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Review article

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Progress in the development of cytokine armoured CAR T cells

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Abstract

The engineering of chimeric antigen receptor (CAR) T cells has evolved from first-generation constructs to sophisticated armoured CAR T cells of the fourth generation. These advanced cellular constructs are engineered to co-express cytokines, chemokines or other immunomodulatory factors alongside CARs, aiming to enhance the efficacy, safety and persistence of CAR T cells within the tumour microenvironment. In particular, the potential for reversion of immunosuppression may allow for the treatment of solid tumours, which are in need of new therapeutic options. Here, we explore clinical and preclinical findings with cytokine-enhanced CAR T cells and discuss strategies for conditional cytokine secretion to mitigate systemic toxicity.

Sections

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Introduction

High tumour-infiltrating lymphocyte (TIL) counts are associated with better prognoses across a spectrum of different types of cancer, highlighting the crucial role of T cells in orchestrating tumour rejection[1–3]. Upon binding to antigen presented by MHC-II molecules, $CD4^+$ T cells amplify immune responses through cytokine secretion, and $CD8^+$ T cells can mediate direct tumour cell killing in response to binding tumour antigens presented via MHCI molecules. Early immunotherapy approaches, notably adoptive cell transfer of expanded intratumoural T cells, showcased the potential, yet variable, efficacy of harnessing T cells against cancer[4].

Chimeric antigen receptor (CAR) T cell therapy emerged as a revolutionary leap in immuno-oncology that enabled the targeted elimination of tumour cells through MHC-independent mechanisms. By engineering patient-derived T cells ex vivo to express synthetic receptors that recognize tumour-associated antigens, CAR T cells have achieved unprecedented clinical outcomes, particularly in haematological malignancies. So far, seven different CAR T cell therapies have received US Food and Drug Administration (FDA) approval, offering effective treatment options for relapsed or refractory B cell acute lymphoblastic leukaemia, B cell non-Hodgkin lymphoma, follicular lymphoma, mantle-cell lymphoma and multiple myeloma[5–7]. Despite these successes, translation of CAR T cell therapy to solid tumours remains a formidable challenge.

The immunosuppressive tumour microenvironment (TME) of solid tumours poses considerable barriers to CAR T cell infiltration, persistence and cytotoxic function, necessitating further innovation[8]. Recent studies using TME modulators, for example, anti-angiogenics such as bevacizumab, demonstrate that it is feasible to remodel the TME and enhance CAR T cell efficacy[9]. Notably, these adjunctive approaches may offer a more tractable and clinically scalable solution for some cancers than intricate CAR engineering, underscoring the potential of combinatorial therapies. However, not all drugs and antibodies can cross the blood–brain barrier or sufficiently diffuse into a large solid tumour mass, so it is likely that combination therapy will require an indication-specific approach. Furthermore, therapeutic resistance and variability in responses among patients with haematological cancers indicate a crucial need to refine CAR T cell design, improve antigen selection and develop biomarkers predictive of response[10]. Continued innovation is essential to broaden the therapeutic reach and durability of CAR-based immunotherapies.

The evolution of CAR T cell design has been a journey of iterative refinement, transitioning from rudimentary constructs to highly engineered cellular systems. First-generation CARs, composed of an antigen-binding domain linked to a transmembrane domain for surface expression, coupled to an intracellular CD3ζ signalling molecule, demonstrated limited efficacy owing to insufficient activation and persistence[11]. The advent of second-generation CARs, which incorporate a costimulatory domain that is commonly derived from CD28, CD134 or CD137 (also known as 4-1BB), marked a pivotal improvement in therapeutic potency[12]. The third generation, with two costimulatory domains, hints at further therapeutic potential, although evidence of conclusive clinical superiority remains elusive.

Building on these foundational designs, a fourth wave of innovation has focused on arming CAR T cells with immunomodulatory payloads to actively remodel the TME. These 'armoured' CAR T cells co-express a CAR and cytokines, cytokine receptors, transcription factors, antibodies or enzymes, thereby enhancing effector function and resistance to immunosuppressive cues. Among these, cytokine-armoured CARs have garnered particular interest owing to their capacity to reshape the immune landscape within tumours (Fig. 1) and are now being explored in models of autoimmunity[13] and neuroinflammation[14].

Harnessing the principles of synthetic biology — the rational design and construction of genetic circuits and signalling molecules — has proved essential to tailor these advanced cell therapies. This approach enables the creation of programmable, logic-gated CAR T cells capable of conditional cytokine secretion, selective activation and adaptive resistance to the immunosuppressive TME. Although this Review focuses on cytokine-armoured CAR strategies, including strategies for conditional cytokine expression, we acknowledge the growing repertoire of complementary approaches, including inverted cytokine receptors[15], dominant-negative receptors[16], single-chain variable fragment (scFv) secretion[17] and transcription factor overexpression[18].

Cytokine enhancements to CAR T cells

Cytokine signalling holds a central role in immune-cell function, with diverse effects mediated through complex networks of shared and heterodimeric receptors. These pathways are not only highly pleiotropic but also context dependent, integrating environmental cues to drive distinct transcriptional and functional responses[19]. Broadly, cytokines are classified as either immunostimulatory or immunosuppressive, based on their effects on immune activation and lineage fate. Immunostimulatory cytokines such as IL-2, IL-7, IL-12, IL-15, IL-18, IL-21 and IL-23 can promote T cell expansion, effector function, TME remodelling and immune memory formation, making them attractive payloads for CAR T cell engineering. These cytokines have been incorporated into CAR T cells using constitutive, inducible or conditional expression systems to augment persistence and antitumour efficacy[20].

Conversely, immunosuppressive cytokines, such as TGFβ, IL-4 and IL-10, are more commonly counteracted by strategies that disrupt their signalling. These include the use of antibody-based ligand traps, small molecule inhibitors targeting cytokine receptors and increasingly, synthetic approaches such as dominant negative or inverted cytokine receptors[21]. Interestingly, and perhaps paradoxically, CAR T cells armed with IL-10 have demonstrated improved function against solid tumours in mouse models, highlighting the context-specific nature of IL-10 biology, as discussed further below. The heterogeneity of cytokine profiles across tumour types necessitates a rational pairing of cytokine enhancements to specific immune contexts.

Evaluating cytokine-armoured CAR T cells requires rigorous preclinical modelling (see Supplementary Table 1 for an overview of preclinical studies with armoured CAR T cells). Animal models using immunocompetent mice remain essential for uncovering systemic toxicities and immune interactions that xenograft models cannot recapitulate. Although models based on humanized mice remain indispensable for antigen specificity studies, they lack the functional immune architecture necessary to assess cytokine-driven effects holistically. Therefore, integrated use of both immunocompetent and immunodeficient mice is crucial to preclinical validation.

Among the most extensively studied strategies so far is the constitutive expression of pro-inflammatory cytokines. Clinical trials have already been initiated for IL-12- and IL-15-armoured CAR T cell therapies, with preliminary findings indicating enhanced persistence and improved tumour control (Table 1). Additional cytokines, including IL-7, IL-10 and IL-18 have also advanced to clinical evaluation, supported by compelling preclinical data that suggest favourable effects on CAR T cell functionality and TME remodelling. Other cytokines, such as

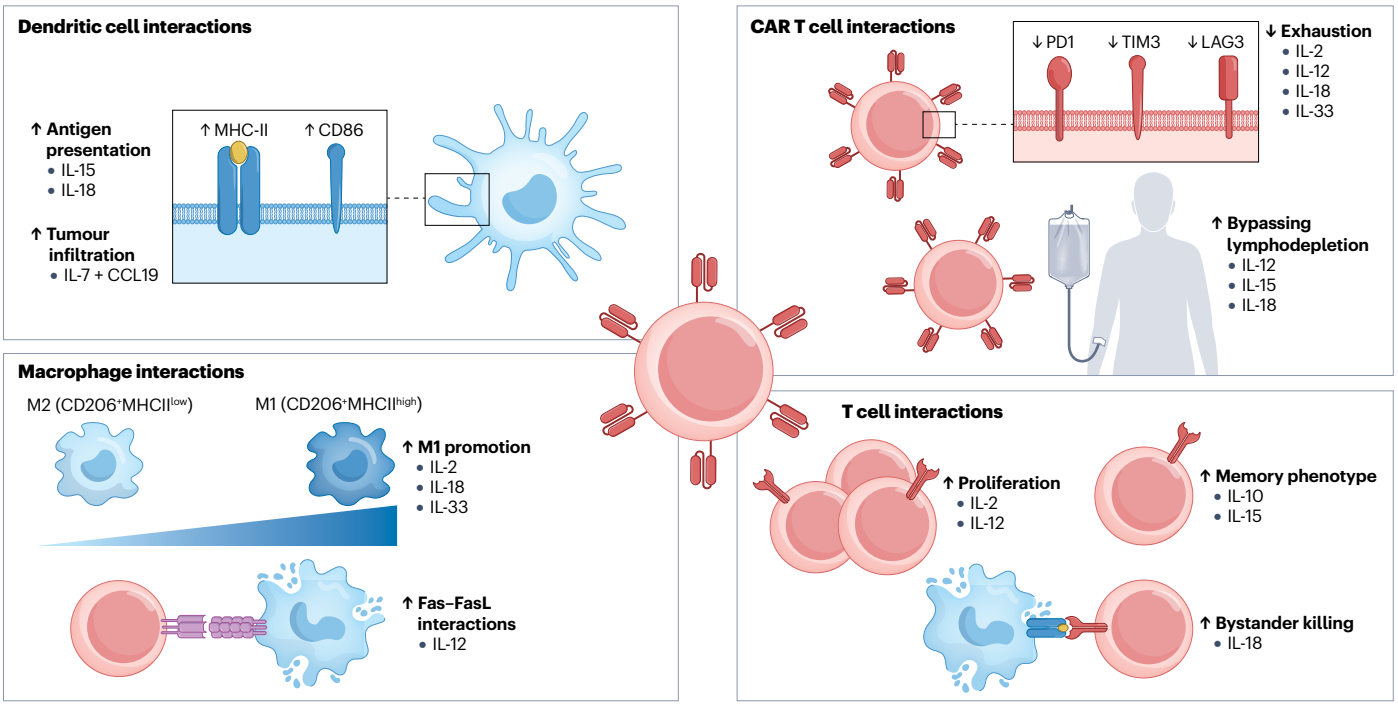


Fig. 1 | Effects of cytokines secreted by armoured CAR T cells on different types of immune cell. Dendritic cells: armouring chimeric antigen receptor (CAR) T cells with IL-15 or IL-18 promotes MHC-II surface expression on dendritic cells, which may increase tumour antigen presentation and recruitment of endogenous T cells[52]. Co-expression of IL-7 and CCL19 in CAR T cells increases dendritic cell infiltration into tumours[71]. CAR T cells: secretion of IL-2 and IL-33, IL-12 or IL-18 induces autocrine and paracrine signalling between CAR T cells that leads to reduced expression of markers of exhaustion such as PD1, LAG3 and TIM3. Moreover, armouring with IL-12 (ref. 24), IL-15 (ref. 86) or IL-18 (ref. 50) obviates the need for lymphodepletion. Other T cells: IL-2, IL-10, IL-12, IL-15 and IL-18 can directly modulate the response of endogenous T cells. IL-10 or IL-15 skews the differentiation of endogenous T cells towards memory phenotypes[86,87,96], IL-2 and IL-12 can increase host T cell proliferation[30,62] and IL-18 facilitates bystander killing in in vitro experiments in a T cell receptor-dependent manner[52]. Macrophages: secretion of IL-2 and IL-33 together, or IL-18 alone, increases the polarization of macrophages towards the inflammatory M1 phenotype[50,52,62]. In addition, IL-12 enhances Fas–FasL-mediated interactions between CAR T cells and macrophages, contributing to macrophage activation and tumour cell killing. Collectively, various (predominantly pro-inflammatory) cytokines demonstrate the ability to alter immune-cell phenotypes in favour of a more robust antitumour response.

IL-23 and IL-33, have shown promise in preclinical models, but their translational readiness requires further investigation.

IL-12 was one of the first cytokines used to armour CAR T cells

IL-12 is a heterodimeric pro-inflammatory cytokine composed of the subunits p35 and p40. Early responses to infection are characterized by stimulation of dendritic cells and macrophages and subsequent activation of MyD88-dependent signalling pathways, which induce the secretion of high levels of IL-12 (ref. 22). The primary responders to IL-12 are cytotoxic lymphocytes, including T cells and natural killer (NK) cells, owing to their expression of the IL-12 receptor complex (IL-12R), which consists of IL-12Rβ1 and IL-12Rβ2 (ref. 23). IL-12R-mediated activation of JAK–STAT signalling pathways promotes T helper 1 (T_H1) cell polarization, enhances cytotoxicity and increases interferon-γ (IFNγ) production and proliferation[23].

The first preclinical studies of IL-12 co-expression in CAR T cells demonstrated remarkable promise: in a 2012 landmark study, constitutive IL-12 co-expression in CD19-targeted CAR T cells led to potent tumour clearance in an immunocompetent mouse model of lymphoma, even in the absence of cyclophosphamide preconditioning[24]. Lymphodepleting chemotherapies such as cyclophosphamide and fludarabine are usually used before CAR T cell infusion to facilitate improved engraftment and are associated with improved survival, but commonly cause cytopenia and carry a risk of infection[25]. Being able to maintain therapeutic responses while avoiding lymphodepletion-induced toxicities is highly attractive for patients undergoing CAR T cell therapy. Subsequent preclinical models, including studies of aggressive B cell lymphomas, showed similar survival benefits[26], highlighting the potential of IL-12 to reduce reliance on lymphodepletion — an advantage given that infection accounts for a substantial proportion (~3.5%) of non-relapse mortality in patients treated with current CAR T products[27].

The success of IL-12 armouring in preclinical models extends to solid tumours. CAR T cells targeting the tumour-associated antigen Muc16 ($Muc16^{ecto}$) induced durable responses in mouse models of ovarian cancer. These effects were mediated by autocrine IL-12 signalling, which, in turn, increased FasL expression on CAR T cells, enabling them to deplete immunosuppressive $F4/80^{+}CD11b^{+}$ tumour-associated macrophages via Fas–FasL signalling[28,29]. Parallel studies in immunodeficient mice bearing human SKOV3-$Muc16^{ecto+}$ tumours confirmed that the activity of IL-12 was intrinsic to the engineered human CAR T cells, as mouse cells lack responsiveness to human IL-12 (refs. 30,31).

These promising data prompted the launch of a first-in-human, dose-escalation phase I trial of $Muc16^{ecto}$-targeted, IL-12-armoured CAR T cells for recurrent ovarian cancer (NCT02498912; see

Table 1 | Outcomes for cytokine-armoured CAR T cell therapies in phase I clinical trials

Construct design (trial number)	Indication	Response, numbers of patients	Toxicities, numbers of patients	Ref.
GPC3-targeted CAR armoured with IL-7 and CCL19	HCC	SD, 1/1 (RECIST 1.1)	None, 1/1	71
GPC3-targeted CAR armoured with IL-7 and CCL19 (NCT03198546)	HCC	CR, 1/6 SD, 2/6 PR, 1/6 PD, 2/6	None, 2/6 Fever, 4/6 Fatigue, 2/6	77
GPC3-targeted CAR armoured with IL-7 and CCL19 (NCT04405778)	HCC (*n*=8) Liposarcoma (*n*=2) Gastric neuroendocrine carcinoma (*n*=1)	Across all cancers: SD, 5/11 Across HCC: SD 4/8 Rest unknown	CRS (any grade), 6/11 Grade 1 CRS, 5/11 Grade 2 CRS, 1/11	78 Terminated (business decision)
CD19-targeted CAR armoured with NFAT-inducible IL-7 and CCL19 (NCT03258047)	R/r large B cell lymphoma	CR, 22/39 PR, 9/39 MPFS, 13 months	Granulocytopenia, 37/39 Anaemia, 34/39 CRS (any grade), 29/39 Grade 1 CRS, 9/39 Grade 2 CRS, 15/39 Grade 3 CRS, 5/39	79
CD19-targeted CAR armoured with IL-10 (NCT06277011)	R/r large B cell lymphoma R/r acute lymphoblastic leukaemia	CR rate at 3 months: 20/20	Neutropenia Thrombocytopenia Anaemia (frequency not stated)	99
CD19-targeted CAR armoured with IL-12 (NCT06343376)	R/r $CD19^+$ haematological malignancies	NA	NA	Terminated (manufacturing issues)
EGFR-targeted CAR armoured with IL-12 (NCT03542799)	Metastatic colorectal cancer	NA	NA	Ongoing
GPC3-targeted CAR armoured with IL-15 and containing inducible caspase 9 safety switch (NCT05103631, NCT04377932)	R/r liver tumours	PR, 4/12 SD, 4/12 PD, 4/12	CRS (any grade), 10/12 Grade 1 CRS, 5/12 Grade 2 CRS, 3/12 Grade 3 CRS, 0/12 Grade 4 CRS, 2/12	92
GD2-targeted CAR armoured with IL-15 and containing inducible caspase 9 safety switch (NCT03721068)	R/r neuroblastoma R/r osteosarcoma	NA	NA	Ongoing
CD19-targeted CAR armoured with IL-18 (NCT04684563)	Rr/r non-Hodgkin lymphoma	CR, 11/21 PR, 6/21 MPFS, 8.7 months	CRS, 13/21 ICANS, 3/21	56
CD371-targeted CAR armoured with IL-18 (NCT06017258)	R/r acute myeloid leukaemia	Responders, 3/5	CRS, 5/5 ICANS, 1/5	57
GD2-targeted CAR armoured with NFAT-inducible IL-18 (EU CT 2022-501725-21-00)	Neuroblastoma Advanced breast cancer Ewing sarcoma Osteosarcoma	NA	NA	Ongoing

For NCT clinical trials, see ClinicalTrials.gov. CAR, chimeric antigen receptor; CR, complete response; CRS, cytokine release syndrome; HCC, hepatocellular carcinoma; ICANS, immune effector cell-associated neurotoxicity syndrome; MPFS, median progression-free survival; NFAT, nuclear factor of activated T cells; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumours; r/r, relapsed or refractory; SD, stable disease.

ClinicalTrials.gov), which remains ongoing[32]. An interim update in 2020 reported no dose-limiting toxicities (DLTs) in 15 heavily pretreated patients who received CAR T cell therapy without lymphodepletion. However, cytokine release syndrome (CRS) occurred at all dose levels and required clinical management[33]. Notably, among three patients who underwent lymphodepletion before infusion, two experienced DLTs, highlighting the additive toxicity risk introduced by preconditioning[33]. In 2024, a phase I trial was initiated that aimed to evaluate IL-12-armoured, CD19-targeted CAR T cells for relapsed or refractory haematological malignancies, but it was recently terminated owing to manufacturing issues (NCT06343376). Another phase I trial is evaluating the maximum tolerated dose for IL-12-armoured, EGFR-targeted CAR T cells being used to treat metastatic colorectal cancer, but its status has not been reported to date (NCT03542799).

Although CRS is common with unarmoured CAR T cell therapies, preclinical data suggest that constitutive IL-12 secretion may exacerbate toxicity in a dose- and context-dependent manner. Factors such as post-infusion CAR T cell expansion, antigen density and tumour burden likely compound the effects, with only dosing being reliably controlled in clinical settings.

Although constitutive IL-12 expression offers potent immunostimulatory capacity, ongoing research is shifting towards transient, inducible, tumour-restricted or tunable systems that preserve efficacy while mitigating systemic toxicity (Fig. 2). For example, mRNA-engineered T cells transiently expressing IL-12 have been administered intratumourally in mouse models of melanoma, where they successfully controlled local and contralateral tumour growth[34]. The benefit of this approach likely reflects a combination of short-lived IL-12 expression and spatially confined delivery, which restricts cytokine exposure to the TME. Within this controlled setting, IL-12 activated dendritic cells and other antigen-presenting cells (APCs), enhancing cross-presentation and promoting the priming or reinvigoration of endogenous tumour-specific T cells. These systematically mobilize and traffic to distant lesions, providing a mechanistic explanation for contralateral tumour control despite unilateral administration. The findings suggest that spatial and temporal confinement of IL-12 activity can preserve therapeutic benefit while mitigating the risk of CRS, and that this strategy may be suitable for tumour types with anatomically confined growth, such as glioblastoma or ovarian cancer, where local administration is feasible and may enhance safety[35].

Other innovations include anchoring IL-12 to the CAR T cell via tethered domains (attIL-12), thus limiting diffusion and reducing systemic toxicity. This approach is currently under clinical evaluation for advanced and/or metastatic soft tissue sarcoma and osteosarcoma (NCT05621668, NCT06474676)[36].

Various technologies are being used to express gene cassettes in primary T cells[37], including transcription factor-responsive promoters that are being explored to achieve antigen-dependent, activation-linked cytokine expression. This strategy exploits endogenous transcriptional programmes induced by T cell receptor or CAR activation, such as those controlled by NFAT[38] (Fig. 2a). This approach has been functionally validated in several models. In a mouse model of gastric adenocarcinoma, affinity-attenuated EpCAM-targeted CAR T cells were engineered with an NFAT-driven IL-12 expression cassette and showed efficacy comparable to that of the original high-affinity CAR T cells, but spared healthy tissues[39]. Similarly, NFAT-driven IL-12 expression in GPC3-targeted CAR T cells enhanced tumour control in mice with hepatocellular carcinoma (HCC) xenografts without overt toxicity[40]. However, although NFAT-mediated IL-12 induction requires antigen-dependent CAR T cell activation, once activated it may retain potential toxicity risks by raising systemic pro-inflammatory cytokine levels. This is also suggested by results from mouse[41] and phase I clinical studies of T cell receptor-engineered T cells and TILs: a phase I clinical trial of TILs enhanced with NFAT-controlled IL-12 in patients with metastatic melanoma resulted in a large increase in objective responses (63% versus 5.9%) in the low-dose cohort, but highlighted a trade-off between antitumour potency and systemic toxicity[42].

In parallel, alternative synthetic promoter systems have been developed[43]. For example, cytokine transgenes have been inserted into endogenous activation-inducible loci, such as *CD25* or *PDCD1*, using transcription activator-like effector nucleases (TALENs)[44]. These loci are transcriptionally upregulated following T cell activation, offering an intrinsically regulated, antigen-dependent, platform for transgene expression. Here, targeted knock-in of an IL-12p70 cassette into the

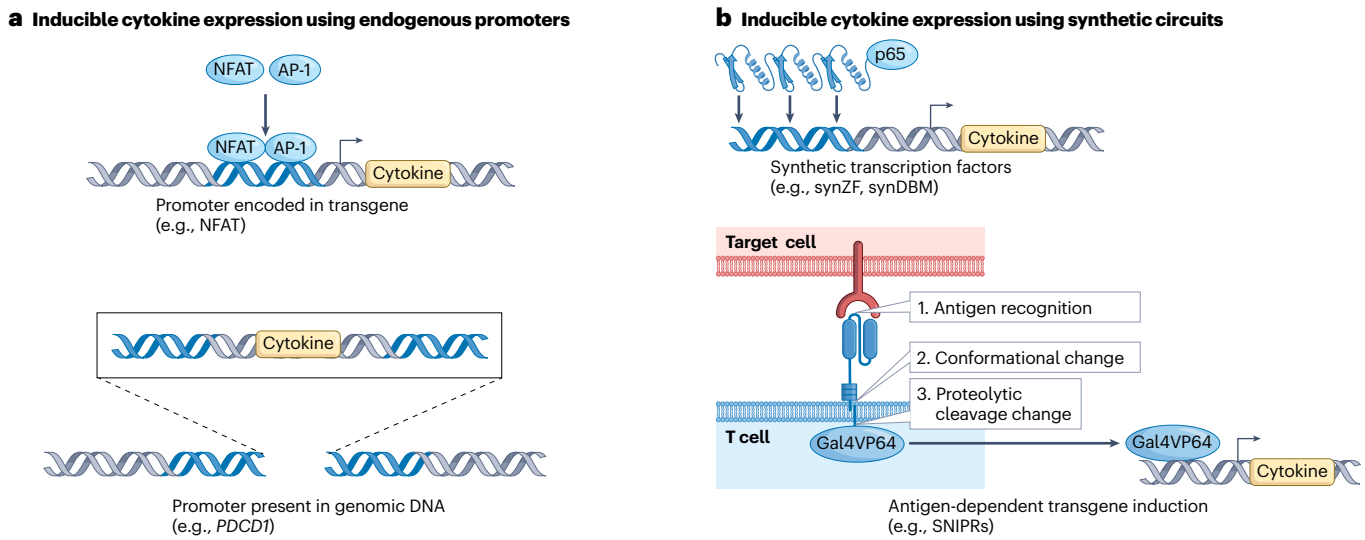


Fig. 2 | Inducible cytokine expression systems to armour chimeric antigen receptor T cell therapies. a, Cytokine expression can be restricted to the tumour microenvironment by placing the transgene encoding the cytokine under the control of transcription factors that are induced in response to T cell activation, such as NFAT and AP-1. The promoter that is bound by these transcription factors can be encoded as part of the transgene. Alternatively, the transgene can be inserted downstream of the corresponding endogenous promoters using transcription activator-like effector nuclease (TALEN) or CRISPR technologies. **b**, Cytokine expression can be uncoupled from endogenous transcriptional programmes through the use of transgene-encoded synthetic transcription factors, such as engineered zinc-finger (synZF) proteins, together with their cognate DNA-binding motifs encoded within the transgene. Antigen-sensing receptors (such as synthetic Notch (synNotch) receptors and synthetic intramembrane proteolysis receptors (SNIPRs)) bind to antigens via an antibody-derived single chain variant fragment (scFv). Antigen recognition induces a conformational change at receptor domains proximal to the transmembrane region, exposing previously hidden protease cleavage sites. When cleaved, these release an intracellular payload (typically a transcription factor). Integration of non-human transcription factors (such as Gal4VP64 or synZF-p65) into antigen-sensing receptors enables programmable, logic-gated control of cytokine expression in T cells. In these systems, cytokine secretion occurs only after synNotch-mediated tumour antigen recognition, thereby confining the cytokine secretion to the tumour site. These systems allow for spatiotemporal control of cytokine expression, with the aim of enhancing antitumour efficacy and limiting systemic toxicity.

CD25 or *PDCD1* locus of human CD22-specific CAR T cells demonstrated effective localization of cytokine release (see Supplementary Table 2 for overview of preclinical studies of CAR T cells with inducible cytokine expression)[44].

To discover promoters that are specifically activated in the tumour environment, an elegant study compared highly expressed genes between intratumoural and splenic T cells and identified *NR4A2* and *RGS16* as highly induced in the former[45]. CAR T cells armoured with an IL-12 construct inserted downstream of *NR4A2* displayed improved efficacy and safety compared with unarmoured CAR T cells in mouse models of ovarian, breast and colorectal cancer[45].

Overall, IL-12 remains a powerful component in the CAR T cell engineering toolkit that is capable of enhancing tumour clearance and bypassing lymphodepletion but only if tightly regulated to avoid life-threatening toxicity.

IL-18 can address tumour heterogeneity

IL-18 is a pro-inflammatory cytokine that is initially synthesized as an inactive monomer (pro-IL-18) and then cleaved into its active form by caspase 1 before secretion[46,47]. Like IL-12, IL-18 is secreted by APCs as well as epithelial cells and is known to synergize with IL-12 to promote T_H1 cell-type immune responses[48]. Accordingly, T cells and NK cells express the IL-18 receptor complex (IL-18Rα and IL-18Rβ), which promotes inflammatory responses and notably, increased IFNγ production[48,49]. IL-18 has emerged as a potent cytokine candidate for CAR T cell armouring owing to its capacity to activate both innate and adaptive immune cells, offering potential to treat heterogeneous tumours[49].

IL-18-armoured CAR T cells have demonstrated promising results in preclinical models, particularly in solid tumours such as small-cell lung cancer (SCLC). In both immunodeficient NOD scid gamma (NSG) mice and immunocompetent B6129SF1/J mice bearing $DLL3^+$ small cell lung cancer tumours, IL-18 expression improved survival and facilitated TME remodelling. This was evidenced by a reduction in immunosuppressive $CD206^+MHC\text{-}II^{low}$ M2-like macrophages and enhanced CD86 expression on APCs, suggesting a pro-inflammatory shift in the TME[50].

Importantly, the effects of IL-18 extend beyond direct CAR T cell cytotoxicity. In tumour-bearing mice treated with mesothelin- or CD19-targeted CAR T cells, IL-18 secretion supported bystander T cell proliferation in an antigen-independent manner[51]. Although these early data implied a capacity for overcoming tumour heterogeneity, definitive evidence for bystander T cell activation came from mouse models of syngeneic lymphoma (EL4) that contained both $CD19^+$ and $CD19^-$ subpopulations: treatment with IL-18-armoured CD19-targeted CAR T cells resulted in durable tumour control, whereas unarmoured CAR T cells failed to eradicate antigen-negative clones[52]. This supports a model whereby IL-18 can act as a paracrine amplifier of antitumour immunity, expanding and activating endogenous T cells and myeloid cells even in the absence of direct CAR-targeted antigen expression.

Recent findings suggest that IL-18 armouring may also enable CAR T cells to overcome antigen density thresholds. In a mouse model of multiple myeloma, BCMA-targeted CAR T cells that were engineered to secrete IL-18 demonstrated the capacity to eliminate tumour cells with low BCMA expression, an effect not observed with unarmoured counterparts[53]. These results highlight the potential of IL-18 to amplify immune responses in antigen-sparse environments.

In clinical trials, systemic administration of recombinant IL-18 has shown limited efficacy, primarily owing to rapid sequestration by IL-18 binding protein (IL-18BP), a soluble high-affinity decoy receptor that neutralizes IL-18 and restricts its bioavailability in circulation. Consequently, the clinical utility of IL-18 likely depends on strategies that tightly regulate cytokine exposure, both spatially and temporally. In particular, localized expression of IL-18 within the TME may circumvent systemic neutralization by limiting diffusion into the circulation and enabling higher concentrations at the tumour site.

To achieve this, inducible IL-18 constructs have been incorporated into TRUCKS (T cells redirected for universal cytokine-mediated killing), a platform designed to couple T cell activation with inducible cytokine release at the tumour site[54]. GD2-targeted IL-18-secreting TRUCKs have been successfully manufactured using good manufacturing practice (GMP)-compliant manufacturing protocols[54], paving the way for clinical implementation. These engineered cells offer the potential to deliver IL-18 in a tumour-restricted manner, and early-stage clinical translation is now under way. Similarly, GD2-targeted CAR T cells armoured with NFAT-inducible IL-18 were successfully used in a mouse model of disseminated neuroblastoma. Unlike the unarmoured therapy, induction of IL-18 facilitated tumour cell eradication from the bone marrow, blood and spleens of these mice, and resulted in the highest T cell frequencies measured within these compartments[55]. Absence of toxicity and demonstration of GMP-compliant manufacture has led to a phase I trial to investigate safety and dosing of this immunotherapy in patients with neuroblastoma and other cancers that express $GD2^+$ (EU CT 2022-501725-21-00)[55].

Remarkably, in 21 patients with refractory or recurrent non-Hodgkin lymphoma who previously received unarmoured CD19 CAR T cell therapy, treatment with IL-18-armoured CD19-targeted CAR T cells achieved complete (52%) and partial (29%) responses at 3 months post-infusion, with some durable responses being reported upon follow-up (NCT04684563)[56]. This strongly highlights that cytokine armouring can be used to strengthen the antitumour potency of existing immunotherapies. Given the challenges of reliably and consistently manufacturing high-quality cell products from patient blood, having a reliable antitumour potency booster will be essential to increase the frequency of responders to CAR T cell therapy.

Several first-in-human trials are currently evaluating IL-18-armoured or IL-12–IL-18 dual-armoured CAR T cells in patients with relapsed or refractory lymphoma, neuroblastoma, ovarian cancer or glioblastoma[55–58]. Although these efforts remain in early phases, they represent a key step towards realizing the therapeutic potential of IL-18 in solid tumour settings.

IL-2 and IL-33 support T cell proliferation and reshape the TME

IL-2 is a monomeric pro-inflammatory cytokine that is secreted predominantly by activated T cells, is essential to sustain effector T cell proliferation and survival, and supports T_H1 cell-type immune responses[59]. IL-2 also expands immunosuppressive $FOXP3^+$ regulatory T (T_{reg}) cells through high-affinity IL-2 receptor signalling, which is essential to maintain immune homeostasis[60]. The binding affinity of the IL-2R complex to IL-2 is determined by whether it comprises IL-2Rα, IL-2Rβ or common γ chains (γc). This differs between IL-2-responding cell types and contributes to whether IL-2 promotes or curtails antitumour efficacy. IL-33, an alarmin released upon epithelial or stromal damage, activates innate and adaptive lymphocytes – including $CD8^+$ T cells, type 2 innate lymphoid cells (ILC2s) and dendritic cells – facilitating immune recruitment and TME modulation in infection, allergy and cancer[61].

The rationale for co-expression of IL-2 and IL-33 lies in their complementary capacity to expand cytotoxic lymphocyte numbers and remodel the TME. In immunocompetent mouse models of melanoma

(B16F10), CAR T cells engineered to secrete both cytokines led to significant TME restructuring, including enhanced infiltration of TILs, a decreased T_{reg} cell to $CD8^+$ T cell ratio, diminished expression of T cell exhaustion markers (PD1 and TIM3) and reduced frequencies of immunosuppressive MHC-II^- tumour-associated macrophages[62]. Notably, these effects were absent in NSG xenograft models in which IL-2–IL-33-armoured human B7-H6-targeted CAR T cells failed to demonstrate similar phenotypic improvements, highlighting a critical dependence on the presence of an intact host immune compartment[62].

The translational potential of this approach is contingent on development of more immunocompetent humanized mouse models or IL-33-responsive transgenic systems to evaluate TME interactions and safety comprehensively. Given the potent immunostimulatory nature of both cytokines and the risk of systemic inflammation, fine-tuning of cytokine release kinetics and their spatial restriction will be essential to mitigate toxicity and to improve the antitumour response by facilitating TME remodelling rather than by changing the CAR T cell phenotype itself. Although the path to clinical translation is complex, these preliminary findings provide a compelling rationale to pursue IL-2–IL-33-based armouring in combination with advanced delivery and safety control platforms.

IL-23 supports sustained antitumour immunity

The pro-inflammatory cytokine IL-23 is a heterodimer that consists of p19 and p40 subunits, with the latter also being a component of IL-12 (ref. 63). IL-23 induces type 3 immune responses, which includes the activation of T_H17 cells, ILCs, NK T cells and mucosal-associated invariant T (MAIT) cells. Although dysregulated IL-23 has been implicated in autoimmune pathologies, including psoriasis, arthritis and inflammatory bowel disease, its ability to promote the generation of tissue-resident and stem-like memory $CD4^+$ T cells with superior persistence and recall capacity are traits that are essential for sustained antitumour activity[63]. Paradoxically, tumour-associated macrophages maintain T_{reg} cells in solid tumours via IL-23 signalling[64]. This suggests that the pro-inflammatory or anti-inflammatory properties of IL-23 are highly context dependent and that its immunostimulatory properties can be strategically harnessed in adoptive cell therapy.

Recent work demonstrates that GD2- and B7-H3-targeted CAR T cells that are engineered to express the p40 subunit exhibit superior tumour control and extend survival in NSG mice bearing neuroblastoma or pancreatic ductal adenocarcinoma (PDAC) xenografts, as well as in immunocompetent PDAC models[65]. Importantly, the decision to overexpress only the p40 subunit was informed by ex vivo profiling of human $CD45RO^+CD27^+CD28^+$ T cells, which revealed strong antigen-induced p19 expression but insufficient endogenous p40, suggesting that selective subunit armouring may rescue incomplete cytokine heterodimer formation in the tumour milieu[65].

This approach exemplifies the concept of strategic engineering — in this case, the precise selection and expression of cytokine subunits instead of entire heterodimers — as a means to amplify functional cytokine availability without inducing off-target effects associated with constitutive cytokine overexpression. Future studies in immunocompetent tumour models are necessary to evaluate the phenotypic consequences of IL-23 subunit armouring and to dissect how it reprogrammes the cytokine landscape within the TME.

IL-7 and CCL19 promote T cell infiltration of tumours

IL-7 is a monomeric cytokine that is predominantly secreted by epithelial cells and stromal cells within the thymus, bone marrow and secondary lymphoid organs[66]. In lymphocytes, IL-7 acts in a paracrine manner through engagement of the heterodimeric IL-7 receptor (IL-7Rα and γc), activating the JAK–STAT signalling cascade[66]. Downstream STAT5 activation promotes the survival and homeostatic proliferation of memory T cells and helps to maintain a less-differentiated T cell phenotype, an attribute associated with long-term persistence and therapeutic efficacy in adoptive cell therapy models[67]. Constitutive expression of IL-7 in CAR T cells has been shown to enhance antitumour activity in several preclinical models, including mouse models of lymphoma[68,69] and solid tumours[70,71].

The rationale for combining IL-7 with the homeostatic chemokine CCL19 is based on the architecture of secondary lymphoid organs. T cell zones within lymph nodes are sustained by fibroblastic reticular cells that co-secrete IL-7 and CCL19 (ref. 67), thereby supporting T cell survival, spatial organization and trafficking. These cytokines not only coordinate the positioning of naive and central memory T cells but also recruit and maintain dendritic cells, enabling efficient antigen presentation[67]. This strategy could augment immune-cell recruitment, enhance T cell longevity and ultimately improve antitumour efficacy.

Clinical observations with unarmoured CAR T cells support the relevance of combining IL-7 and CCL19: in comparison to CAR T cell products that predominantly contain terminal effector T cells, products that are enriched for naive and central memory phenotypes have been associated with improved responses[72,73]. In preclinical models, CD20-targeted CAR T cells engineered to co-express IL-7 and CCL19 induced complete tumour regression and facilitated robust infiltration of both T cells and dendritic cells in a mouse model of mastocytoma[74]. This result is unlikely to be model dependent given that similar findings were reported with IL-7–CCL19-armoured GPC3-targeted CAR T cells in mouse models of HCC[71]. A likely explanation is that CCL19 is a potent immune-cell attractant that promotes the migration of dendritic cells, $CD4^+$ T cells and $CD8^+$ T cells[75]. Furthermore, IL-7–CCL19 overexpression in mesothelin-targeted CAR T cells improved overall survival in mouse models of orthotopic mesothelioma and subcutaneous pancreatic cancer[76], further supporting its clinical potential for treatment of solid tumours.

Early reports from ongoing phase I clinical trials support these observations (NCT03198546, NCT03258047). A case study of a patient with advanced pancreatic cancer reported a resolution of lymph node metastases after five infusions of IL-7–CCL19-armoured mesothelin-targeted CAR T cells, each administered 1–2 months apart[77]. Separately, a patient with advanced HCC showed resolution of a metastatic lesion after a single intratumoural injection of IL-7–CCL19-armoured GPC3-targeted CAR T cells, although the primary tumour did not regress[77]. Radiographic scans of another patient with HCC who received a single infusion of IL-17–CCL19-armoured GPC3-targeted CAR T cells demonstrated regression of two metastatic lymph lesions[71]. Early results of TAK-102, another IL-7–CCL19-armoured GPC3-targeting CAR T cell product, reported stable disease in four of eight patients with HCC and had a manageable safety profile with no high-grade CRS occurrence (NCT04405778)[78]. Unfortunately, business decisions unrelated to patient safety led to this phase I trial being terminated.

Promising clinical findings also extend to haematological cancers. In a phase I clinical trial (NCT03258047), 39 adults with relapsed or refractory diffuse large B cell lymphoma (DLBCL) were treated with CD19-targeted CAR T cells engineered to express IL-7 and CCL19 (ref. 79). The IL-7–CCL19 cassette in this study was under the control of an NFAT-responsive promoter, ensuring that transgene expression was restricted to antigen-activated CAR T cells. CRS of any grade occurred

in 74.4% of patients, with grade 3 CRS reported in 12.8% and no cases of grade 4 CRS[79]. At 3 months post-infusion, complete remission and partial response rates were 56.4% and 23.1%, respectively. No DLTs were observed, supporting the tolerability of this armoured design.

Although direct cross-trial comparisons are inherently limited, these results appear favourable when benchmarked against data from the pivotal JULIET study (NCT02445248), in which unarmoured CD19-targeted CAR T cells (tisagenlecleucel) elicited complete and partial response rates of 32% and 5%, respectively, at the same post-infusion time point[80]. Together, these findings suggest that IL-7–CCL19 armouring may meaningfully augment clinical efficacy without exacerbating toxicity, potentially through improved T cell persistence and enhanced recruitment of dendritic cells and endogenous T cells within the TME. Notably, conditional IL-7–CCL19 expression may have mitigated excessive systemic cytokine release, contributing to the lower incidence of high-grade CRS (12.8%) compared with that reported for unarmoured tisagenlecleucel (22.5%) in the JULIET trial.

Alternative strategies to enhance IL-7 signalling have emerged, including cytokine switch receptors that transduce immunostimulatory IL-7 signals in response to suppressive cues within the TME. For example, chimeric cytokine receptors that combine either IL-4R or TGFβR ectodomains with the IL-7R endodomain have shown promise in reversing CAR T cell dysfunction and augmenting antitumour immunity in preclinical studies[15,81–83]. Together, these preclinical insights and emerging translational data position IL-7 armouring, either alone, in combination with chemokines or via synthetic signalling pathways, as a viable approach to address the challenges of CAR T cell expansion, limited persistence and intratumoural infiltration for solid and haematological malignancies

IL-15 enhances T cell persistence and memory

APCs are the primary source of IL-15; however, unlike most other cytokines that are secreted, IL-15 complexes with high-affinity IL-15Rα intracellularly and remains membrane bound on the producing cell[84]. IL-15–IL-15Rα-mediated signalling in lymphocytes occurs via trans-presentation, by binding to a receptor complex composed of IL-2–IL-15Rβ (also known as CD122) and the γc on the interacting cell[84]. IL-15 has gained attention for its potential in armouring CAR T cells owing to its role in priming APCs, stimulating IFNγ secretion by $CD4^+$ T cells and for promoting the differentiation of activated $CD8^+$ T cells into memory T cells[85].

Indeed, preclinical studies have shown that IL-15 armouring can enhance claudin 18.2-targeted CAR T cell persistence and enrich for central memory ($CD62L^{high}CD44^{high}$) CAR T cell populations in mouse models of melanoma (B16F10) and PDAC, compared with the unarmoured therapy[86]. However, therapeutic benefits in solid tumour models have been modest, with only partial responses and limited tumour clearance observed, even when used with preconditioning[86,87].

More concerningly, IL-15 overexpression has consistently been associated with toxicity. In models of leukaemia in NSG mice, IL-15-armoured CD19-targeted CAR T cells caused hepatotoxicity and decreased survival[88], which was linked to excessive systemic levels of IL-15. These can be reduced by co-expressing IL-15Rα, which may act as a cytokine sink and stabilize IL-15 on the cell surface[88]. However, although this strategy alleviated liver toxicity, it failed to improve survival, indicating that merely modulating cytokine levels is insufficient. In an orthotopic mouse model of glioblastoma, IL-15 armouring of GD2-targeting CAR T cells slightly improved overall survival and prevented development of neurological symptoms typical of this tumour model, but also made mice susceptible to developing poor physical condition including splenomegaly[89].

Similar toxicity profiles were observed in mouse models of acute myeloid leukaemia, where IL-15 armouring of CLL1-targeted CAR T cells triggered lethal TNF-mediated inflammation, which was counteracted using sequential anti-TNF therapy and delayed activation of a suicide switch[90]. These results highlight that IL-15 requires tight pharmacodynamic control[90]. Notably, membrane-tethered IL-15 reduced systemic cytokine levels in mouse models of neuroblastoma but still caused unacceptable toxicities — unless co-expressed with membrane-tethered IL-21, a cytokine that promotes $CD8^+$ T cell expansion and inhibits T_{reg} cell differentiation, which improves safety and tumour control synergistically[91].

Despite these observations, several phase I clinical trials have proceeded in adult and paediatric patients with cancer (NCT02905188, NCT02932956, NCT05103631, NCT04377932, NCT03721068). Notably, all have included an inducible caspase 9 (iC9) suicide switch in the CAR constructs, patient selection criteria are stringent and participants are closely monitored for adverse events. A particularly informative study of IL-15-armoured GPC3-targeted CAR T cells in patients with solid tumours, including HCC and paediatric sarcomas, found significant efficacy, with half (6 out of 12) of patients experiencing a >20% tumour volume reduction with IL-15 armouring, which was not observed in any of the patients who were treated with unarmoured CAR T cells (0 out of 6)[92]. However, higher rates of CRS and grade 1–3 toxicities were observed in patients treated with IL-15-armoured CAR T cells. Notably, three patients required activation of the caspase 9 suicide switch, highlighting the need for built-in safety mechanisms[92]. Phase I clinical trials have commenced to investigate the safety of GPC3-targeted CAR T cells that overexpress IL-15 in patients with glioblastoma (NCT06815432). Moreover, GPC3-targeted CAR T cells that overexpress both IL-15 and IL-21 are being tested in patients with solid tumours (NCT04715191, NCT06198296).

These findings strongly suggest that conditional IL-15 expression — whether via drug-inducible systems, tumour-specific promoters or logic-gated circuits — will be essential for clinical translation. Without such refinement, the benefits of IL-15 with regard to persistence may be outweighed by unacceptable toxicity.

IL-10 imparts pleiotropic effects

IL-10 is a homodimer and is traditionally viewed as a key anti-inflammatory cytokine that is predominantly associated with limiting excessive inflammation and maintaining homeostasis[93]. It is produced by epithelial cells and a range of immune cells, including T_{reg} cells, T_H1, T_H2 and T_H17 cell subsets, activated macrophages (particularly M2-polarized macrophages), as well as tolerogenic dendritic cells and other APCs[94]. On responding cells, the IL-10R1 subunit specifically binds to IL-10 with high affinity, then complexes with IL-10R2 to permit STAT3 phosphorylation[93]. Canonical functions of IL-10 include the suppression of antigen presentation, downregulation of pro-inflammatory cytokine expression and limiting effector T cell responses. However, accumulating evidence suggests that IL-10 exhibits context-dependent pleiotropy, functioning not merely as an immunosuppressant, but also as a modulator of inflammation with the capacity to enhance T cell metabolic fitness, survival, effector function and memory formation, as well as reducing cellular exhaustion under specific conditions[95].

Given its ability to modulate the TME and potentially enhance T cell persistence in a hostile environment, there is renewed interest in IL-10 as a candidate for armouring CAR T cells. A recent study

demonstrated that constitutive IL-10 secretion by CAR T cells improved tumour control in models of colon cancer (MC38-HER2), melanoma (B16F10-TRP1) and metastatic breast cancer (4T1-EGFRvIII) in immunocompetent mice. IL-10-armoured CAR T cells but not unarmoured controls (even when combined with exogenous IL-10 infusion), induced tumour regression, supported memory responses upon tumour rechallenge and exhibited transcriptional signatures consistent with memory or stem-like T cell phenotypes, including enhanced mitochondrial metabolism and reduced exhaustion[96].

However, these tumour models warrant careful interpretation. It is well established that human antigens (such as HER2$^+$ or EGFRvIII$^+$) provoke immune rejection when expressed in mouse tumours, necessitating the use of transgenic mouse models to achieve tolerance and enable tumour growth[97,98]. The reported ability to engraft human HER2$^+$ or EGFRvIII$^+$ tumours into non-transgenic immunocompetent mice suggests potential alterations in antigen immunogenicity or uncharacterized tolerance-breaking mechanisms, possibly as a consequence of lymphodepletion. As such, although this study provides compelling evidence for IL-10 armouring, the translational relevance of these models may need to be interpreted with caution.

Nevertheless, preliminary clinical data support the approach. In a recent phase I clinical trial (NCT06277011), ten adults with relapsed or refractory DLBCL and ten patients with B cell acute lymphoblastic leukaemia (B-ALL) were treated with a single infusion of IL-10-armoured CD19-targeted CAR T cells, and a complete response rate of 100% was reported at 3 months post-infusion[99]. Although these data await peer review and long-term follow-up, they highlight the potential of IL-10 as an unexpected yet promising immunomodulatory payload in CAR T cell therapy.

Taken together, these findings challenge the conventional binary classification of IL-10 as strictly immunosuppressive and instead support its re-evaluation as a context-specific immune modulator in adoptive cell therapy. Nonetheless, the unexpected nature of these findings — both mechanistically and in terms of tumour biology — demands further rigorous investigation to delineate the pathways involved, clarify the contribution of host immunity, and determine the safety and reproducibility of IL-10-based strategies in clinical settings.

Advances in conditional gene expression systems

To restrict cytokine expression to the TME, several strategies for conditional gene expression have been developed. These conditional expression strategies represent two broad conceptual frameworks. The first is transcriptional regulation via context-dependent promoters: cytokine expression is induced by promoters (such as *NFAT*[39,41], *NR4A2*[45], *PDCD1*[44]) that are known to promote gene expression in the context of T cell activation, ensuring that cytokine secretion is limited to activated CAR T cells. These promoters can be encoded in the transgene or are present in the genomic DNA (Fig. 2a), and they have been shown to improve the safety profile of many cytokine-armoured CAR T cells[39,44,45,55,79]. However, for CAR T cells that show a degree of non-specific activation, a second strategy, based on synthetic logic-gated circuits, can ensure more precise control over cytokine secretion: here, engineered CAR T cells integrate multiple environmental inputs (such as antigen presence or anatomical location) to activate cytokine expression. This antigen-sensing architecture is particularly suited to heterogeneous TMEs, providing both specificity and safety. Receptors that are designed for antigen-sensing circuits include synthetic Notch (synNotch) receptors and synthetic intramembrane proteolysis receptors (SNIPRs) (Fig. 2b). Studies exploring antigen-sensing receptor and promoter-mediated circuits have shown that large, polycistronic gene constructs can be stably expressed in individual T cells, laying the foundation for multi-layered cellular programming[100–102]. Progress is being made towards reducing the predicted immunogenicity of synNotch receptor and SNIPR protein domains[100,103].

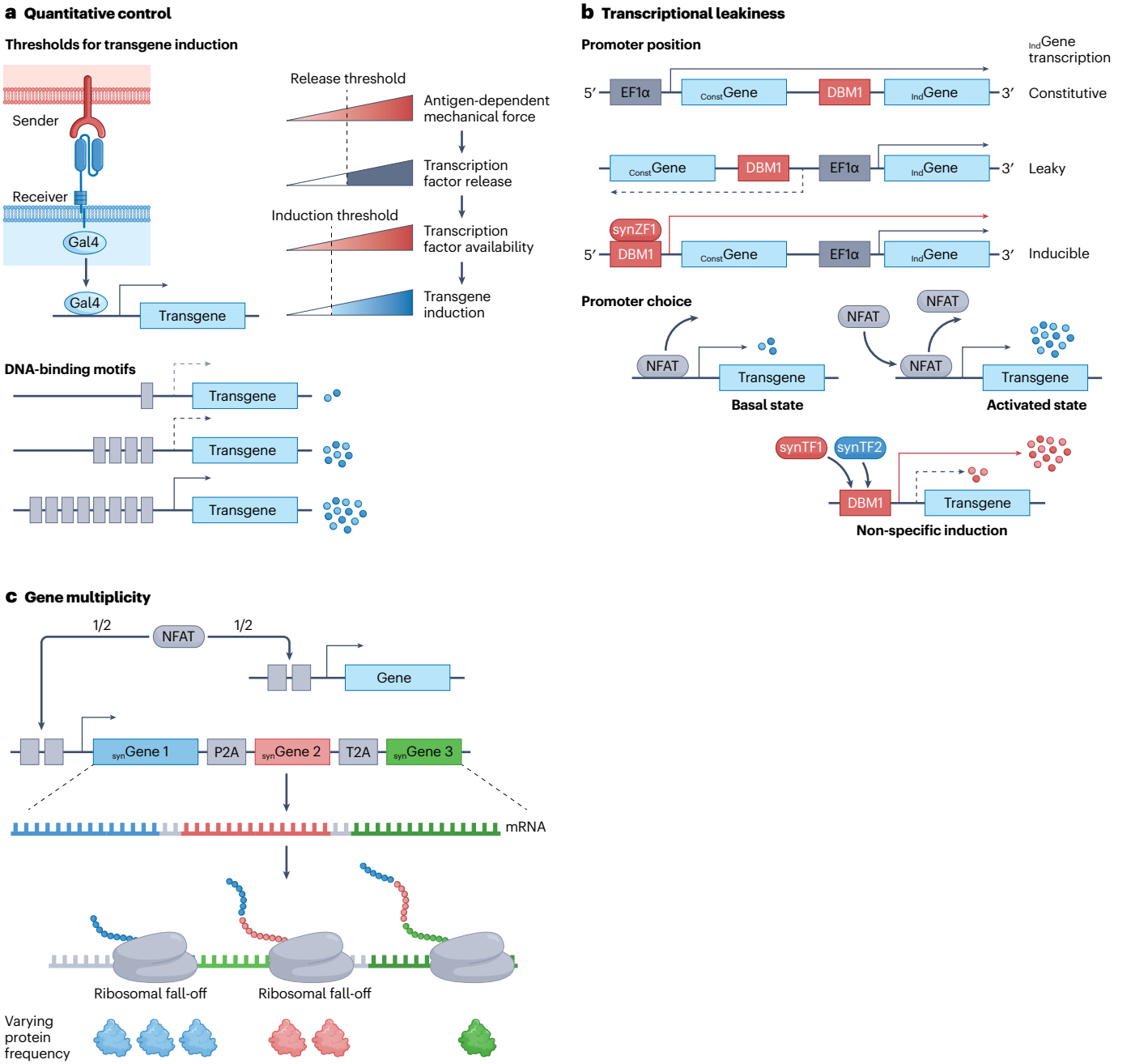
In the context of CAR T cell therapy, a synNotch-based GPC3-responsive inducible IL-12 circuit enhanced CAR T cell tumour infiltration and tumour control, without detectable off-target organ toxicity, in a mouse model of liver cancer[104]. Moreover, mesothelin-specific CAR T cells with a synNotch-inducible IL-2 circuit, significantly enhanced survival in a mouse model of pancreatic cancer compared with CAR T cells armed non-tumour-localized IL-2 (ref. 105). These approaches build on earlier work using synNotch-induced CAR expression, which showed promising antitumour activity and safety profiles across multiple preclinical tumour models[106–109] and are now beginning to enter clinical trials (NCT06186401). To date, SNIPRs have been used to achieve tumour-localized CAR induction but not cytokine induction and have yet to be clinically tested[103,110]. Beyond synNotch-based systems, alternative antigen-sensing synthetic receptor circuits have also been developed. For example, a HER2-responsive inducible CRISPR activation system was used to drive endogenous IL-12 expression selectively upon tumour recognition, thereby restricting cytokine production to the TME, with no constitutive expression or systemic IL-12 detected in the peripheral circulation cells[111].

A major step towards programmable cell therapies is the development of orthogonal, humanized transcription factor–promoter pairs[100]. This creates an expansive framework for parallel gene induction, constrained only by the limits of construct size and cellular tolerance to expression burden. Proof of concept for such systems has been provided by a study using sequential drug-mediated induction of a CD122-biased IL-2 variant ('super IL-2') and a HER2-targeted CAR. This dual regulation strategy improved tumour control and survival in a mouse model of leukaemia[100], highlighting the utility of temporal gene control to fine-tune both T cell expansion and antigen targeting. Alongside this, a better understanding of the quantitative aspects of transgene induction[100,112], the relevance of promoter choice and position with respect to transcriptional leakiness[113,114] and how transgene copy number alterations affect protein output[115] will help to facilitate the development of synthetic circuits that achieve desired cellular phenotypes (Fig. 3).

Future iterations may combine autonomous, antigen-responsive elements with externally regulated cytokine release, creating highly adaptable cell therapies capable of executing complex logic and responding dynamically to patient-specific tumour environments. Such dual-mode systems may ultimately be necessary to safely explore potent cytokine combinations that are otherwise limited by systemic toxicity. By merging synthetic gene regulation with advances in immunotherapy, parallel gene induction circuits will underpin the next generation of precision-engineered, armoured CAR T cell platforms.

Conclusion and outlook

The integration of cytokine payloads into CAR T cells represents a significant frontier in cellular immunotherapy. When appropriately calibrated, cytokine armouring can endow CAR T cells with highly favourable therapeutic features including enhanced persistence, bystander immune activation and durable TME remodelling, as well as reducing the need for lymphodepletion before therapy. These attributes are particularly valuable in the context of solid tumours, where conventional CAR T cells have had limited success. However,



preclinical and emerging clinical data reveal that their therapeutic impact and safety profiles are highly context dependent — shaped by variables such as CAR architecture, cytokine selection, tumour histotype and the immune composition of the TME. As a result, outcomes observed in preclinical studies exhibit marked heterogeneity, and rigorous evaluation across both syngeneic and xenograft models is essential to discern their translational relevance. Moreover, achieving a therapeutic window remains a crucial challenge, as constitutive cytokine expression can lead to systemic toxicity, off-tumour effects or T cell exhaustion.

As discussed above, IL-7-, IL-10-, IL-12-, IL-15- and IL-18-armoured CAR T cells have progressed into phase I clinical trials, with published results available for IL-7, IL-15 and IL-18 constructs[56,57,71,77,79,92] (Table 1). Overexpression of cytokines such as IL-7, IL-12 and IL-18 in preclinical models enhanced antitumour responses by promoting T cell proliferation, reducing exhaustion and, in the case of IL-18, inducing a bystander endogenous T cell response towards CAR-antigen-negative tumour cells[52]. IL-15-armoured CAR T cells increased the frequency of memory T cell subsets and T cell persistence, but exhibited toxicity in mouse models, necessitating strategies to attenuate IL-15 signalling. Overexpression of additional cytokines including IL-2, IL-23 and IL-33 improved tumour control in preclinical models; however, these constructs have not yet entered clinical testing, and rigorous evaluation of their toxicity profiles will be essential before translation to

Fig. 3 | Inducible gene circuits for next-generation armoured CAR T cell therapies. Different strategies for conditional gene expression have unique features that affect the phenotype of the engineered cells. **a**, Quantitative control: this considers the thresholds that must be met in a gene induction circuit to achieve a particular response and how these thresholds can be tuned through design. Synthetic Notch (synNotch) receptor and synthetic intramembrane proteolysis receptor (SNIPR)-mediated AND logic-gated circuits require sufficient force generation during antigen binding to cause a conformational change of the receptor, intracellular proteolytic cleavage and subsequent transcription factor release[102,103,116]. The transcription factor must also be present at sufficient levels to successfully induce transgene expression, thus creating two threshold events. Altering the number of upstream response elements allows the level of gene induction to be tuned[100]. **b**, Transcriptional leakiness: cytokine-armoured chimeric antigen receptor (CAR) T cells can contain both a constitutive gene ($_{Const}$Gene) and an inducible gene ($_{Ind}$Gene). Constitutive expression is typically driven by strong promoters (such as EF1α), whereas inducible gene expression is regulated by promoter–transcription factor pairs (such as DBM1 synZF1 or NFAT–AP-1), the activity of which varies across T cell activation states. To reduce leakiness of the inducible gene, the promoters must be correctly arranged in the transgene because EF1α can override inducibility if positioned inappropriately[113]. Many endogenous activation-responsive promoters used in T cell engineering (such as NFAT-responsive promoters) drive progressively higher levels of transgene expression with increasing T cell activation but exhibit basal activity in resting cells owing to low-level availability or activity of the cognate transcription factors, resulting in transcriptional 'leakiness'. Cross-reactive synthetic transcription factors (synTF) can also contribute to transcriptional leakiness. **c**, Gene multiplicity: this refers to the number of different genes that are induced by a given transcription factor. When synthetic constructs are placed under the control of endogenous promoters, induction strength is likely reduced as the transcription factor can bind to the promoters of different genes, unless promoter occupancy becomes saturated[112]. Moreover, in polycistronic vectors, genes positioned downstream of sites encoding the self-cleaving P2A and T2A peptide sequences often exhibit reduced protein expression owing to ribosomal fall-off during translation[114]. Despite this limitation, P2A and/or T2A sequences are commonly incorporated into cytokine-armoured CAR designs to enable expression of multiple transgenes within in a single, compact vector.

human studies. Such assessments will be a prerequisite for regulatory approval, particularly given concerns around off-tumour effects and cytokine-mediated toxicity.

Findings from immunocompetent mouse models highlight a central challenge: although IL-12, IL-15 and IL-18 armouring can enhance tumour control, these gains are often offset by increased systemic toxicity, resulting in no net survival advantage. To overcome this, technologies enabling conditional cytokine expression, such as NFAT promoters, synthetic transcriptional activators, synNotch receptors and SNIPRs, are being developed to restrict cytokine release to the TME. NFAT-driven IL-12 cassettes in CAR T cells targeting EpCAM, ICAM1 and GPC3 have demonstrated tumour-selective activity and limit off-target effects in humanized mouse models[39,40], supporting the feasibility of this strategy for safer clinical translation. From a clinical perspective, the ability to restrict cytokine secretion to the TME, safely administer higher therapeutic doses and immediately activate safety switches in response to toxicity are highly desirable features for next-generation CAR T cell therapies.

Promoter-driven cytokine expression provides a mechanistically elegant and clinically promising framework for next-generation CAR T cell engineering. However, its safe implementation and translation will likely depend on tailoring promoter choice to a patient's tumour and integrating such systems with additional safety layers, including suicide switches, antigen affinity modulation or multi-input logic control circuits to ensure efficacy is not undermined by toxicity. Although such personalized approaches are currently very expensive, their potential to considerably enhance or initiate clinical responses and reduce toxicities justifies further development. These strategies promise to make potent immunotherapy safer, more precise and potentially transformative across a wide range of cancers.

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References

1. Fuchs, T. L. et al. Assessment of tumor-infiltrating lymphocytes using International TILs Working Group (ITWG) system is a strong predictor of overall survival in colorectal carcinoma: a study of 1034 patients. *Am. J. Surg. Pathol.* **44**, 536–544 (2020).
2. Luen, S. J. et al. Tumour-infiltrating lymphocytes in advanced HER2-positive breast cancer treated with pertuzumab or placebo in addition to trastuzumab and docetaxel: a retrospective analysis of the CLEOPATRA study. *Lancet Oncol.* **18**, 52–62 (2017).
3. Hwang, C. et al. Stromal tumor-infiltrating lymphocytes evaluated on H&E-stained slides are an independent prognostic factor in epithelial ovarian cancer and ovarian serous carcinoma. *Oncol. Lett.* **17**, 4557–4565 (2019).
4. Paijens, S. T., Vledder, A., de Bruyn, M. & Nijman, H. W. Tumor-infiltrating lymphocytes in the immunotherapy era. *Cell Mol. Immunol.* **18**, 842–859 (2021).
5. Zhang, J., Huang, D., Saw, P. E. & Song, E. Turning cold tumors hot: from molecular mechanisms to clinical applications. *Trends Immunol.* **43**, 523–545 (2022).
6. 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).
7. June, C. H. & Sadelain, M. Chimeric antigen receptor therapy. *N. Engl. J. Med.* **379**, 64–73 (2018).
8. Sterner, R. C. & Sterner, R. M. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J.* **11**, 69 (2021).
9. Dong, X. et al. Anti-VEGF therapy improves EGFR-vIII-CAR-T cell delivery and efficacy in syngeneic glioblastoma models in mice. *J. Immunother. Cancer* **11**, e005583 (2023).
10. Neelapu, S. S. et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N. Engl. J. Med.* **377**, 2531–2544 (2017).
11. Eshhar, Z., Waks, T., Gross, G. & Schindler, D. G. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc. Natl Acad. Sci. USA* **90**, 720–724 (1993).
12. Globerson Levin, A., Rivière, I., Eshhar, Z. & Sadelain, M. CAR T cells: building on the CD19 paradigm. *Eur. J. Immunol.* **51**, 2151–2163 (2021).
13. Reddy, N. R. et al. Engineering synthetic suppressor T cells that execute locally targeted immunoprotective programs. *Science* **386**, eadl4793 (2024).
14. Simic, M. S. et al. Programming tissue-sensing T cells that deliver therapies to the brain. *Science* **386**, eadl4237 (2024).
15. Mohammed, S. et al. Improving chimeric antigen receptor-modified T cell function by reversing the immunosuppressive tumor microenvironment of pancreatic cancer. *Mol. Ther.* **25**, 249–258 (2017).
16. 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).
17. 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).
18. Lynn, R. C. et al. c-Jun overexpression in CAR T cells induces exhaustion resistance. *Nature* **576**, 293–300 (2019).
19. Majzner, R. G. & Mackall, C. L. Tumor antigen escape from CAR t-cell therapy. *Cancer Discov.* **8**, 1219–1226 (2018).
20. Berraondo, P. et al. Cytokines in clinical cancer immunotherapy. *Br. J. Cancer* **120**, 6–15 (2019).
21. Kim, B. G., Malek, E., Choi, S. H., Ignatz-Hoover, J. J. & Driscoll, J. J. Novel therapies emerging in oncology to target the TGF-β pathway. *J. Hematol. Oncol.* **14**, 55 (2021).
22. Medzhitov, R. Toll-like receptors and innate immunity. *Nat. Rev. Immunol.* **1**, 135–145 (2001).
23. Tugues, S. et al. New insights into IL-12-mediated tumor suppression. *Cell Death Differ.* **22**, 237–246 (2015).
24. 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).
25. Canelo-Vilaseca, M. et al. Lymphodepletion chemotherapy in chimeric antigen receptor-engineered T (CAR-T) cell therapy in lymphoma. *Bone Marrow Transpl.* **60**, 559–567 (2025).
26. Pegram, H. J. et al. IL-12-secreting CD19-targeted cord blood-derived T cells for the immunotherapy of B-cell acute lymphoblastic leukemia. *Leukemia* **29**, 415–422 (2015).
27. Cordas dos Santos, D. M. et al. A systematic review and meta-analysis of nonrelapse mortality after CAR T cell therapy. *Nat. Med.* **30**, 2667–2678 (2024).

28. Aithal, A. et al. MUC16 as a novel target for cancer therapy. *Expert Opin. Ther. Targets* **22**, 675–686 (2018).
29. Yeku, O. O., Purdon, T. J., Koneru, M., Spriggs, D. & Brentjens, R. J. Armored CAR T cells enhance antitumor efficacy and overcome the tumor microenvironment. *Sci. Rep.* **7**, 10541 (2017).
30. Koneru, M., Purdon, T. J., Spriggs, D., Koneru, S. & Brentjens, R. J. IL-12 secreting tumor-targeted chimeric antigen receptor T cells eradicate ovarian tumors in vivo. *Oncoimmunology* **4**, e994446 (2015).
31. Zou, J. J. et al. Structure-function analysis of the p35 subunit of mouse interleukin 12. *J. Biol. Chem.* **270**, 5864–5871 (1995).
32. Koneru, M., O'Cearbhaill, R., Pendharkar, S., Spriggs, D. R. & Brentjens, R. J. A phase I clinical trial of adoptive T cell therapy using IL-12 secreting MUC-16(ecto) directed chimeric antigen receptors for recurrent ovarian cancer. *J. Transl. Med.* **13**, 102 (2015).
33. O'Cearbhaill, R. E. et al. A phase I clinical trial of autologous chimeric antigen receptor (CAR) T cells genetically engineered to secrete IL-12 and to target the MUC16ecto antigen in patients (pts) with MUC16ecto+ recurrent high-grade serous ovarian cancer (HGSOC). *Gynecol. Oncol.* **159** (Suppl. 1), 42 (2020).
34. Etxeberria, I. et al. Intratumor adoptive transfer of IL-12 mRNA transiently engineered antitumor CD8$^+$ T cells. *Cancer Cell* **36**, 613–629.e617 (2019).
35. Jia, Z. et al. IL12 immune therapy clinical trial review: Novel strategies for avoiding CRS-associated cytokines. *Front. Immunol.* **13**, 952231 (2022).
36. Hu, J. et al. Cell membrane-anchored and tumor-targeted IL-12 (attIL12)-T cell therapy for eliminating large and heterogeneous solid tumors. *J. Immunother. Cancer* **10**, e003633 (2022).
37. Atsavapranee, E. S., Billingsley, M. M. & Mitchell, M. J. Delivery technologies for T cell gene editing: applications in cancer immunotherapy. *EBioMedicine* **67**, 103354 (2021).
38. Vaeth, M. & Feske, S. NFAT control of immune function: new frontiers for an abiding trooper. *F1000Res* **7**, 260 (2018).
39. Yang, Y. et al. Inducible expression of interleukin-12 augments the efficacy of affinity-tuned chimeric antigen receptors in murine solid tumor models. *Nat. Commun.* **14**, 2068 (2023).
40. 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).
41. Kunert, A. et al. Intra-tumoral production of IL18, but not IL12, by TCR-engineered T cells is non-toxic and counteracts immune evasion of solid tumors. *Oncoimmunology* **7**, e1378842 (2017).
42. Zhang, L. et al. Tumor-infiltrating lymphocytes genetically engineered with an inducible gene encoding interleukin-12 for the immunotherapy of metastatic melanoma. *Clin. Cancer Res.* **21**, 2278–2288 (2015).
43. Guo, T., Ma, D. & Lu, T. K. Sense-and-Respond payload delivery using a novel antigen-inducible promoter improves suboptimal car-t activation. *ACS Synth. Biol.* **11**, 1440–1453 (2022).
44. Sachdeva, M. et al. Repurposing endogenous immune pathways to tailor and control chimeric antigen receptor T cell functionality. *Nat. Commun.* **10**, 5100 (2019).
45. Chen, A. X. Y. et al. Rewiring endogenous genes in CAR T cells for tumour-restricted payload delivery. *Nature* **644**, 241–251 (2025).
Genes that are highly expressed in intratumoural T cells relative to splenic T cells are used to rationally select the promoters *NR4A2* and *RGS16* for tumour-localized induction of cytokine payloads. This enhanced CAR T cell efficacy and safety across multiple solid tumour models.
46. Ghayur, T. et al. Caspase-1 processes IFNγ-inducing factor and regulates LPS-induced IFNγ production. *Nature* **386**, 619–623 (1997).
47. Fantuzzi, G., Puren, A. J., Harding, M. W., Livingston, D. J. & Dinarello, C. A. Interleukin-18 regulation of interferon gamma production and cell proliferation as shown in interleukin-1beta-converting enzyme (caspase-1)-deficient mice. *Blood* **91**, 2118–2125 (1998).
48. Tsutsumi, N. et al. The structural basis for receptor recognition of human interleukin-18. *Nat. Commun.* **5**, 5340 (2014).
49. Ihim, S. A. et al. Interleukin-18 cytokine in immunity, inflammation, and autoimmunity: biological role in induction, regulation, and treatment. *Front. Immunol.* **13**, 919973 (2022).
50. 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).
51. Hu, B. et al. Augmentation of antitumor immunity by human and mouse car T cells secreting IL-18. *Cell Rep.* **20**, 3025–3033 (2017).
52. 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).
53. Ng, B. D. et al. IL-18-secreting multiantigen targeting CAR T cells eliminate antigen-low myeloma in an immunocompetent mouse model. *Blood* **144**, 171–186 (2024).
54. Glienke, W. et al. GMP-compliant manufacturing of TRUCKs: car T cells targeting GD(2) and releasing inducible IL-18. *Front. Immunol.* **13**, 839783 (2022).
55. Fischer-Riepe, L. et al. Preclinical development of CAR T cells with antigen-inducible IL18 enforcement to treat GD2-positive solid cancers. *Clin. Cancer Res.* **30**, 3564–3577 (2024).
56. Svoboda, J. et al. Enhanced CAR T-cell therapy for lymphoma after previous failure. *N. Engl. J. Med.* **392**, 1824–1835 (2025).
Here, IL-18 armoured CD19 CAR T cells are shown to be safe for patients with relapsed or refractory lymphoma who have previously received unarmoured CD19 CAR T cell therapy. For the 21 patients enrolled, complete and partial response rates at 3 months post-infusion were 52% and 29%, respectively.
57. Geyer, M. B. et al. CD371-targeted CAR T-cells secreting interleukin-18 exhibit robust expansion and disease clearance in patients with refractory acute myeloid leukemia. *Blood* **144**, 2070 (2024).
58. Kessel, C., Rossig, C. & Abken, H. Weal and woe of interleukin-18 in the T cell therapy of cancer. *J. Immunother. Cancer* **13**, e010545 (2025).
59. Shouse, A. N., LaPorte, K. M. & Malek, T. R. Interleukin-2 signaling in the regulation of T cell biology in autoimmunity and cancer. *Immunity* **57**, 414–428 (2024).
60. Chinen, T. et al. An essential role for the IL-2 receptor in T_{reg} cell function. *Nat. Immunol.* **17**, 1322–1333 (2016).
61. Yi, X. M., Lian, H. & Li, S. Signaling and functions of interleukin-33 in immune regulation and diseases. *Cell Insight* **1**, 100042 (2022).
62. 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).
63. Krueger, J. G. et al. IL-23 past, present, and future: a roadmap to advancing IL-23 science and therapy. *Front. Immunol.* **15**, 1331217 (2024).
64. Wertheimer, T. et al. IL-23 stabilizes an effector T_{reg} cell program in the tumor microenvironment. *Nat. Immunol.* **25**, 512–524 (2024).
65. Ma, X. et al. Interleukin-23 engineering improves CAR T cell function in solid tumors. *Nat. Biotechnol.* **38**, 448–459 (2020).
66. Chen, D., Tang, T. X., Deng, H., Yang, X. P. & Tang, Z. H. Interleukin-7 biology and its effects on immune cells: mediator of generation, differentiation, survival, and homeostasis. *Front. Immunol.* **12**, 747324 (2021).
67. Link, A. et al. Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. *Nat. Immunol.* **8**, 1255–1265 (2007).
68. Li, L. et al. Transgenic expression of IL-7 regulates CAR-T cell metabolism and enhances in vivo persistence against tumor cells. *Sci. Rep.* **12**, 12506 (2022).
69. Markley, J. C. & Sadelain, M. IL-7 and IL-21 are superior to IL-2 and IL-15 in promoting human T cell-mediated rejection of systemic lymphoma in immunodeficient mice. *Blood* **115**, 3508–3519 (2010).
70. Wu, H. et al. Interleukin-7 expression by CAR-T cells improves CAR-T cell survival and efficacy in chordoma. *Cancer Immunol. Immunother.* **73**, 188 (2024).
71. Lu, L. L. et al. GPC3-IL7-CCL19-CAR-T primes immune microenvironment reconstitution for hepatocellular carcinoma therapy. *Cell Biol. Toxicol.* **39**, 3101–3119 (2023).
72. Cappell, K. M. & Kochenderfer, J. N. Long-term outcomes following CAR T cell therapy: what we know so far. *Nat. Rev. Clin. Oncol.* **20**, 359–371 (2023).
73. Brown, C. E. et al. Locoregional delivery of IL-13Rα2-targeting CAR-T cells in recurrent high-grade glioma: a phase 1 trial. *Nat. Med.* **30**, 1001–1012 (2024).
74. 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).
75. Yan, Y. et al. CCL19 and CCR7 expression, signaling pathways, and adjuvant functions in viral infection and prevention. *Front. Cell Dev. Biol.* **7**, 212 (2019).
76. Goto, S. et al. Enhanced anti-tumor efficacy of IL-7/CCL19-producing human CAR-T cells in orthotopic and patient-derived xenograft tumor models. *Cancer Immunol. Immunother.* **70**, 2503–2515 (2021).
77. Pang, N. et al. IL-7 and CCL19-secreting CAR-T cell therapy for tumors with positive glypican-3 or mesothelin. *J. Hematol. Oncol.* **14**, 118 (2021).
78. Nakajima, T. E. et al. Updated results from first-in-human phase 1 dose-escalation trial of TAK-102, a GPC3-targeted armored CAR T cells, in patients with advanced solid tumors. *J. Clin. Oncol.* **42**, 2543 (2024).
79. 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).
80. Schuster, S. J. et al. Tisagenlecleucel in adult relapsed or refractory diffuse large b-cell lymphoma. *N. Engl. J. Med.* **380**, 45–56 (2019).
81. Leen, A. M. et al. Reversal of tumor immune inhibition using a chimeric cytokine receptor. *Mol. Ther.* **22**, 1211–1220 (2014).
82. Weimin, S., Abula, A., Qianghong, D. & Wenguang, W. Chimeric cytokine receptor enhancing PSMA-CAR-T cell-mediated prostate cancer regression. *Cancer Biol. Ther.* **21**, 570–580 (2020).
83. Noh, K. E. et al. TGF-β/IL-7 chimeric switch receptor-expressing car-t cells inhibit recurrence of cd19-positive b cell lymphoma. *Int. J. Mol. Sci.* **22**, 8706 (2021).
84. Perera, P. Y., Lichy, J. H., Waldmann, T. A. & Perera, L. P. The role of interleukin-15 in inflammation and immune responses to infection: implications for its therapeutic use. *Microbes Infect.* **14**, 247–261 (2012).
85. Cai, M. et al. Research progress of interleukin-15 in cancer immunotherapy. *Front. Pharmacol.* **14**, 1184703 (2023).
86. Shi, H. et al. IL-15 armoring enhances the antitumor efficacy of claudin 18.2-targeting CAR-T cells in syngeneic mouse tumor models. *Front. Immunol.* **14**, 1165404 (2023).
87. Lanitis, E. et al. Optimized gene engineering of murine CAR-T cells reveals the beneficial effects of IL-15 coexpression. *J. Exp. Med.* **218**, e20192203 (2021).
88. Zhang, Y. et al. Co-expression IL-15 receptor alpha with IL-15 reduces toxicity via limiting IL-15 systemic exposure during CAR-T immunotherapy. *J. Transl. Med.* **20**, 432 (2022).
89. Gargett, T. et al. GD2-targeting CAR-T cells enhanced by transgenic IL-15 expression are an effective and clinically feasible therapy for glioblastoma. *J. Immunother. Cancer* **10**, e005187 (2022).
90. Ataca Atilla, P. et al. Modulating TNFα activity allows transgenic IL15-expressing CLL-1 CAR T cells to safely eliminate acute myeloid leukemia. *J. Immunother. Cancer* **8**, e001229 (2020).

91. Nguyen, R. et al. Cooperative armoring of CAR and TCR T cells by T cell-restricted IL15 and IL21 universally enhances solid tumor efficacy. *Clin. Cancer Res.* **30**, 1555–1566 (2024).
92. Steffin, D. et al. Interleukin-15-armoured GPC3 CAR T cells for patients with solid cancers. *Nature* **637**, 940–946 (2024).
The antitumour efficacy of GPC3-targeted CAR T cells towards solid tumours is shown to be dependent on co-expression of IL-15 in phase I clinical trials in adult or paediatric patients and it is shown that inducible safety switches can be implemented to successfully mitigate toxicities.
93. Saraiva, M., Vieira, P. & O'Garra, A. Biology and therapeutic potential of interleukin-10. *J. Exp. Med.* **217**, e20190418 (2020).
94. Saraiva, M. & O'Garra, A. The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* **10**, 170–181 (2010).
95. Carlini, V. et al. The multifaceted nature of IL-10: regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions. *Front. Immunol.* **14**, 1161067 (2023).
96. Zhao, Y. IL-10-expressing CAR T cells resist dysfunction and mediate durable clearance of solid tumors and metastases. *Nat. Biotechnol.* **42**, 1693–1704 (2024).
97. Piechocki, M. P., Ho, Y. S., Pilon, S. & Wei, W. Z. Human ErbB-2 (Her-2) transgenic mice: a model system for testing Her-2 based vaccines. *J. Immunol.* **171**, 5787–5794 (2003).
98. Yong, C. S. et al. Embryonic lethality in homozygous human Her-2 transgenic mice due to disruption of the Pds5b gene. *PLoS ONE* **10**, e0136817 (2015).
99. Guo, Y. et al. IL-10 expressing CD19 CAR-T cells induce complete remission and improve long-term protection in relapsed or refractory B-Cell Hematological malignancies. *Blood* **144**, 92–92 (2024).
100. Li, H. S. et al. Multidimensional control of therapeutic human cell function with synthetic gene circuits. *Science* **378**, 1227–1234 (2022).
101. Webber, B. R. et al. Cas9-induced targeted integration of large DNA payloads in primary human T cells via homology-mediated end-joining DNA repair. *Nat. Biomed. Eng.* **8**, 1553–1570 (2024).
102. Roybal, K. T. et al. Engineering T cells with customized therapeutic response programs using synthetic notch receptors. *Cell* **167**, 419–432.e416 (2016).
The first demonstration that the synNotch system can be used to restrict antitumour responses to the tumour site based on the requirement for antigen detection, inspiring further development of logic-gated, armoured T cell therapies.
103. Zhu, I. et al. Modular design of synthetic receptors for programmed gene regulation in cell therapies. *Cell* **185**, 1431–1443.e1416 (2022).
104. Luo, H. et al. Target-dependent expression of IL12 by synNotch receptor-engineered NK92 cells increases the antitumor activities of CAR-T cells. *Front. Oncol.* **9**, 1448 (2019).
105. Allen, G. M. et al. Synthetic cytokine circuits that drive T cells into immune-excluded tumors. *Science* **378**, eaba1624 (2022).
106. Choe, J. H. et al. SynNotch-CAR T cells overcome challenges of specificity, heterogeneity, and persistence in treating glioblastoma. *Sci. Transl. Med.* **13**, eabe7378 (2021).
107. Srivastava, S. et al. Logic-gated ROR1 chimeric antigen receptor expression rescues T cell-mediated toxicity to normal tissues and enables selective tumor targeting. *Cancer Cell* **35**, 489–503.e488 (2019).
108. Hyrenius-Wittsten, A. et al. SynNotch CAR circuits enhance solid tumor recognition and promote persistent antitumor activity in mouse models. *Sci. Transl. Med.* **13**, eabd8836 (2021).
109. Hernandez-Lopez, R. A. et al. T cell circuits that sense antigen density with an ultrasensitive threshold. *Science* **371**, 1166–1171 (2021).
110. Piraner, D. I. et al. Engineered receptors for soluble cellular communication and disease sensing. *Nature* **638**, 805–813 (2025).
111. Yang, Z. et al. Nanoscale, antigen encounter-dependent, IL-12 delivery by CAR T cells plus PD-L1 blockade for cancer treatment. *J. Transl. Med.* **21**, 158 (2023).
112. Das, D., Dey, S., Brewster, R. C. & Choubey, S. Effect of transcription factor resource sharing on gene expression noise. *PLoS Comput. Biol.* **13**, e1005491 (2017).
113. Reichenbach, P. et al. A lentiviral vector for the production of T cells with an inducible transgene and a constitutively expressed tumour-targeting receptor. *Nat. Biomed. Eng.* **7**, 1063–1080 (2023).
114. Liu, Z. et al. Systematic comparison of 2A peptides for cloning multi-genes in a polycistronic vector. *Sci. Rep.* **7**, 2193 (2017).
115. Ayala Ceja, M., Khericha, M., Harris, C. M., Puig-Saus, C. & Chen, Y. Y. CAR-T cell manufacturing: major process parameters and next-generation strategies. *J. Exp. Med.* **221**, e20230903 (2024).
116. Sloas, D. C., Tran, J. C., Marzilli, A. M. & Ngo, J. T. Tension-tuned receptors for synthetic mechanotransduction and intercellular force detection. *Nat. Biotechnol.* **41**, 1287–1295 (2023).

Author contributions

All authors researched data for the article. All authors contributed substantially to discussion of the content. K.P. and M.R.J. wrote the article. All authors reviewed and/or edited the manuscript before submission.

Competing interests

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