., Gross, G. & Schindler, D. G. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the γ or ζ subunits of the immunoglobulin and T-cell receptors. *Proc. Natl Acad. Sci. USA* **90**, 720–724 (1993).
4. Gross, G., Waks, T. & Eshhar, Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc. Natl Acad. Sci. USA* **86**, 10024–10028 (1989).
5. Ochi, T. et al. A single-chain antibody generation system yielding CAR-T cells with superior antitumor function. *Commun. Biol.* **4**, 273 (2021).
6. Long, A. H. et al. 4-1BB costimulation ameliorates T-cell exhaustion induced by tonic signaling of chimeric antigen receptors. *Nat. Med.* **21**, 581–590 (2015).
7. Chen, J. et al. Tuning charge density of chimeric antigen receptor optimizes tonic signaling and CAR-T cell fitness. *Cell Res.* **33**, 341–354 (2023).
8. Kozani, P. S., Kozani, P. S. & O'Connor, R. S. Humanized chimeric antigen receptor (CAR) T cells. *J. Cancer Immunol.* **3**, 183–187 (2021).
9. Hamers-Casterman, C. et al. Naturally occurring antibodies devoid of light chains. *Nature* **363**, 446–448 (1993).
10. Sarén, T. et al. Complementarity-determining region clustering may cause CAR-T cell dysfunction. *Nat. Commun.* **14**, 4732 (2023).
11. Zhou, J. et al. The persistence and antitumor efficacy of CAR-T cells are modulated by tonic signaling within the CDR. *Int. Immunopharmacol.* **126**, 111239 (2024).
12. Berdeja, J. G. et al. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study. *Lancet* **398**, 314–324 (2021).
13. Siegler, E., Li, S., Kim, Y. J. & Wang, P. Designed ankyrin repeat proteins as Her2 targeting domains in chimeric antigen receptor-engineered T cells. *Hum. Gene Ther.* **28**, 726–736 (2017).
14. Balakrishnan, A. et al. Multispecific targeting with synthetic ankyrin repeat motif chimeric antigen receptors. *Clin. Cancer Res.* **25**, 7506–7516 (2019).
15. Walsh, S. T. R., Cheng, H., Bryson, J. W., Roder, H. & Degrado, W. F. Solution structure and dynamics of a de novo designed three-helix bundle protein. *Proc. Natl Acad. Sci. USA* **96**, 5486–5491 (1999).
16. Buonato, J. M. et al. Preclinical efficacy of BCMA-directed CAR T cells incorporating a novel D domain antigen recognition domain. *Mol. Cancer Ther.* **21**, 1171–1183 (2022).
17. Qin, H. et al. Chimeric antigen receptors incorporating D domains targeting CD123 direct potent mono- and bi-specific antitumor activity of T cells. *Mol. Ther.* **27**, 1262–1274 (2019).
18. Frigault, M. J. et al. Phase 1 study of CART-ddBCMA for the treatment of subjects with relapsed and refractory multiple myeloma. *Blood Adv.* **7**, 768–777 (2023).
19. Shaffer, D. R. et al. T cells redirected against CD70 for the immunotherapy of CD70-positive malignancies. *Blood* **117**, 4304–4314 (2011).
20. Wang, Q. J. et al. Preclinical evaluation of chimeric antigen receptors targeting CD70-expressing cancers. *Clin. Cancer Res.* **23**, 2267–2276 (2017).
21. Sauer, T. et al. CD70-specific CAR T cells have potent activity against acute myeloid leukemia without HSC toxicity. *Blood* **138**, 318–330 (2021).
22. Ochi, F. et al. Gene-modified human α/β-T cells expressing a chimeric CD16-CD3ζ receptor as adoptively transferable effector cells for anticancer monoclonal antibody therapy. *Cancer Immunol. Res.* **2**, 249–262 (2014).
23. Kudo, K. et al. T lymphocytes expressing a CD16 signaling receptor exert antibody-dependent cancer cell killing. *Cancer Res.* **74**, 93–103 (2014).
24. Clémenceau, B. et al. Antibody-dependent cellular cytotoxicity (ADCC) is mediated by genetically modified antigen-specific human T lymphocytes. *Blood* **107**, 4669–4677 (2006).
25. Van Cutsem, E. et al. Phase 1 studies assessing the safety and clinical activity of autologous and allogeneic NKG2D-based CAR-T therapy in metastatic colorectal cancer. *Ann. Oncol.* **30**, iv124–iv125 (2019).
26. Dai, H. et al. Development of NKG2D chimeric antigen receptor-T cells as targeted therapy of liver cancer. *J. Clin. Oncol.* **36**, e15057 (2018).

27. Chen, N. et al. Targeting of IL-10R on acute myeloid leukemia blasts with chimeric antigen receptor-expressing T cells. *Blood Cancer J.* **11**, 144 (2021).
28. Huang, G. et al. Genetically modified T cells targeting interleukin-11 receptor α-chain kill human osteosarcoma cells and induce the regression of established osteosarcoma lung metastases. *Cancer Res.* **72**, 271–281 (2012).
29. Brown, C. E. et al. Optimization of IL13Rα2-targeted chimeric antigen receptor T cells for improved anti-tumor efficacy against glioblastoma. *Mol. Ther.* **26**, 31–44 (2018).
30. Kahlon, K. S. et al. Specific recognition and killing of glioblastoma multiforme by interleukin 13-zetakine redirected cytolytic T cells. *Cancer Res.* **64**, 9160–9166 (2004).
31. Hombach, A. et al. IL12 integrated into the CAR exodomain converts $CD8^+$ T cells to poly-functional NK-like cells with superior killing of antigen-loss tumors. *Mol. Ther.* **30**, 593–605 (2022).
32. Han, X. et al. Adnectin-based design of chimeric antigen receptor for T cell engineering. *Mol. Ther.* **25**, 2466–2476 (2017).
33. Salzer, B. et al. Engineering AvidCARs for combinatorial antigen recognition and reversible control of CAR function. *Nat. Commun.* **11**, 4166 (2020).
34. Zajc, C. U. et al. A conformation-specific ON-switch for controlling CAR T cells with an orally available drug. *Proc. Natl Acad. Sci. USA* **117**, 14926–14935 (2020).
35. Kuwana, Y. et al. Expression of chimeric receptor composed of immunoglobulin-derived V resions and T-cell receptor-derived C regions. *Biochem. Biophys. Res. Commun.* **149**, 960–968 (1987).
36. Irving, B. A. & Weiss, A. The cytoplasmic domain of the T cell receptor ζ chain is sufficient to couple to receptor-associated signal transduction pathways. *Cell* **64**, 891–901 (1991).
37. Feucht, J. et al. Calibration of CAR activation potential directs alternative T cell fates and therapeutic potency. *Nat. Med.* **25**, 82–88 (2019).
38. Schoutrop, E. et al. Tuned activation of MSLN-CAR T cells induces superior antitumor responses in ovarian cancer models. *J. Immunother. Cancer* **11**, e005691 (2023).
39. Park, J. H. et al. A phase I study of CD19-targeted 19(T2)28z1xx CAR T cells in adult patients with relapsed or refractory diffuse large B-cell lymphoma. *Blood* **140**, 403–404 (2022).
40. Krause, A. et al. Antigen-dependent CD28 signaling selectively enhances survival and proliferation in genetically modified activated human primary T lymphocytes. *J. Exp. Med.* **188**, 619–626 (1998).
41. Xu, T. et al. A novel TREM1/DAP12-based multiple chain CAR-T cell targets PTK7 in ovarian cancer therapy. *Med. Oncol.* **40**, 226 (2023).
42. Velasco Cárdenas, R. M. H. et al. Harnessing CD3 diversity to optimize CAR T cells. *Nat. Immunol.* **24**, 2135–2149 (2023).
43. Gudipati, V. et al. Inefficient CAR-proximal signaling blunts antigen sensitivity. *Nat. Immunol.* **21**, 848–856 (2020).
44. Mansilla-Soto, J. et al. HLA-independent T cell receptors for targeting tumors with low antigen density. *Nat. Med.* **28**, 345–352 (2022).
45. Zhong, X. S., Matsushita, M., Plotkin, J., Riviere, I. & Sadelain, M. Chimeric antigen receptors combining 4-1BB and CD28 signaling domains augment PI 3 kinase/AKT/Bcl-X L activation and CD8 T cell-mediated tumor eradication. *Mol. Ther.* **18**, 413–420 (2010).
46. Textor, A. et al. Cd28 co-stimulus achieves superior CAR T cell effector function against solid tumors than 4-1BB co-stimulus. *Cancers (Basel)* **13**, 1050 (2021).
47. Porter, D. L. et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci. Transl. Med.* **7**, 303ra139 (2015).
48. Davila, M. L. et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci. Transl. Med.* **6**, 224ra25 (2014).
49. Lee, D. W. et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet* **385**, 517–528 (2015).
50. Zhao, Z. et al. Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells. *Cancer Cell* **28**, 415–428 (2015).
51. van der Stegen, S. J. C., Hamieh, M. & Sadelain, M. The pharmacology of second-generation chimeric antigen receptors. *Nat. Rev. Drug Discov.* **14**, 499–509 (2015).
52. Guedan, S. et al. Single residue in CD28-costimulated CAR-T cells limits long-term persistence and antitumor durability. *J. Clin. Invest.* **130**, 3087–3097 (2020).
53. Milone, M. C. et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. *Mol. Ther.* **17**, 1453–1464 (2009).
54. Melenhorst, J. J. et al. Decade-long leukaemia remissions with persistence of $CD4^+$ CAR T cells. *Nature* **602**, 503–509 (2022).
55. Boroughs, A. C. et al. A distinct transcriptional program in human CAR T cells bearing the 4-1BB signaling domain revealed by scRNA-Seq. *Mol. Ther.* **28**, 2577–2592 (2020).
56. Kawalekar, O. U. et al. Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. *Immunity* **44**, 380–390 (2016).
57. Carpenito, C. et al. Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. *Proc. Natl Acad. Sci. USA* **106**, 3360–3365 (2009).
58. Del Bufalo, F. et al. GD2-CART01 for relapsed or refractory high-risk neuroblastoma. *N. Engl. J. Med.* **388**, 1284–1295 (2023).
59. Kagoya, Y. et al. A novel chimeric antigen receptor containing a JAK-STAT signaling domain mediates superior antitumor effects. *Nat. Med.* **24**, 352–359 (2018).
60. Guedan, S. et al. ICOS-based chimeric antigen receptors program bipolar TH17/TH1 cells. *Blood* **124**, 1070–1080 (2014).
61. Guedan, S. et al. Enhancing CAR T cell persistence through ICOS and 4-1BB costimulation. *JCI Insight* **3**, e96976 (2018).
62. Moreno-Cortes, E. et al. ICOS and OX40 tandem co-stimulation enhances CAR T-cell cytotoxicity and promotes T-cell persistence phenotype. *Front. Oncol.* **13**, 1200914 (2023).
63. Song, D. G. et al. CD27 costimulation augments the survival and antitumor activity of redirected human T cells in vivo. *Blood* **119**, 696–706 (2012).
64. Prinzing, B. et al. MyD88/CD40 signaling retains CAR T cells in a less differentiated state. *JCI Insight* **5**, e136093 (2020).
65. Collinson-Pautz, M. R. et al. Constitutively active MyD88/CD40 costimulation enhances expansion and efficacy of chimeric antigen receptor T cells targeting hematological malignancies. *Leukemia* **33**, 2195–2207 (2019).
66. Hudecek, M. et al. Receptor affinity and extracellular domain modifications affect tumor recognition by ROR1-specific chimeric antigen receptor T cells. *Clin. Cancer Res.* **19**, 3153–3164 (2013).
67. Levin-Piaeda, O. et al. The intracellular domain of CD40 is a potent costimulatory element in chimeric antigen receptors. *J. Immunother.* **44**, 209–213 (2021).
68. Jonnalagadda, M. et al. Chimeric antigen receptors with mutated IgG4 Fc spacer avoid fc receptor binding and improve T cell persistence and antitumor efficacy. *Mol. Ther.* **23**, 757–768 (2015).
69. Alabanza, L. et al. Function of novel anti-CD19 chimeric antigen receptors with human variable regions is affected by hinge and transmembrane domains. *Mol. Ther.* **25**, 2452–2465 (2017).
70. Li, S. et al. DAP10 integration in CAR-T cells enhances the killing of heterogeneous tumors by harnessing endogenous NKG2D. *Mol. Ther. Oncolytics* **26**, 15–26 (2022).

https://doi.org/10.1038/s41551-025-01492-8

71. Singh, N. et al. Antigen-independent activation enhances the efficacy of 4-1BB-costimulated CD22 CAR T cells. *Nat. Med.* **27**, 842–850 (2021).
72. Rodriguez-Marquez, P. et al. CAR density influences antitumoral efficacy of BCMA CAR T cells and correlates with clinical outcome. *Sci. Adv.* **8**, eabo0514 (2022).
73. Fraietta, J. A. et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. *Nature* **558**, 307–312 (2018).
74. Glaser, V. et al. Combining different CRISPR nucleases for simultaneous knock-in and base editing prevents translocations in multiplex-edited CAR T cells. *Genome Biol.* **24**, 89 (2023).
75. Liao, C. et al. CD38-specific CAR integrated into CD38 locus driven by different promoters causes distinct antitumor activities of T and NK cells. *Adv. Sci.* **10**, e2207394 (2023).
76. Tipanee, J., Samara-Kuko, E., Gevaert, T., Chuah, M. K. & VandenDriessche, T. Universal allogeneic CAR T cells engineered with Sleeping Beauty transposons and CRISPR-CAS9 for cancer immunotherapy. *Mol. Ther.* **30**, 3155–3175 (2022).
77. Eyquem, J. et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. *Nature* **543**, 113–117 (2017).
78. Deep, G. & Panigrahi, G. K. Hypoxia-induced signaling promotes prostate cancer progression: exosomes role as messenger of hypoxic response in tumor microenvironmen. *Crit. Rev. Oncog.* **20**, 419–434 (2015).
79. Kosti, P. et al. Hypoxia-sensing CAR T cells provide safety and efficacy in treating solid tumors. *Cell Rep. Med.* **2**, 100227 (2021).
80. Gu, X., He, D., Li, C., Wang, H. & Yang, G. Development of inducible CD19-CAR T cells with a Tet-on system for controlled activity and enhanced clinical safety. *Int. J. Mol. Sci.* **19**, 3455 (2018).
81. Agarwal, S. et al. In vivo generation of CAR T cells selectively in human CD4$^+$ lymphocytes. *Mol. Ther.* **28**, 1783–1794 (2020).
82. Da Vià, M. C. et al. Homozygous BCMA gene deletion in response to anti-BCMA CAR T cells in a patient with multiple myeloma. *Nat. Med.* **27**, 616–619 (2021).
83. Rasche, L. et al. Spatial genomic heterogeneity in multiple myeloma revealed by multi-region sequencing. *Nat. Commun.* **8**, 268 (2017).
84. Liu, Y. et al. Chimeric STAR receptors using TCR machinery mediate robust responses against solid tumors. *Sci. Transl. Med.* **13**, eabb5191 (2021).
85. Simon, S. et al. Design of sensitive monospecific and bispecific synthetic chimeric T cell receptors for cancer therapy. *Nat. Cancer* **6**, 647–665 (2025).
86. Larrayoz, M. et al. Preclinical models for prediction of immunotherapy outcomes and immune evasion mechanisms in genetically heterogeneous multiple myeloma. *Nat. Med.* **29**, 632–645 (2023).
87. Denk, D. et al. Expansion of T memory stem cells with superior anti-tumor immunity by Urolithin A-induced mitophagy. *Immunity* **55**, 2059–2073.e8 (2022).
88. Vogt, M. et al. Targeting MYC effector functions in pancreatic cancer by inhibiting the ATPase RUVBL1/2. *Gut* **73**, 1509–1528 (2024).
89. Van Der Windt, G. J. W. et al. CD8 memory T cells have a bioenergetic advantage that underlies their rapid recall ability. *Proc. Natl Acad. Sci. USA* **110**, 14336–14341 (2013).
90. Panetti, S. et al. Engineering amino acid uptake or catabolism promotes CAR T-cell adaption to the tumor environment. *Blood Adv.* **7**, 1754–1761 (2023).
91. Quintana, F. J. et al. Control of T_{reg} and T_H17 cell differentiation by the aryl hydrocarbon receptor. *Nature* **453**, 65–71 (2008).
92. Macintyre, A. N. et al. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab.* **20**, 61–72 (2014).
93. Navarro, F. et al. Overcoming T cell dysfunction in acidic pH to enhance adoptive T cell transfer immunotherapy. *Oncoimmunology* **11**, 2070337 (2022).
94. Fultang, L. et al. Metabolic engineering against the arginine microenvironment enhances CAR-T cell proliferation and therapeutic activity. *Blood* **136**, 1155–1160 (2020).
95. Hickman, T. L. et al. BOXR1030, an anti-GPC3 CAR with exogenous GOT2 expression, shows enhanced T cell metabolism and improved anti-cell line derived tumor xenograft activity. *PLoS ONE* **17**, e0266980 (2022).
96. Si, X. et al. Mitochondrial isocitrate dehydrogenase impedes CAR T cell function by restraining antioxidant metabolism and histone acetylation. *Cell Metab.* **36**, 176–192.e10 (2024).
97. Xu, W. et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. *Cancer Cell* **19**, 17–30 (2011).
98. Schmidt, N. M. et al. Targeting human Acyl-CoA:cholesterol acyltransferase as a dual viral and T cell metabolic checkpoint. *Nat. Commun.* **12**, 2814 (2021).
99. Jung, I. Y. et al. CRISPR/Cas9-mediated knockout of DGK improves antitumor activities of human T cells. *Cancer Res.* **78**, 4692–4703 (2018).
100. Zhao, L. et al. Inhibition of cholesterol esterification enzyme enhances the potency of human chimeric antigen receptor T cells against pancreatic carcinoma. *Mol. Ther. Oncolytics* **16**, 262–271 (2020).
101. Lontos, K. et al. Metabolic reprogramming via an engineered PGC-1α improves human chimeric antigen receptor T-cell therapy against solid tumors. *J. Immunother. Cancer* **11**, e006522 (2023).
102. Laletin, V. et al. Negative intracellular regulators of T-cell receptor (TCR) signaling as potential antitumor immunotherapy targets. *J. Immunother. Cancer* **11**, e005845 (2023).
103. Shui, J. W. et al. Hematopoietic progenitor kinase 1 negatively regulates T cell receptor signaling and T cell-mediated immune responses. *Nat. Immunol.* **8**, 84–91 (2007).
104. Wang, X. et al. Attenuation of T cell receptor signaling by serine phosphorylation-mediated lysine 30 ubiquitination of SLP-76 protein. *J. Biol. Chem.* **287**, 34091–34100 (2012).
105. Liu, M. et al. CRISPR/Cas9-mediated knockout of intracellular molecule SHP-1 enhances tumor-killing ability of CD133-targeted CAR T cells in vitro. *Exp. Hematol. Oncol.* **12**, 88 (2023).
106. Du, X., Wiede, F., Darcy, P. K. & Tiganis, T. Abstract PR04: CRISPR-mediated PTPN2 deletion in CAR T cells enhances anti-tumor efficacy. *Cancer Immunol. Res.* **10**, PR04 (2022).
107. Naramura, M. et al. c-Cbl and Cbl-b regulate T cell responsiveness by promoting ligand-induced TCR down-modulation. *Nat. Immunol.* **3**, 1192–1199 (2002).
108. Wiede, F. et al. PTPN 2 phosphatase deletion in T cells promotes anti-tumour immunity and CAR T-cell efficacy in solid tumours. *EMBO J.* **39**, e103637 (2020).
109. Wu, L. et al. CD28-CAR-T cell activation through FYN kinase signaling rather than LCK enhances therapeutic performance. *Cell Rep. Med.* **4**, 100917 (2023).
110. Kalinin, R. S. et al. Engineered removal of PD-1 from the surface of CD19 CAR-T cells results in increased activation and diminished survival. *Front. Mol. Biosci* **8**, 745286 (2021).
111. Mestermann, K. et al. The tyrosine kinase inhibitor dasatinib acts as a pharmacologic on/off switch for CAR T cells. *Sci. Transl. Med.* **11**, eaau5907 (2019).
112. Zhang, Y. et al. CRISPR-Cas9 mediated LAG-3 disruption in CAR-T cells. *Front. Med.* **11**, 554–562 (2017).
113. Hu, B. et al. Nucleofection with plasmid DNA for CRISPR/Cas9-mediated inactivation of programmed cell death protein 1 in CD133-specific CAR T cells. *Hum. Gene Ther.* **30**, 446–458 (2019).

114. Cui, Q. et al. CD38-directed CAR-T cell therapy: a novel immunotherapy strategy for relapsed acute myeloid leukemia after allogeneic hematopoietic stem cell transplantation. *J. Hematol. Oncol.* **14**, 82 (2021).
115. Agarwal, S. et al. Deletion of the inhibitory co-receptor CTLA-4 enhances and invigorates chimeric antigen receptor T cells. *Immunity* **56**, 2388–2407.e9 (2023).
116. Masoumi, E. et al. Genetic and pharmacological targeting of A2a receptor improves function of anti-mesothelin CAR T cells. *J. Exp. Clin. Cancer Res.* **39**, 49 (2020).
117. Tang, N. et al. TGF-β inhibition via CRISPR promotes the long-term efficacy of CAR T cells against solid tumors. *JCI Insight* **5**, e133977 (2020).
118. Chen, X. et al. Secretion of bispecific protein of anti-PD-1 fused with TGF-β trap enhances antitumor efficacy of CAR-T cell therapy. *Mol. Ther. Oncolytics* **21**, 144–157 (2021).
119. Kloss, C. C. et al. Dominant-negative TGF-β receptor enhances PSMA-targeted human CAR T cell proliferation and augments prostate cancer eradication. *Mol. Ther.* **26**, 1855–1866 (2018).
120. Narayan, V. et al. PSMA-targeting TGFβ-insensitive armored CAR T cells in metastatic castration-resistant prostate cancer: a phase 1 trial. *Nat. Med.* **28**, 724–734 (2022).
121. Liu, G. et al. Disruption of adenosine 2A receptor improves the anti-tumor function of anti-mesothelin CAR T cells both in vitro and in vivo. *Exp. Cell. Res.* **409**, 112886 (2021).
122. Li, N. et al. Improving the anti-solid tumor efficacy of CAR-T cells by inhibiting adenosine signaling pathway. *Oncoimmunology* **9**, 1824643 (2020).
123. Künkele, A. et al. Functional tuning of CARs reveals signaling threshold above which CD8^{+} CTL antitumor potency is attenuated due to cell fas-FasL-dependent AICD. *Cancer Immunol. Res.* **3**, 368–379 (2015).
124. Tschumi, B. O. et al. CART cells are prone to Fas- and DR5-mediated cell death. *J. Immunother. Cancer* **6**, 92 (2018).
125. Ren, J. et al. A versatile system for rapid multiplex genome-edited CAR T cell generation. *Oncotarget* **8**, 17002–17011 (2017).
126. Prinzing, B. et al. Deleting DNMT3A in CAR T cells prevents exhaustion and enhances antitumor activity. *Sci. Transl. Med.* **13**, eabh0272 (2021).
127. Lopez-Cobo, S. et al. SUV39H1 ablation enhances long-term CAR T function in solid tumors. *Cancer Discov* **14**, 120–141 (2023).
128. Someya, K. et al. Improvement of Foxp3 stability through CNS2 demethylation by TET enzyme induction and activation. *Int. Immunol.* **29**, 365–375 (2017).
129. Yue, X. et al. Control of Foxp3 stability through modulation of TET activity. *J. Exp. Med.* **213**, 377–397 (2016).
130. Jain, N. et al. Disruption of SUV39H1-mediated H3K9 methylation sustains CAR T-cell function. *Cancer Discov.* **14**, 142–157 (2024).
131. Seo, H. et al. TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8^{+} T cell exhaustion. *Proc. Natl Acad. Sci. USA* **116**, 12410–12415 (2019).
132. Chen, J. et al. NR4A transcription factors limit CAR T cell function in solid tumours. *Nature* **567**, 530–534 (2019).
133. Jung, I. Y. et al. BLIMP1 and NR4A3 transcription factors reciprocally regulate antitumor CAR T cell stemness and exhaustion. *Sci. Transl. Med.* **14**, eabn7336 (2022).
134. Wei, J. et al. Targeting REGNASE-1 programs long-lived effector T cells for cancer therapy. *Nature* **576**, 471–476 (2019).
135. Zhao, H. et al. Genome-wide fitness gene identification reveals Roquin as a potent suppressor of CD8 T cell expansion and anti-tumor immunity. *Cell Rep.* **37**, 110083 (2021).
136. Zheng, W. et al. Regnase-1 suppresses TCF-1^{+} precursor exhausted T-cell formation to limit CAR–T-cell responses against ALL. *Blood* **138**, 122–135 (2021).
137. Behrens, G. et al. Disrupting Roquin-1 interaction with Regnase-1 induces autoimmunity and enhances antitumor responses. *Nat. Immunol.* **22**, 1563–1576 (2021).
138. Wang, D. et al. CRISPR screening of CAR T cells and cancer stem cells reveals critical dependencies for cell-based therapies. *Cancer Discov.* **11**, 1192–1211 (2021).
139. Bandyopadhyay, S., Valdor, R. & Macian, F. Tle4 regulates epigenetic silencing of gamma interferon expression during effector T helper cell tolerance. *Mol. Cell. Biol.* **34**, 233–245 (2014).
140. Gogishvili, T. et al. SLAMF7-CAR T cells eliminate myeloma and confer selective fratricide of SLAMF7^{+} normal lymphocytes. *Blood* **130**, 2838–2847 (2017).
141. Prommersberger, S. et al. CARAMBA: a first-in-human clinical trial with SLAMF7 CAR-T cells prepared by virus-free Sleeping Beauty gene transfer to treat multiple myeloma. *Gene Ther.* **28**, 560–571 (2021).
142. Georgiadis, C. et al. Base-edited CAR T cells for combinational therapy against T cell malignancies. *Leukemia* **35**, 3466–3481 (2021).
143. Srinivasan, S. et al. Investigation of ALLO-316: a fratricide-resistant allogeneic CAR T targeting CD70 as a potential therapy for the treatment of AML. *Blood* **136**, 23 (2020).
144. Breman, E. et al. Overcoming target driven fratricide for T cell therapy. *Front. Immunol.* **9**, 2940 (2018).
145. Wellhausen, N. et al. Epitope base editing CD45 in hematopoietic cells enables universal blood cancer immune therapy. *Sci. Transl. Med.* **15**, eadi1145 (2023).
146. Moon, E. K. et al. Expression of a functional CCR2 receptor enhances tumor localization and tumor eradication by retargeted human T cells expressing a mesothelin-specific chimeric antibody receptor. *Clin. Cancer Res.* **17**, 4719–4730 (2011).
147. Di Stasi, A. et al. T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. *Blood* **113**, 6392–6402 (2009).
148. Jin, L. et al. CXCR1- or CXCR2-modified CAR T cells co-opt IL-8 for maximal antitumor efficacy in solid tumors. *Nat. Commun.* **10**, 4016 (2019).
149. Li, G. et al. CXCR5 guides migration and tumor eradication of anti-EGFR chimeric antigen receptor T cells. *Mol. Ther. Oncolytics* **22**, 507–517 (2021).
150. Grover, N. S. et al. CD30-directed CAR-T cells co-expressing CCR4 in relapsed/refractory Hodgkin Lymphoma and CD30^{+} cutaneous T cell lymphoma. *Blood* **138**, 742 (2021).
151. Nicolas-Boluda, A. et al. Tumor stiffening reversion through collagen crosslinking inhibition improves T cell migration and anti-PD-1 treatment. *eLife* **10**, e58688 (2021).
152. Caruana, I. et al. Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. *Nat. Med.* **21**, 524–529 (2015).
153. Liu, X. et al. A chimeric switch-receptor targeting PD1 augments the efficacy of second-generation CAR T cells in advanced solid tumors. *Cancer Res.* **76**, 1578–1590 (2016).
154. Hoogi, S. et al. A TIGIT-based chimeric co-stimulatory switch receptor improves T-cell anti-tumor function. *J. Immunother. Cancer* **7**, 243 (2019).
155. Ma, Q. et al. A PD-L1-targeting chimeric switch receptor enhances efficacy of CAR-T cell for pleural and peritoneal metastasis. *Signal Transduct. Target. Ther.* **7**, 380 (2022).
156. Liu, H. et al. CD19-specific CAR T cells that express a PD-1/CD28 chimeric switch-receptor are effective in patients with PD-L1⇓positive B-cell lymphoma. *Clin. Cancer Res.* **27**, 473–484 (2021).

157. Brog, R. A. et al. Superkine IL-2 and IL-33 armored CAR T cells reshape the tumor microenvironment and reduce growth of multiple solid tumors. *Cancer Immunol. Res.* **10**, 962–977 (2022).
158. Hou, A. J. et al. IL-13Rα2/TGF-β bispecific CAR-T cells counter TGF-β-mediated immune suppression and potentiate anti-tumor responses in glioblastoma. *Neuro Oncol.* **26**, 1850–1866 (2024).
159. Liu, X. et al. A novel dominant-negative PD-1 armored anti-CD19 CAR T cell is safe and effective against refractory/relapsed B cell lymphoma. *Transl. Oncol.* **14**, 101085 (2021).
160. Yamamoto, T. N. et al. T cells genetically engineered to overcome death signaling enhance adoptive cancer immunotherapy. *J. Clin. Invest.* **129**, 1551–1565 (2019).
161. Yang, F. et al. Self-delivery of TIGIT-blocking scFv enhances CAR-T immunotherapy in solid tumors.*Front. Immunol.* **14**, 1175920 (2023).
162. Rafiq, S. et al. Targeted delivery of a PD-1-blocking scFV by CAR-T cells enhances anti-tumor efficacy in vivo. *Nat. Biotechnol.* **36**, 847–856 (2018).
163. Huang, S. W. et al. BiTE-secreting CAR-γδT as a dual targeting strategy for the treatment of solid tumors. *Adv. Sci* **10**, e2206856 (2023).
164. Choi, B. D. et al. CAR-T cells secreting BiTEs circumvent antigen escape without detectable toxicity. *Nat. Biotechnol.* **37**, 1049–1058 (2019).
165. Porter, C. E. et al. Oncolytic adenovirus armed with BiTE, cytokine, and checkpoint inhibitor enables CAR T cells to control the growth of heterogeneous tumors. *Mol. Ther.* **28**, 1251–1262 (2020).
166. Yin, Y. et al. Locally secreted BiTEs complement CAR T cells by enhancing killing of antigen heterogeneous solid tumors. *Mol. Ther.* **30**, 2537–2553 (2022).
167. Li, G. et al. Fn14-targeted BiTE and CAR-T cells demonstrate potent preclinical activity against glioblastoma. *Oncoimmunology* **10**, 1983306 (2021).
168. Choi, B. D. et al. Intraventricular CARv3-TEAM-E T cells in recurrent glioblastoma. *N. Engl. J. Med.* **390**, 1290–1298 (2024).
169. Adachi, K. et al. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. *Nat. Biotechnol.* **36**, 346–351 (2018).
170. Liu, L. et al. Enhanced CAR-T activity against established tumors by polarizing human T cells to secrete interleukin-9. *Nat. Commun.* **11**, 5902 (2020).
171. Jaspers, J. E. et al. IL-18-secreting CAR T cells targeting DLL3 are highly effective in small cell lung cancer models. *J. Clin. Invest.* **133**, e166028 (2023).
172. Ma, X. et al. Interleukin-23 engineering improves CAR T cell function in solid tumors. *Nat. Biotechnol.* **38**, 448–459 (2020).
173. Krenciute, G. et al. Transgenic expression of IL15 improves antiglioma activity of IL13Rα2-CAR T cells but results in antigen loss variants. *Cancer Immunol. Res.* **5**, 571–581 (2017).
174. Kueberuwa, G., Kalaitsidou, M., Cheadle, E., Hawkins, R. E. & Gilham, D. E. CD19 CAR T cells expressing IL-12 eradicate lymphoma in fully lymphoreplete mice through induction of host immunity. *Mol. Ther. Oncolytics* **8**, 41–51 (2018).
175. Lynn, R. C. et al. c-Jun overexpression in CAR T cells induces exhaustion resistance. *Nature* **576**, 293–300 (2019).
176. Blaeschke, F. et al. Modular pooled discovery of synthetic knockin sequences to program durable cell therapies. *Cell* **186**, 4216–4234.e33 (2023).
177. Zhang, X. et al. Depletion of BATF in CAR-T cells enhances antitumor activity by inducing resistance against exhaustion and formation of central memory cells. *Cancer Cell* **40**, 1407–1422.e7 (2022).
178. Ohno, M. et al. Expression of miR-17-92 enhances anti-tumor activity of T-cells transduced with the anti-EGFRvIII chimeric antigen receptor in mice bearing human GBM xenografts. *J. Immunother. Cancer* **1**, 21 (2013).
179. Johnson, L. R. et al. The immunostimulatory RNA RN7SL1 enables CAR-T cells to enhance autonomous and endogenous immune function. *Cell* **184**, 4981–4995.e14 (2021).
180. Zhang, J. et al. Co-expression of miR155 or LSD1 shRNA increases the anti-tumor functions of CD19 CAR-T cells. *Front. Immunol* **12**, 811364 (2022).
181. Stepanov, A. V. et al. Control of the antitumour activity and specificity of CAR T cells via organic adapters covalently tethering the CAR to tumour cells. *Nat. Biomed. Eng.* **8**, 529–543 (2024).
182. Micklethwaite, K. P. et al. Investigation of product-derived lymphoma following infusion of piggyBac-modified CD19 chimeric antigen receptor T cells. *Blood* **138**, 1391–1405 (2021).
183. Elsallab, M. et al. Second primary malignancies after commercial CAR T-cell therapy: analysis of the FDA Adverse Events Reporting System. *Blood* **143**, 2099–2105 (2024).
184. Ghilardi, G. et al. T cell lymphoma and secondary primary malignancy risk after commercial CAR T cell therapy. *Nat. Med.* **30**, 984–989 (2024).
185. Tsai, S. Q. et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nat. Biotechnol.* **33**, 187–197 (2015).
186. Głów, D. et al. LATE–a novel sensitive cell-based assay for the study of CRISPR/Cas9-related long-term adverse treatment effects. *Mol. Ther. Methods Clin. Dev.* **22**, 249–262 (2021).
187. Nobles, C. L. et al. IGUIDE: an improved pipeline for analyzing CRISPR cleavage specificity Jin-Soo Kim. *Genome Biol.* **20**, 14 (2019).
188. Donnadieu, E. et al. Time to evolve: predicting engineered T cell-associated toxicity with next-generation models. *J. Immunother. Cancer* **10**, e003486 (2022).
189. Guedan, S. et al. Time 2EVOLVE: predicting efficacy of engineered T-cells—how far is the bench from the bedside?. *J. Immunother. Cancer* **10**, e003487 (2022).
190. O'Rourke, D. M. et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci. Transl. Med.* **9**, eaaa0984 (2017).
191. Wadman, M. FDA no longer needs to require animal tests before human drug trials. *Science* **379**, 127–128 (2023).
192. Qasim, W. et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. *Sci. Transl. Med.* **9**, eaaj2013 (2017).
193. Stadtmauer, E. A. et al. CRISPR-engineered T cells in patients with refractory cancer. *Science* **367**, eaba7365 (2020).
194. Diorio, C. et al. Cytosine base editing enables quadruple-edited allogeneic CART cells for T-ALL. *Blood* **140**, 619–629 (2022).
195. Bengsch, B. et al. Bioenergetic insufficiencies due to metabolic alterations regulated by the inhibitory receptor PD-1 are an early driver of $CD8^+$ T cell exhaustion. *Immunity* **45**, 358–373 (2016).
196. Wang, X. et al. Engineering tolerance toward allogeneic CAR-T cells by regulation of MHC surface expression with Human Herpes Virus-8 proteins. *Mol. Ther.* **29**, 718–733 (2021).
197. Bishop, D. C. et al. Development of CAR T-cell lymphoma in 2 of 10 patients effectively treated with piggyBac-modified CD19 CAR T cells. *Blood* **138**, 1504–1509 (2021).
198. Wang, B. et al. Generation of hypoimmunogenic T cells from genetically engineered allogeneic human induced pluripotent stem cells. *Nat. Biomed. Eng.* **5**, 429–440 (2021).
199. Kagoya, Y. et al. Genetic ablation of HLA class I, class II, and the T-cell receptor enables allogeneic T cells to be used for adoptive T-cell therapy. *Cancer Immunol. Res.* **8**, 926–936 (2020).
200. Perica, K. et al. HIV immune evasin Nef enhances allogeneic CAR T cell potency. *Nature* **640**, 793–801 (2025).

201. Grosskopf, A. et al. Delivery of CAR-T cells in a transient injectable stimulatory hydrogel niche improves treatment of solid tumors. *Sci. Adv.* **8**, eabn8264 (2022).
202. Frank, A. M. et al. Combining T-cell-specific activation and in vivo gene delivery through CD3-targeted lentiviral vectors. *Blood Adv.* **4**, 5702–5715 (2020).
203. Nicolai, C. J. et al. In vivo CAR T-cell generation in nonhuman primates using lentiviral vectors displaying a multidomain fusion ligand. *Blood* **144**, 977–987 (2024).
204. Hamilton, J. R. et al. In vivo human T cell engineering with enveloped delivery vehicles. *Nat. Biotechnol.* **42**, 1684–1692 (2024).
205. Haghikia, A. et al. Anti-CD19 CAR T cells for refractory myasthenia gravis. *Lancet Neurol.* **15**, 2542 (2024).
206. Müller, F. et al. CD19 CAR T-cell therapy in autoimmune disease—a case series with follow-up. *N. Engl. J. Med.* **390**, 687–700 (2024).
207. Staudt, S. et al. Microbial metabolite-guided CAR T cell engineering enhances anti-tumor immunity via epigenetic-metabolic crosstalk. Preprint at https://www.biorxiv.org/content/10.1101/2024.08.19.608538v1 (2024).
208. Gottschlich, A. et al. Single-cell transcriptomic atlas-guided development of CAR-T cells for the treatment of acute myeloid leukemia. *Nat. Biotechnol.* **41**, 1618–1632 (2023).
209. Hort, S. et al. Deciphering and advancing CAR T-cell therapy with single-cell sequencing technologies. *Mol. Cancer* **9**, 1–25 (2022).
210. Qiu, S. et al. CAR-Toner: an AI-driven approach for CAR tonic signaling prediction and optimization. *Cell Res.* **34**, 386–388 (2023).
211. Hort, S. et al. Toward rapid, widely available autologous CAR-T cell therapy—artificial intelligence and automation enabling the smart manufacturing hospital. *Front. Med.* **9**, 913287 (2022).
212. Tousley, A. M. et al. Co-opting signalling molecules enables logic-gated control of CAR T cells. *Nature* **615**, 507–516 (2023).
213. Sharifzadeh, Z. et al. Genetically engineered T cells bearing chimeric nanoconstructed receptors harboring TAG-72-specific camelid single domain antibodies as targeting agents. *Cancer Lett.* **334**, 237–244 (2013).
214. Li, N., Fu, H., Hewitt, S. M., Dimitrov, D. S. & Ho, M. Therapeutically targeting glypican-2 via single-domain antibody-based chimeric antigen receptors and immunotoxins in neuroblastoma. *Proc. Natl Acad. Sci. USA* **114**, E6623–E6631 (2017).
215. An, N. et al. Anti-multiple myeloma activity of nanobody-based anti-CD38 chimeric antigen receptor T cells. *Mol. Pharm.* **15**, 4577–4588 (2018).
216. De Munter, S. et al. Nanobody based dual specific CARs. *Int. J. Mol. Sci.* **19**, 403 (2018).
217. Shadman, M. et al. CD20 targeted CAR-T for high-risk B-cell non-Hodgkin lymphomas. *Blood* **134**, 3235 (2019).
218. Xie, Y. J. et al. Nanobody-based CAR T cells that target the tumor microenvironment inhibit the growth of solid tumors in immunocompetent mice. *Proc. Natl Acad. Sci. USA* **116**, 7624–7631 (2019).
219. McComb, S. et al. Programmable attenuation of antigenic sensitivity for a nanobody-based EGFR chimeric antigen receptor through hinge domain truncation. *Front. Immunol.* **13**, 864868 (2022).
220. Zhang, M. et al. A single-arm, open-label, pilot trial of autologous CD7-CAR-T cells for CD7 positive relapsed and refractory T-lymphoblastic leukemia/lymphoma. *Blood* **138**, 3829 (2021).
221. Pan, J. et al. Donor-derived CD7 chimeric antigen receptor T cells for T-cell acute lymphoblastic leukemia: first-in-human, phase I trial. *J. Clin. Oncol.* **39**, 3340–3351 (2021).
222. Mo, F. et al. Nanobody-based chimeric antigen receptor T cells designed by CRISPR/Cas9 technology for solid tumor immunotherapy. *Signal Transduct. Target. Ther.* **6**, 80 (2021).
223. Li, D. et al. Camel nanobody-based B7-H3 CAR-T cells show high efficacy against large solid tumours. *Nat. Commun.* **14**, 5920 (2023).
224. Hammill, J. A. et al. Designed ankyrin repeat proteins are effective targeting elements for chimeric antigen receptors. *J. Immunother. Cancer* **3**, 55 (2015).
225. Stepanov, A. V. et al. Switchable targeting of solid tumors by BsCAR T cells. *Proc. Natl Acad. Sci. USA* **119**, e2210562119 (2022).
226. Wang, Y. et al. Targeting FLT3 in acute myeloid leukemia using ligand-based chimeric antigen receptor-engineered T cells. *J. Hematol. Oncol.* **11**, 60 (2018).
227. Kubo, H. et al. Development of non-viral, ligand-dependent, EPHB4-specific chimeric antigen receptor T cells for treatment of rhabdomyosarcoma. *Mol. Ther. Oncolytics* **20**, 646–658 (2021).
228. Wang, D. et al. Chlorotoxin-directed CAR T cells for specific and effective targeting of glioblastoma. *Sci. Transl. Med.* **12**, eaaw2672 (2020).
229. Davies, D. M. et al. Flexible targeting of ErbB dimers that drive tumorigenesis by using genetically engineered T cells. *Mol. Med.* **18**, 565–576 (2012).
230. Schmidts, A. et al. Rational design of a trimeric April-based CAR-binding domain enables efficient targeting of multiple myeloma. *Blood Adv.* **3**, 3248–3260 (2019).
231. Wong, D. P. et al. A BAFF ligand-based CAR-T cell targeting three receptors and multiple B cell cancers. *Nat. Commun.* **13**, 217 (2022).
232. Vedvyas, Y. et al. Manufacturing and preclinical validation of CAR T cells targeting ICAM-1 for advanced thyroid cancer therapy. *Sci. Rep.* **9**, 10634 (2019).
233. Urbanska, K., Stashwick, C., Poussin, M. & Powell, D. J. Follicle-stimulating hormone receptor as a target in the redirected T-cell therapy for cancer. *Cancer Immunol. Res.* **3**, 1130–1137 (2015).
234. Zoine, J. T. et al. Thrombopoietin-based CAR-T cells demonstrate in vitro and in vivo cytotoxicity to MPL positive acute myelogenous leukemia and hematopoietic stem cells. *Gene Ther.* **29**, 1–12 (2022).
235. Hasegawa, A. et al. Mutated GM-CSF-based CAR-T cells targeting CD116/CD131 complexes exhibit enhanced anti-tumor effects against acute myeloid leukaemia. *Clin. Transl. Immunol.* **10**, e1282 (2021).
236. Chinsuwan, T. et al. Ligand-based, piggyBac-engineered CAR-T cells targeting EGFR are safe and effective against non-small cell lung cancers. *Mol. Ther. Oncolytics* **31**, 100728 (2023).
237. D'aloia, M. M. et al. T lymphocytes engineered to express a CD16-chimeric antigen receptor redirect T-cell immune responses against immunoglobulin G-opsonized target cells. *Cytotherapy* **18**, 278–290 (2016).
238. Wu, M. R., Zhang, T., DeMars, L. R. & Sentman, C. L. B7H6-specific chimeric antigen receptors lead to tumor elimination and host antitumor immunity. *Gene Ther.* **22**, 675–684 (2015).
239. Zhang, T., Wu, M.-R. & Sentman, C. L. An NKp30-based chimeric antigen receptor promotes T cell effector functions and antitumor efficacy in vivo. *J. Immunol.* **189**, 2290–2299 (2012).
240. Butler, S. E. et al. Engineering a natural ligand-based CAR: directed evolution of the stress-receptor NKp30. *Cancer Immunol. Immunother.* **71**, 165–176 (2022).
241. Quintarelli, C. et al. Choice of costimulatory domains and of cytokines determines CAR T-cell activity in neuroblastoma. *Oncoimmunology* **7**, e1433518 (2018).
242. Hombach, A. A., Heiders, J., Foppe, M., Chmielewski, M. & Abken, H. OX40 costimulation by a chimeric antigen receptor abrogates CD28 and IL-2 induced IL-10 secretion by redirected $CD4^+$ T cells. *Oncoimmunology* **1**, 458–466 (2012).
243. Pulè, M. A. et al. A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells. *Mol. Ther.* **12**, 933–941 (2005).

244. M, G. et al. CD28.OX40 co-stimulatory combination is associated with long in vivo persistence and high activity of CAR.CD30 T-cells. *Haematologica* **106**, 987–999 (2021).
245. Zhang, H. et al. A chimeric antigen receptor with antigen-independent OX40 signaling mediates potent antitumor activity. *Sci. Transl. Med.* **13**, eaba7308 (2021).
246. Duong, C. P. M. et al. Engineering T cell function using chimeric antigen receptors identified using a DNA library approach. *PLoS One* **8**, e63037 (2013).
247. Han, Y., Xie, W., Song, D. G. & Powell, D. J. Control of triple-negative breast cancer using ex vivo self-enriched, costimulated NKG2D CAR T cells. *J. Hematol. Oncol.* **11**, 92 (2018).
248. Yu, L. et al. GD2-specific chimeric antigen receptor-modified T cells for the treatment of refractory and/or recurrent neuroblastoma in pediatric patients. *J. Cancer Res. Clin. Oncol.* **148**, 2643–2652 (2021).
249. Posey, A. D. & June, C. H. 211. CD2, the first identified T cell co-stimulator, demonstrates more effective chimeric antigen receptor activity over CD28 and 4-1BB. *Mol. Ther.* 10.1016/S1525-0016(16)33816-3 (2015).
250. Weng, J. et al. A novel generation 1928zT2 CAR T cells induce remission in extramedullary relapse of acute lymphoblastic leukemia. *J. Hematol. Oncol.* **11**, 25 (2018).
251. Lai, Y. et al. Toll-like receptor 2 costimulation potentiates the antitumor efficacy of CAR T Cells. *Leukemia* **32**, 801–808 (2018).
252. Nair, S. et al. Functional improvement of chimeric antigen receptor through intrinsic interleukin-15Rα signaling. *Curr. Gene Ther.* **19**, 40–53 (2018).
253. Ma, Y.-J. et al. Successful application of PD-1 knockdown CLL-1 CAR-T therapy in two AML patients with post-transplant relapse and failure of anti-CD38 CAR-T cell treatment. *Am. J. Cancer Res* **12**, 615–621 (2022).
254. Zhou, J. E. et al. ShRNA-mediated silencing of PD-1 augments the efficacy of chimeric antigen receptor T cells on subcutaneous prostate and leukemia xenograft. *Biomed. Pharmacother.* **137**, 111339 (2021).
255. Liu, G., Rui, W., Zhao, X. & Lin, X. Enhancing CAR-T cell efficacy in solid tumors by targeting the tumor microenvironment. *Cell. Mol. Immunol.* **18**, 1085–1095 (2021).
256. Simon, B. et al. Enhancing lentiviral transduction to generate melanoma-specific human T cells for cancer immunotherapy. *J. Immunol. Methods* **472**, 55–64 (2019).
257. Lee, Y. H. et al. PD-1 and TIGIT downregulation distinctly affect the effector and early memory phenotypes of CD19-targeting CAR T cells. *Mol. Ther.* **30**, 579–592 (2022).
258. Jafarzadeh, L. et al. Targeted knockdown of Tim3 by short hairpin RNAs improves the function of anti-mesothelin CAR T cells. *Mol. Immunol.* **139**, 1–9 (2021).
259. Beavis, P. A. et al. Targeting the adenosine 2A receptor enhances chimeric antigen receptor T cell efficacy. *J. Clin. Invest.* **127**, 929–941 (2017).
260. Jain, N. et al. TET2 guards against unchecked BATF3-induced CAR T cell expansion. *Nature* **615**, 315–322 (2023).
261. López-Cobo, S. et al. SUV39H1 ablation enhances long-term CAR T function in solid tumors. *Cancer Discov.* **14**, 120–141 (2024).
262. Mai, D. et al. Combined disruption of T cell inflammatory regulators regnase-1 and roquin-1 enhances antitumor activity of engineered human T cells. *Proc. Natl Acad. Sci. USA* **120**, e2218632120 (2023).
263. Craddock, J. A. et al. Enhanced tumor trafficking of GD2 chimeric antigen receptor T cells by expression of the chemokine receptor CCR2b. *J. Immunother.* **33**, 780–788 (2010).
264. Kuhn, N. F. et al. CD40 ligand-modified chimeric antigen receptor T cells enhance antitumor function by eliciting an endogenous antitumor response. *Cancer Cell* **35**, 473–488.e6 (2019).
265. Hu, W. et al. Chimeric antigen receptor modified T cell (CAR-T) co-expressed with ICOSL-41BB promote CAR-T proliferation and tumor rejection. *Biomed. Pharmacother.* **118**, 109333 (2019).
266. Kalbasi, A. et al. Publisher Correction: Potentiating adoptive cell therapy using synthetic IL-9 receptors. *Nature* **612**, E10 (2022).
267. Chen, C. et al. Construction of PD1/CD28 chimeric-switch receptor enhances anti-tumor ability of c-Met CAR-T in gastric cancer. *Oncoimmunology* **10**, 1901434 (2021).
268. Supimon, K. et al. Cytotoxic activity of anti-mucin 1 chimeric antigen receptor T cells expressing PD-1-CD28 switch receptor against cholangiocarcinoma cells. *Cytotherapy* **25**, 148–161 (2023).
269. Zhao, S. et al. Switch receptor T3/28 improves long-term persistence and antitumor efficacy of CAR-T cells. *J. Immunother. Cancer* **9**, e003176 (2021).
270. Beck, C. et al. Development of a TGFβ–IL-2/15 switch receptor for use in adoptive cell therapy. *Biomedicines* **11**, 459 (2023).
271. Leen, A. M. et al. Reversal of tumor immune inhibition using a chimeric cytokine receptor. *Mol. Ther.* **22**, 1211–1220 (2014).
272. Lange, S. et al. A chimeric GM-CSF/IL18 receptor to sustain CAR T-cell function. *Cancer Discov.* **11**, 1661–1671 (2021).
273. Durgin, J. S. et al. Enhancing CAR T function with the engineered secretion of *C. perfringens* neuraminidase. *Mol. Ther.* **30**, 1201–1214 (2022).
274. Qu, Y. et al. Adenosine deaminase 1 overexpression enhances the antitumor efficacy of chimeric antigen receptor-engineered T cells. *Hum. Gene Ther.* **33**, 223–236 (2022).
275. Bai, Y. et al. Enhancement of the in vivo persistence and antitumor efficacy of CD19 chimeric antigen receptor T cells through the delivery of modified TERT mRNA. *Cell Discov.* **1**, 15040 (2015).
276. Xie, Y. J. et al. Improved antitumor efficacy of chimeric antigen receptor T cells that secrete single-domain antibody fragments. *Cancer Immunol. Res.* **8**, 518–529 (2020).
277. Suarez, E. R. et al. Chimeric antigen receptor T cells secreting anti-PD-L1 antibodies more effectively regress renal cell carcinoma in a humanized mouse model. *Oncotarget* **7**, 34341–34355 (2016).
278. Zhang, Y. et al. Chimeric antigen receptor T cells engineered to secrete CD40 agonist antibodies enhance antitumor efficacy. *J. Transl. Med.* **19**, 82 (2021).
279. Yi, Y. et al. CRISPR-edited CART with GM-CSF knockout and auto secretion of IL6 and IL1 blockers in patients with hematologic malignancy. *Cell Discov.* **7**, 27 (2021).
280. Cao, G. et al. GPC3-targeted CAR-T cells secreting B7H3-targeted BiTE exhibit potent cytotoxicity activity against hepatocellular carcinoma cell in the in vitro assay. *Biochem. Biophys. Rep.* **31**, 101324 (2022).
281. Wehrli, M. et al. Abstract 569: Mesothelin CAR T cells secreting FAP specific T cell engaging molecule (TEAM) target pancreatic cancer and its tumor microenvironment (TME). *Cancer Res.* **82**, 569 (2022).
282. Luo, H. et al. Coexpression of IL7 and CCL21 increases efficacy of CAR-T cells in solid tumors without requiring preconditioned lymphodepletion. *Clin. Cancer Res.* **26**, 5494–5505 (2020).
283. Lei, W. et al. Safety and feasibility of anti-CD19 CAR T cells expressing inducible IL-7 and CCL19 in patients with relapsed or refractory large B-cell lymphoma. *Cell Discov.* **10**, 5 (2024).
284. Swan, S. L. et al. IL7 and IL7 Flt3L co-expressing CAR T cells improve therapeutic efficacy in mouse EGFRvIII heterogeneous glioblastoma. *Front. Immunol* **14**, 1085547 (2023).
285. Liu, Y. et al. Armored inducible expression of IL-12 enhances antitumor activity of glypican-3-targeted chimeric antigen receptor-engineered T cells in hepatocellular carcinoma. *J. Immunol.* **203**, 198–207 (2019).

286. Pegram, H. J. et al. Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. *Blood* **119**, 4133–4141 (2012).
287. Avanzi, M. P. et al. Engineered tumor-targeted T cells mediate enhanced anti-tumor efficacy both directly and through activation of the endogenous immune system. *Cell Rep.* **23**, 2130–2141 (2018).
288. Wang, D., Shao, Y., Zhang, X., Lu, G. & Liu, B. IL-23 and PSMA-targeted duo-CAR T cells in prostate cancer eradication in a preclinical model. *J. Transl. Med* **18**, 23 (2020).
289. Li, X., Daniyan, A. F., Lopez, A. V., Purdon, T. J. & Brentjens, R. J. Cytokine IL-36γ improves CAR T-cell functionality and induces endogenous antitumor response. *Leukemia* **35**, 506–521 (2021).
290. Seo, H. et al. BATF and IRF4 cooperate to counter exhaustion in tumor-infiltrating CAR T cells. *Nat. Immunol.* **22**, 983–995 (2021).

Acknowledgements

The study was supported by the Innovative Medicines Initiative 2 Joint Undertaking, from the European Union's Horizon 2020 research and innovation programme and EFPIA (grant agreement no. 116026, T2EVOLVE to M.H. and M.L.), the Wilhelm-Sander-Stiftung (grant no. 2022.134.1 to A.V., K.Z.-M. and M.L.), ERA-NET TRANSCAN-3 (EC co-funded call 2021, SmartCAR-T to T.T.N., J.C., M.H., K.Z.-M. and M.L.), the Paula & Rodger Riney Foundation (to M.H. and M.L.), IZKF Würzburg (S-511 to S.S. and M.L., C-543 to M.L.), the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG, TRR 221 (subproject A03 to M.H., H.E. and M.L.); TRR338 (subproject A02 to M.H. and M.L., C04 to M.L.); and CRC1525 (seed grant to M.L.)), the Bavarian Cancer Research Center (BZKF; TANGO to M.L. and M.H.), INCA Award by Novartis (to M.L.), the Fonds de la Recherche Scientifique (Postdoctoral fellowship to T.T.N. and funding to J.C.), Fondation Contre le Cancer (C/2020/1447), Université de Liège (J.C.) and FIRS CHU de Liège (J.C.).

Author contributions

Article design was carried out by T.T.N. and M.L. Figures and tables were produced by M.J., A.B. and T.T.N. The paper was written by C.G., J.J.M., J.C., S.S., K.Z.-M., T.T.N. and M.L. Editing was carried out by C.G., J.J.M., M.H., P.H. and M.L.

Competing interests

M.L. and M.H. are listed as inventors on patent application WO2021/058811A1. M.H. is listed as an inventor on patent applications and granted patents related to CAR T technologies that have been filed by the Fred Hutchinson Cancer Research Center, Seattle, WA and by the University of Würzburg, Würzburg, Germany. M.H. is a co-founder and equity owner of T-CURX GmbH, Würzburg, Germany. M.H. received honoraria from Celgene/BMS, Janssen, Kite/Gilead.

Additional information

Correspondence and requests for materials should be addressed to Maik Luu.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

© Springer Nature Limited 2025