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Review

Rewriting CAR-T fate through cytokine engineering

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Chimeric antigen receptor (CAR)-engineered T (CAR-T) cell therapy has transformed the treatment of hematologic malignancies, yet its efficacy in solid tumors is constrained by limited persistence, functional exhaustion, toxicity, and an immunosuppressive tumor microenvironment. Cytokine engineering has emerged as a versatile strategy to reprogram CAR-T cell fate and overcome these barriers. This review highlights recent preclinical and clinical advances in cytokine-armored CAR-T cells, focusing on cytokines such as interleukin-10 (IL-10), IL-12, IL-15, IL-18, IL-21, IL-23, and others that enhance proliferation, metabolic fitness, persistence, and antitumor activity while reshaping the tumor milieu. The authors discuss mechanistic insights into engineered cytokine circuits that mitigate dysfunction and promote durable responses, and they consider future directions for safely unlocking the full therapeutic potential of CAR-T therapy.

Significance

Cytokine engineering is redefining the future of chimeric antigen receptor-engineered T cell therapy by enhancing persistence, metabolic fitness, and antitumor activity while addressing key safety challenges. By integrating controlled cytokine expression, synthetic circuits, and *in vivo* delivery strategies, next-generation chimeric antigen receptor platforms show improved efficacy in both hematologic malignancies and solid tumors. These advances highlight a pivotal shift from simple cell targeting to programmable immune modulation, accelerating the development of safer and more durable cancer immunotherapies.

Cytokine engineering to overcome CAR-T cell dysfunction

Chimeric antigen receptor (CAR)-engineered T (CAR-T) cell therapy has revolutionized the treatment landscape for hematologic malignancies and select B cell-mediated autoimmune diseases, yet its translation to solid tumors remains a significant challenge [1–5]. Major barriers include limited trafficking and infiltration into tumor sites, antigen heterogeneity and loss, T cell exhaustion, metabolic insufficiency, and the immunosuppressive tumor microenvironment (TME) [6–9]. In addition, treatment-associated toxicities, such as **cytokine release syndrome (CRS)** (see Glossary) and **immune effector cell-associated neurotoxicity syndrome (ICANS)**, require careful management [10,11]. To address these limitations, diverse strategies have been developed, including CAR structural optimization, dual-targeting approaches, armored CAR-T cells, combination therapies with checkpoint blockade or oncolytic viruses, and modulation of intracellular signaling pathways [12–16]. Among these, cytokine engineering has emerged as a particularly promising approach, with encouraging preclinical and early clinical results.

Currently, a broad spectrum of cytokines has been explored to enhance CAR-T cell therapy, including interleukin-2 (IL-2), IL-4, IL-7, IL-9, IL-10, IL-12, IL-15, IL-18, IL-21, IL-23, IL-24, IL-33, and IL-36γ (Table 1), which have been incorporated into CAR constructs through constitutive, inducible, or synthetic circuit-based expression strategies (Figure 1). Constitutive expression systems provide continuous cytokine support to enhance T cell persistence and expansion but may increase the risk of systemic toxicity, particularly with highly potent cytokines. In contrast, inducible expression platforms, such as those driven by nuclear factor of activated T cells (NFAT)-responsive promoters, restrict cytokine release to sites of antigen engagement, thereby promoting localized immune activation within the TME while limiting off-target effects [17,18]. Membrane-bound or tethered cytokines, such as IL-15/IL-15Rα complexes, further refine this approach by enabling **juxtacrine signaling** that supports CAR-T survival without significant

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systemic exposure [19,20]. More advanced designs incorporate synthetic gene circuits, including synNotch-based systems, which allow programmable and context-dependent cytokine expression [21]. Additionally, switch or inverted cytokine receptors can rewire immunosuppressive signals into stimulatory outputs, helping CAR-T cells overcome inhibitory cues in solid tumors [22] (Figure 1). Collectively, these platforms illustrate the evolving sophistication of cytokine engineering strategies aimed at improving CAR-T cell function.

In this review, the authors systematically examine the mechanistic rationale and therapeutic potential of these cytokine-engineered CAR-T platforms, integrating insights from preclinical studies and emerging clinical trials, including IL-15- and IL-18-augmented CAR-T therapies. The authors further discuss design considerations, safety challenges, and innovative approaches such as synthetic **cytokine circuits**, combinatorial cytokine programming, and integration with multi-armored and gene-edited CAR systems, thereby providing a comprehensive framework to guide the rational development and clinical translation of next-generation cytokine-engineered CAR-T cell therapies.

Preclinical development of cytokine-augmented CAR-T cells

Different cytokines contribute to distinct aspects of T cell function, persistence, and tumor control. The common γ-chain (γc) family, including IL-2, IL-15, and IL-21, primarily regulates T cell proliferation, survival, and memory formation through signal transducer and activator of transcription 5 (STAT5)- and STAT3-mediated pathways. The IL-1 family cytokines, such as IL-18, IL-33, and IL-36γ, are key mediators of inflammation and play critical roles in activating innate and adaptive immunity, as well as reshaping the TME. In contrast, members of the IL-10 family, including IL-10 and IL-24, exhibit context-dependent immunomodulatory functions that can enhance CAR-T cell metabolic fitness and directly target tumor cell biology (Table 1).

Building on this classification, the authors further organize cytokine-based CAR-T engineering strategies according to their dominant functional roles: (i) cytokines that enhance effector function (IL-2, IL-4, IL-10, and IL-23), (ii) cytokines that promote stemlike memory and quiescence (IL-7, IL-9, IL-15, and IL-21), and (iii) cytokines that remodel the TME and facilitate **epitope spreading** (IL-12, IL-18, IL-24, IL-33, and IL-36γ) (Table 1). This functional grouping highlights shared mechanisms of action and provides a clearer framework for comparing emerging cytokine engineering approaches in CAR-T cell therapy.

Effector-potentiating cytokine: IL-2

IL-2 is a central cytokine in T cell biology, critically regulating the expansion, survival, and cytotoxic function of cluster of differentiation 8 $(CD8)^+$ T cells and natural killer (NK) cells [23]. Although IL-2 is primarily produced by activated $CD4^+$ T cells, its therapeutic administration has been limited by dose-dependent systemic toxicities and the preferential expansion of regulatory T cells (Tregs). To overcome these challenges, recent strategies have focused on engineering modified IL-2 variants and optimizing delivery systems to enhance effector responses while minimizing adverse effects [23,24].

One approach involves engineering CAR-T cells to secrete modified IL-2 variants. For example, human epidermal growth factor receptor 2 (HER2)-directed CAR-T cells expressing IL2m, a mutant IL-2 designed to reduce Treg expansion while enhancing $CD8^+$ T cell activity, demonstrated superior functional properties compared with conventional CAR-T cells [25]. IL2m-CAR-T cells exhibited enhanced proliferation, increased STAT5 phosphorylation, reduced programmed cell death protein 1 (PD-1) expression, and improved *in vitro* cytotoxicity, accompanied by elevated $CD8^+$ and granzyme B expression. *In vivo*, treatment led to increased $CD3^+$ T cell infiltration and enhanced tumor control in xenograft models, supporting the capacity of IL-2-based engineering to improve CAR-T expansion, persistence, and antitumor efficacy [25].

Glossary

Anemia: a deficiency in red blood cells or hemoglobin, resulting in decreased oxygen-carrying capacity and symptoms such as fatigue and shortness of breath.
Cyclophosphamide: an alkylating chemotherapeutic and immunosuppressive agent commonly used for lymphodepletion prior to adoptive cell therapy to enhance immune cell engraftment and antitumor efficacy.
Cytokine circuits: genetically engineered regulatory systems in immune cells that control the production, sensing, or signaling of cytokines to modulate cellular function in a spatially and temporally defined manner.
Cytokine release syndrome (CRS): a systemic inflammatory toxicity characterized by the excessive and rapid release of proinflammatory cytokines following immune activation, commonly observed after CAR-T cell therapy.
Epitope spreading: a process by which an immune response initially directed against a single antigenic epitope broadens to target additional epitopes on the same or different antigens, thereby expanding the scope of immune recognition.
Immune effector cell-associated neurotoxicity syndrome (ICANS): a therapy-related neurotoxic complication of immune effector cell treatments, particularly CAR-T cells, marked by symptoms ranging from mild confusion and aphasia to seizures and cerebral edema.
Juxtacrine signaling: a form of cell-to-cell communication in which signaling occurs through direct physical contact between neighboring cells via membrane-bound ligands and receptors.
Leukopenia: a reduction in the total number of circulating white blood cells, resulting in increased susceptibility to infections.
Lymphodepletion: a preconditioning treatment typically involving chemotherapy or radiation used to reduce endogenous lymphocyte populations prior to adoptive cell therapy in order to enhance the expansion and persistence of infused cells.
Neutropenia: a decrease in circulating neutrophils, which increases susceptibility to bacterial and fungal infections.

Additional studies have explored combinatorial strategies using IL-2 variants with enhanced receptor affinity. The IL-2 superkine (Super2), engineered for increased binding to the intermediate-affinity IL-2 receptor, showed synergistic activity when combined with IL-33 [26]. In murine B16-F10 tumor models, Super2-expressing CAR-T cells plus IL-33 significantly reduced tumor burden and prolonged survival. Mechanistically, this combination reshaped the TME, reducing M2-like macrophages and increasing effector $CD8^+$ T cells, endogenous lymphocyte recruitment, and CAR-T persistence [26]. Importantly, the therapeutic benefit was observed across multiple CAR constructs, suggesting a platform-level effect.

Suicide switch: a genetically engineered safety mechanism incorporated into modified cells that allows selective and rapid elimination of those cells in the event of severe toxicity or uncontrolled activity.
Thrombocytopenia: a reduced platelet count in the blood, leading to an elevated risk of bleeding and impaired clot formation.

To further mitigate systemic toxicity, targeted IL-2 delivery strategies have been developed. A CAR enhancer (CAR-E), consisting of a low-affinity IL-2 mutant fused to a B-cell maturation antigen (BCMA) ectodomain, selectively activated BCMA CAR-T cells and promoted tumor clearance at low doses [27]. Similarly, orthogonal IL-2/IL-2 receptor systems have been engineered to restrict cytokine signaling to genetically modified T cells, enhancing Interferon gamma (IFN-γ) production and reducing exhaustion markers without increasing systemic exposure [28].

Effector-potentiating cytokine: IL-4

IL-4, a canonical type 2 cytokine, plays a multifaceted role in lymphocyte biology, including the regulation of T and B cell survival, differentiation, and function. Although traditionally associated with type 2 immunity, recent studies have highlighted its unexpected capacity to enhance CAR-T cell fitness and antitumor activity, particularly in the context of T cell exhaustion.

Emerging evidence indicates that IL-4 can rejuvenate dysfunctional tumor-infiltrating T cells. Single-cell analyses of CAR-T infusion products from pediatric acute lymphoblastic leukemia patients revealed that enhanced type 2 signaling signatures correlate with prolonged B cell aplasia, a surrogate marker of durable CAR-T persistence [29]. Incorporation of IL-4 during *ex vivo* activation or as a priming signal prior to infusion improves CAR-T cell functionality by alleviating exhaustion and enhancing cellular fitness at both transcriptional and epigenetic levels. Notably, restoring type 2 functionality in IL-4-low CAR-T products rescues antitumor efficacy, supporting a direct role for IL-4 in optimizing therapeutic potency [29].

Mechanistically, IL-4-based strategies also act on metabolically constrained T cells within the TME. A fusion cytokine (Fc-IL-4) has been shown to directly target $CD8^+$ T cells, promoting the expansion of functionally competent, terminally exhausted subsets [30]. This effect is mediated through coordinated activation of STAT6 and mammalian target of rapamycin (mTOR) signaling pathways, leading to enhanced glycolytic activity and increased intracellular NAD^+ levels in a lactate dehydrogenase A-dependent manner. These metabolic adaptations are critical for restoring effector function under chronic antigen exposure.

Collectively, IL-4 represents a promising adjunct in CAR-T cell engineering, capable of reprogramming exhausted T cells and synergizing with type 1-driven antitumor immunity to achieve durable therapeutic responses.

Effector-potentiating cytokine: IL-10

IL-10, classically defined as an immunoregulatory cytokine with anti-inflammatory functions, has recently been recognized for its unexpected capacity to enhance CAR-T cell durability and function in tumor settings [31]. Rather than simply suppressing immune activation, IL-10 signaling within CAR-T cells supports sustained antitumor responses, particularly under conditions of persistent antigen exposure. Preclinical studies in both syngeneic (e.g., MC38 cell line overexpressing HER2 and 4T1 cell line overexpressing EGFRvIII) and xenograft (e.g., Raji

Table 1. Preclinical evaluation of cytokine-engineered CAR-T cells

Cytokine classification	Cytokine	Cytokine type and biology	Engineering strategy	Key functional effects on CAR-T cells
Effector-potentiating cytokines	IL-2	Common γc cytokine; 15–16-kDa single-chain protein that signals via IL-2Rα/β/γ and activates STAT5.	Engineered variants (mutant IL-2 and 'Super2'), orthogonal IL-2/IL-2R pairs, targeted/low-affinity fusions, CAR-restricted delivery to limit systemic exposure and Treg expansion.	Potently drives proliferation, effector differentiation and cytotoxicity; when optimized, enhances $CD8^+$ expansion and function while reducing Treg expansion and systemic toxicity.
	IL-4	Type 2 cytokine; single chain (~15–20 kDa) that signals via IL-4Rα/γc or IL-4Rα/IL-13Rα1 complexes, activating STAT6 and metabolic pathways associated with T cell differentiation and survival.	Incorporation of IL-4 during CAR-T cell manufacturing or *ex vivo* priming to enhance type 2 functionality; development of IL-4 fusion proteins (e.g., Fc–IL-4) to improve stability and bioactivity.	Reverses T cell dysfunction and enhances cellular fitness at transcriptional and epigenetic levels; promotes survival and persistence; enriches functional exhausted $CD8^+$ T cell subsets; enhances glycolytic metabolism and NAD^+ production via STAT6 and mTOR signaling; improves antitumor efficacy; and supports durable responses, particularly under chronic antigen exposure.
	IL-10	IL-10 family (anti-inflammatory); homodimeric cytokine (~20-kDa monomer, signals via IL-10Rα/β and STAT3).	CAR-T cells engineered to secrete IL-10 locally (constitutive or inducible) to reprogram metabolism without broad systemic immunosuppression.	Stabilizes mitochondrial architecture, enhances oxidative phosphorylation and metabolic resilience, reduces exhaustion, and sustains effector function during chronic stimulation.
	IL-23	Heterodimeric cytokine (p19 + p40); signals via IL-23R/IL-12Rβ1 and promotes effector programs.	Engineering to express p40 (or activation-regulated p19/p40 expression) so IL-23 secretion is CAR-activation dependent and confined to TME.	Activation-restricted enhancement of cytotoxicity, sustained IFN-γ/TNF-α production, reduced exhaustion marker expression and improved persistence with limited systemic exposure.
Memory-supporting cytokines	IL-7	Common γc cytokine; single chain (~20 kDa) that signals via IL-7Rα/γc to activate STAT5 and antiapoptotic programs.	Fc-fusion/extended-half-life IL-7, CAR supplementation or coexpression to increase systemic persistence and CAR-proximal availability.	Promotes survival, antiapoptotic signaling, expansion and enrichment of stem cell-memory and central-memory T cell subsets and increases persistence and resistance to apoptosis.
	IL-9	Common γc cytokine; single-chain protein that engages IL-9R and activates STAT1/3/4 pathways.	CAR-intrinsic IL-9R expression or IL-9 supply (secretion/codelivery) to render CAR-T cells responsive to IL-9.	Enhances expansion, persistence and tumor infiltration under chronic antigen exposure; supports less-differentiated, proliferative $CD4^+$ and memory-associated $CD8^+$ programs; and limits exhaustion.
	IL-15	γc family member with IL-15Rα transpresentation; supports STAT5 signaling for survival and memory.	Membrane-bound IL-15 (mbIL-15), constitutive or inducible coexpression, or combination with IL-7/IL-21 to provide autonomous cytokine support.	Promotes stem cell-memory and central-memory T cell and memory programs; increases proliferation, persistence, and *in vivo* durability; and improves cytotoxicity with generally favorable safety versus IL-2.
	IL-21	Common γc cytokine; signals predominantly via STAT3 (single chain ~15 kDa).	Coexpression with IL-15 or alone in CAR-T constructs; combinatorial pairing or context-dependent delivery.	Favors memory-like phenotypes, supports long-term persistence and self-renewal (TCF7/TCF-1 dependent), and balances proliferation with reduced apoptosis.
TME-modulating cytokines	IL-12	Heterodimeric p70 cytokine (p35 + p40), IL-12 family; potently induces IFN-γ via STAT4 and NK/T cell activation.	Localized/activation-restricted secretion (CAR-linked IL-12, fusion to tumor-targeting domains, and immunocytokines) to limit systemic toxicity.	Drives IFN-γ production, recruits endogenous immunity and epitope spreading, polarizes TAMs toward M1, and enhances antigen presentation and antigen-independent tumor clearance.

Table 1. (continued)

Cytokine classification	Cytokine	Cytokine type and biology	Engineering strategy	Key functional effects on CAR-T cells
	IL-18	IL-1 family cytokine (single chain, requires IL-18Rα/β); induces IFN-γ and inflammatory programs.	Constitutive or inducible secretion by CAR-T cells to modulate local inflammation and recruit host immunity.	Amplifies CAR- and host TIL activation, increases IFN-γ/TNF-α production, reduces exhaustion, promotes T-bet^{high}FoxO1^{low} effector polarization, and enables epitope spreading.
	IL-24	IL-10 family member; secreted cytokine that can modulate tumor cell survival and stemness (single chain).	CAR-T cells engineered to secrete IL-24 (lentiviral coexpression) to target tumor cell biology.	Suppresses cancer stemness (↓SOX2/OCT4/NANOG), inhibits STAT3 in tumor cells, enhances CAR-T cytotoxicity, and reduces tumor relapse driven by CSCs.
	IL-33	Alarmin (IL-1 family-like); released upon cell stress, signals via ST2 (IL-1RL1) to recruit and activate innate/adaptive cells.	Coexpression with other cytokine variants (e.g., Super2) or local secretion to remodel TME and recruit host immunity.	Improves immune cell recruitment (CD8$^+$ and NK), reshapes intratumoral leukocyte composition, increases infiltration and persistence, and reduces suppressive populations.
	IL-36γ	IL-1 family proinflammatory cytokine (single chain) that signals via IL-36R to activate epithelial/immune cells.	CAR-T armoring with IL-36γ to elicit TME remodeling and innate immune mobilization.	Reprograms neutrophils to tumoricidal and antigen-presenting phenotypes, mobilizes endogenous CD8$^+$ responses, enables tumor eradication often without lymphodepletion and supports durable host-engaged immunity.

lymphoma and CD19$^+$ PANC1 pancreatic cancer) models demonstrate that IL-10-expressing CAR-T cells achieve superior tumor control, prolonged persistence, and durable remission, including in metastatic disease contexts [32].

At the mechanistic level, IL-10 acts by stabilizing mitochondrial architecture and reinforcing oxidative metabolism in chronically stimulated CAR-T cells. IL-10-engineered CAR-T cells exhibit improved mitochondrial membrane potential, reduced structural abnormalities, and enhanced oxidative phosphorylation capacity, as reflected by increased oxygen consumption rates [32]. These metabolic advantages depend on mitochondrial pyruvate carrier-mediated substrate utilization, establishing a direct link between IL-10 signaling and metabolic resilience. By maintaining bioenergetic integrity, IL-10 enables CAR-T cells to resist exhaustion and sustain effector activity over time.

Importantly, IL-10-mediated enhancement appears most prominent during strong antigen stimulation, suggesting a context-dependent mechanism that may reduce unintended off-tumor activation. Consistent with this notion, preclinical models report potent antitumor efficacy without significant systemic toxicity or weight loss [32].

Effector-potentiating cytokine: IL-23

IL-23 is a heterodimeric cytokine composed of p19 and p40 subunits. Following T cell receptor (TCR) stimulation, T cells upregulate IL-23 receptor expression and induce transcription of the p19 subunit; however, they do not express p40. Consequently, functional IL-23 cannot be produced unless T cells are genetically engineered to express p40 [33]. Recent studies using p40-engineered CAR-T cells demonstrate that activation-dependent IL-23 production enhances effector function, augments antitumor cytotoxicity, and reduces T cell exhaustion.

Compared with other cytokines incorporated into CAR-T platforms, IL-23 displays potent antitumor activity with limited systemic toxicity due to its activation-restricted expression. Production of

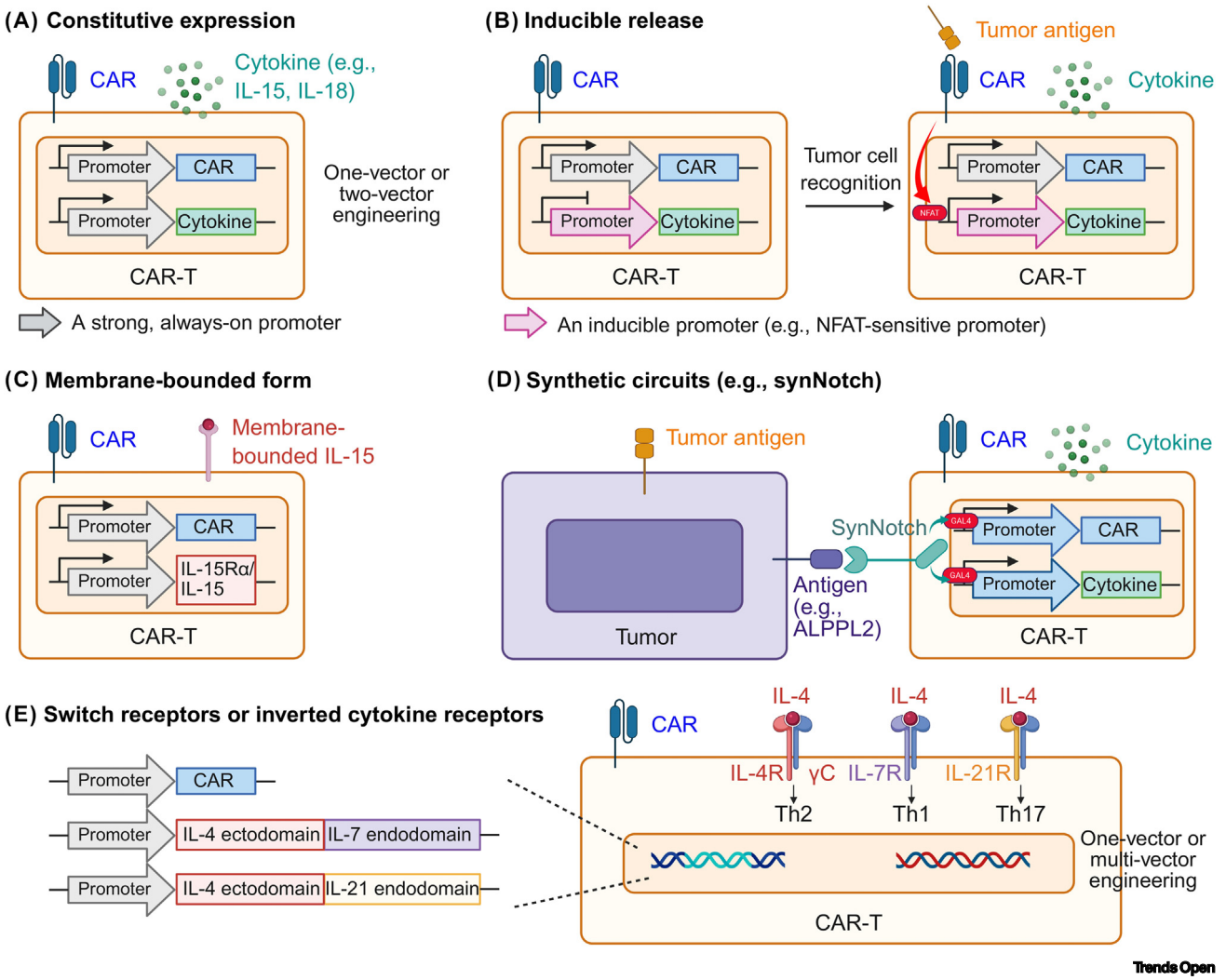


Figure 1. Cytokine engineering strategies for CAR-T cells. Schematic illustration of major design platforms used to incorporate cytokine support into CAR-T cells. (A) Constitutive expression: cytokine genes (e.g., IL-7, IL-15, and IL-18) are placed under strong constitutive promoters, enabling continuous secretion independent of antigen engagement. While this strategy enhances persistence, it may pose systemic toxicity risks for highly potent cytokines. (B) Inducible release: cytokine expression is driven by antigen-activated promoters such as NFAT-responsive elements, restricting cytokine production to sites of CAR engagement and promoting localized tumor microenvironment remodeling. (C) Membrane-bound or tethered cytokines: cytokines (commonly IL-15/IL-15Rα complexes) are expressed in a surface-anchored form to enable juxtacrine signaling, enhancing CAR-T survival while minimizing systemic exposure. (D) Synthetic circuits (e.g., synNotch): logic-gated systems couple tumor antigen recognition to customized cytokine expression programs, allowing highly precise spatial and contextual control. (E) Switch/inverted cytokine receptors: chimeric receptors convert immunosuppressive signals (e.g., IL-4) into stimulatory outputs (e.g., IL-7R signaling), thereby neutralizing inhibitory cues within the tumor microenvironment. Together, these strategies enable spatially and temporally controlled cytokine support to enhance CAR-T efficacy and safety. ALPPL2: alkaline phosphatase, placental-like 2; CAR: chimeric antigen receptor; IL: interleukin; IL-15Rα: interleukin-15 receptor alpha; NFAT: nuclear factor of activated T cells; synNotch: synthetic Notch receptor; Th2: T helper type 2 cell; Th17: T helper type 17 cell; TME: tumor microenvironment. This figure was created using BioRender (www.biorender.com).

the p19 subunit is induced by antigen recognition through the TCR or CAR, thereby confining IL-23 secretion to the TME when p40 is present [34]. In TCR stimulation assays using anti-CD3/CD28 antibodies to mimic antigen engagement, activated CAR-T cells upregulated p19 expression, whereas resting T cells did not, indicating that IL-23 production is activation dependent rather than constitutive [35]. Similarly, CAR-T cells cocultured with antigen-positive tumor cells secreted IL-23 only upon CAR engagement, supporting the specificity and safety of this strategy.

Repeated *in vitro* tumor coculture assays further demonstrated that IL-23-engineered CAR-T cells (CAR.p40-Td) exhibit superior tumor control compared with control CAR-T cells. These cells showed increased granzyme B expression and sustained production of IFN-γ and TNF-α following repeated tumor exposure, consistent with enhanced cytotoxic capacity and functional persistence [35]. Moreover, CAR.p40-Td cells retained CAR expression, demonstrated improved survival, and maintained tumor-killing activity across multiple stimulation cycles [35]. Reduced expression of exhaustion markers, including PD-1 and CD101, was also observed. *In vivo* tumor rechallenge models confirmed enhanced proliferation and persistence, collectively indicating that IL-23 signaling supports durable CAR-T cell functionality under chronic antigen stimulation.

Memory-supporting cytokine: IL-7

IL-7, a common γc cytokine critical for T cell proliferation, differentiation, and survival, has emerged as a promising strategy to enhance durable antitumor immunity, particularly in solid tumors where T cell exhaustion and depletion limit therapeutic efficacy. Mechanistically, IL-7 sustains antitumor responses through STAT5-mediated antiapoptotic signaling [36], downregulation of the exhaustion marker PD-1 [37], and promotion of stem cell-memory and central-memory T cell subsets associated with long-term persistence [38].

Despite its therapeutic potential, native human IL-7 has a short circulating half-life and primarily functions in autocrine and paracrine contexts, limiting its durability and clinical efficacy [37]. To address this limitation, engineered IL-7 variants incorporating a hybrid Fc domain derived from IgD and IgG4 have been developed to extend serum half-life and enhance bioactivity in CAR-T cells [39]. *In vitro* studies using $CD19^+$ Ramos B lymphoma cells demonstrate that CAR-T cells supplemented with Fc-engineered IL-7 exhibit enhanced expansion, preserved effector function, and increased resistance to apoptosis [39]. *In vivo*, xenograft models confirm superior CAR-T cell persistence and antitumor efficacy, with prolonged survival observed in treated mice [39]. Notably, no evidence of xenogeneic graft-versus-host disease was reported. Furthermore, evaluation in immunocompetent syngeneic murine models revealed no excessive toxicity, supporting the safety profile of engineered IL-7 in CAR-T cell therapy [39].

Interestingly, IL-7-mediated differentiation appears context dependent, promoting less differentiated memory phenotypes in some settings while favoring effector-memory populations in others. Clarifying these context-specific effects will be essential for optimizing combinatorial immunotherapeutic strategies that leverage IL-7-driven T cell expansion while maintaining durable, functional immunity.

Memory-supporting cytokine: IL-9

Recent studies have identified IL-9 as a potent regulator of CAR-T cell fate under chronic antigen stimulation. Engineering CAR-T cells to express the IL-9 receptor (IL-9R) enables cytokine-responsive signaling that enhances expansion, persistence, and tumor infiltration, resulting in superior tumor control at reduced cell doses [40]. These effects have been demonstrated in multiple preclinical models, including gp100-specific pmel-1 T cells in the B16-F10 melanoma model and CAR-T cells evaluated in murine pancreatic ductal adenocarcinoma systems [40].

Mechanistically, IL-9 signaling reshapes the transcriptional and differentiation landscape of CAR-T cells, promoting less differentiated, proliferative $CD4^+$ states and memory-associated $CD8^+$ phenotypes while limiting exhaustion under continuous antigen exposure. Transcriptomic and single-cell trajectory analyses reveal enrichment of STAT1, STAT4, and STAT3 pathways associated with stemlike and durable functional programs [40,41]. Importantly, IL-9-based engineering may offer safety advantages compared with other cytokines, as the

relative orthogonality and restricted expression of the IL-9/IL-9R axis could reduce off-target systemic toxicity. Nevertheless, IL-9-augmented CAR-T therapies have not yet been evaluated clinically, and comprehensive assessment of safety, dosing parameters, and potential toxicities will be essential for translation.

Memory-supporting cytokine: IL-15

IL-15 is a pleiotropic cytokine with broad immunomodulatory functions spanning both the innate and adaptive immune systems. Its role in enhancing T cell survival, proliferation, and memory differentiation has generated considerable interest in the context of CAR-T cell therapy [42]. In particular, IL-15 has been shown to promote central-memory and stem cell-like memory T cell phenotypes, improve cellular expansion and persistence, and augment antitumor activity.

IL-15, especially in combination with IL-7, has been demonstrated to facilitate the *in vitro* generation of memory stem T cells [43–45]. Building on this concept, studies have evaluated membrane-bound IL-15 (mbIL-15) coexpressed in CAR-T cells to provide autonomous cytokine support without repeated exogenous stimulation [46]. *In vitro*, mbIL-15-boosted CAR-T cells maintained cytotoxic function even in the absence of antigen or supplemental cytokines. Transcriptomic analyses revealed a pro-memory signature characterized by increased expression of Transcription factor 7 (TCF7), Eomesodermin (EOMES), and B-cell lymphoma 2 (BCL2), alongside reduced expression of effector differentiation-associated transcription factors. *In vivo* validation in murine models demonstrated that mbIL-15 CAR-T cells persisted significantly longer than conventional CAR-T cells under antigen- and cytokine-limited conditions. Phenotypic analysis indicated enrichment of less differentiated $CD45RO^-$ C-C chemokine receptor type 7 $(CCR7)^+$ cells in peripheral compartments, supporting the role of IL-15 in sustaining memory-like populations [46].

Beyond memory induction, IL-15 is a critical regulator of T cell activation, expansion, and differentiation. A recent study investigated the combined expression of IL-15 and IL-21 in glypican-3 (GPC3)-targeted CAR-T cells (21.15.GBBz) for hepatocellular carcinoma (HCC) [47]. These engineered cells exhibited enhanced proliferation, reduced apoptosis, and sustained expansion following repeated antigen stimulation compared with single-cytokine or conventional constructs. In xenograft NSG mouse models, dual-cytokine-expressing CAR-T cells demonstrated superior persistence and increased $CD8^+$ T cell frequencies. Mechanistically, IL-15-mediated upregulation of STAT5 phosphorylation likely contributes to improved survival and expansion [47]. These findings address key limitations of CAR-T therapy in solid tumors, namely insufficient persistence and limited durability of response.

Clinically, IL-15-expressing GPC3 CAR-T cells have shown encouraging activity. In a Phase 1 trial, patients receiving IL-15-modified CAR-T therapy achieved higher stable disease and objective response rates compared with conventional CAR-T treatment [48,49]. Enhanced cytotoxicity may be attributable to IL-15-induced upregulation of IFN-γ, CD107a, IL-2, TNF-α, and granzyme B [19,50].

Safety considerations remain critical. Preclinical studies in nonhuman primates reported dose-dependent toxicities with frequent IL-15 administration, whereas intermittent dosing demonstrated improved tolerability [51]. Importantly, IL-15 is generally associated with a more favorable safety profile than IL-2. Incorporation of safety mechanisms, such as inducible caspase-9 **suicide switches**, provides an additional safeguard. Collectively, IL-15 represents a promising strategy to enhance CAR-T cell persistence, functionality, and therapeutic efficacy [49,52,53].

Memory-supporting cytokine: IL-21

In the context of CAR-T cell therapy, IL-21 promotes a memory-like phenotype and enhances cellular durability, positioning it as a promising strategy to mitigate relapse and secondary tumor progression [54]. Preclinical studies highlight the distinct functional contributions of IL-21 to $CD8^+$ T cell biology. Unlike cytokines that activate the STAT5 pathway, which drives T cell proliferation, IL-21 activates STAT3, thereby promoting naive and memory T cell states. This distinct mechanism positions IL-21 as functionally unique and synergistically compatible with STAT5-directed cytokines, such as IL-2 and IL-15 [55]. In syngeneic thymoma models, IL-21 augmented and sustained $CD8^+$ T cell responses in a manner not replicated by IL-2 or IL-15 alone [55]. While IL-2 robustly drives T cell proliferation, it is also associated with increased apoptosis of $CD8^+$ T cells. IL-21 induces comparatively moderate proliferation but does not promote $CD8^+$ T cell depletion, likely due to STAT3-mediated activation of antiapoptotic pathways. Although IL-15 supports T cell persistence at levels comparable to IL-21, it does not preferentially promote a central-memory phenotype. Thus, IL-21 functionally overlaps with IL-2 in supporting expansion and with IL-15 in sustaining persistence, yet demonstrates advantages in maintaining both short-term viability and long-term memory characteristics [55].

Emerging evidence suggests that IL-21 may be particularly effective when combined with other cytokines. In preclinical solid tumor models, including HCC (Huh-7 and Hep3B), rhabdoid tumor (G401), and lung carcinoma (A549-GPC3), CAR-T cells coexpressing IL-15 and IL-21 displayed superior proliferation, persistence, and effector function compared with either cytokine alone [47]. Notably, IL-15/IL-21 coexpression did not elevate IL-1 or IL-6 levels, which are cytokines associated with CRS and neurotoxicity. Mechanistically, dual-cytokine expression increased TCF7 transcription and TCF-1 protein levels, which are key regulators of T cell self-renewal and proliferation. TCF7 knockout abrogated this proliferative advantage, underscoring its functional importance [47].

The activity of IL-21 is context dependent. When coexpressed with C-X-C motif chemokine ligand 9 (CXCL9), a chemokine involved in tumor homing, CAR-T cells demonstrated enhanced effector differentiation and cytokine secretion rather than enrichment of central-memory subsets [56]. However, antigen-independent cytokine production in this setting raises concerns regarding potential exhaustion.

TME-modulating cytokine: IL-12

IL-12 enhances cytotoxic T lymphocyte and NK cell function, promotes IFN-γ production, facilitates epitope spreading to target antigen-negative tumor populations, and remodels the TME toward a proinflammatory state. These properties make IL-12 an attractive payload for engineered cellular immunotherapies [57,58].

Preclinical studies have demonstrated the therapeutic potential of IL-12-armored CAR-T cells. In a syngeneic orthotopic glioblastoma model, EGFRvIII-targeted CAR-T cells engineered to secrete single-chain IL-12 achieved complete tumor eradication without prior **lymphodepletion** [58]. Notably, therapeutic efficacy extended to heterogeneous tumors containing both antigen-positive and antigen-negative cells, with a substantial proportion of mice achieving long-term survival [58]. These findings highlight the ability of IL-12 to recruit endogenous $CD8^+$ T cells and promote antigen-independent tumor clearance through epitope spreading. Similar antigen-independent antitumor activity has been observed in other models, where IL-12-secreting CAR-T cells eliminated both carcinoembryonic antigen (CEA)-positive and CEA-negative colorectal cancer tumor populations [59].

The importance of endogenous immune engagement is underscored by studies demonstrating that lymphodepletion can influence therapeutic outcomes. While conventional CAR-T cells

often require **cyclophosphamide**-based conditioning to achieve optimal efficacy, IL-12-secreting CAR-T cells have been shown to mediate tumor control even in the absence of lymphodepletion [60]. Enhanced cytotoxic function in these cells is associated with increased expression of perforin and granzyme B. Conversely, in certain combination strategies, such as mesothelin-directed CAR-T cells administered with IL-12-expressing oncolytic viruses, preceding lymphodepletion may attenuate epitope spreading by eliminating endogenous $CD8^+$ T cells, thereby limiting efficacy against antigen-negative tumors [61].

Beyond direct immune activation, IL-12 exerts profound effects on the TME. It promotes the polarization of tumor-associated macrophages from an immunosuppressive M2 phenotype toward a proinflammatory M1 phenotype and increases the expression of major histocompatibility complex (MHC) class II molecules, thereby enhancing antigen presentation and T cell priming [62,63]. These immunomodulatory effects further contribute to durable antitumor immunity.

Despite its therapeutic promise, systemic administration of IL-12 is associated with significant toxicities, including **leukopenia**, **thrombocytopenia**, and CRS, largely driven by excessive systemic IFN-γ production [63]. Accordingly, multiple engineering strategies have been developed to localize IL-12 activity and reduce off-target effects. One approach involves the expression of IL-12 as part of a fusion protein linked to tumor-targeting domains, such as αPD-L1–IL-12, enabling preferential delivery to PD-L1-expressing tumor sites and reducing systemic cytokine exposure [63]. Alternative strategies include integrating IL-12 into the CAR extracellular domain to restrict cytokine activity to sites of antigen engagement [59], or employing IL-12-based immunocytokines that combine tumor-specific binding domains with cytokine functionality [64].

TME-modulating cytokine: IL-18

Recent studies across multiple cancer models have identified IL-18 as a promising strategy to enhance the therapeutic efficacy of CAR-T cells. IL-18 augments immune activation by stimulating both engineered CAR-T cells and endogenous tumor-infiltrating lymphocytes (TILs), promoting memory differentiation, limiting functional exhaustion, and polarizing CAR-T cells toward a T-$bet^{high}FoxO1^{low}$ effector phenotype [65].

Preclinical leukemia xenograft models demonstrate that IL-18-secreting CAR-T cells significantly reduce tumor burden and improve overall survival compared with conventional CAR-T cells. Notably, therapeutic benefit is maintained at lower cell doses, and IL-18-engineered CAR-T cells persist in circulation for up to 28 days, whereas conventional CAR-T cells decline more rapidly [66]. In immunocompetent mouse models, similar survival advantages have been observed, indicating that IL-18 enhances antitumor activity in the context of an intact immune system [66].

IL-18 also reshapes the TME to favor antitumor immunity. Single-cell RNA sequencing of bone marrow-derived macrophages reveals that IL-18 promotes polarization toward an inflammatory M1-like phenotype, characterized by enhanced antigen presentation and immune activation [67]. Furthermore, IL-18 broadens antitumor immunity by recruiting endogenous T cells capable of recognizing tumor antigens beyond the CAR target, as evidenced by the clearance of mixed $CD19^+$ and $CD19^-$ tumor populations. This capacity to induce epitope spreading addresses a major limitation of conventional CAR-T therapies, which are restricted to single-antigen targeting [66].

Mechanistically, IL-18 enhances cytokine production and effector function. IL-18-secreting CAR-T cells produce elevated levels of IFN-γ and TNF-α upon antigen stimulation, while cocultured

endogenous T cells similarly exhibit increased cytokine secretion. Endogenous CD4 and CD8 T cells isolated from treated mice demonstrate heightened tumor reactivity, indicating amplification of host antitumor responses [67–69].

In addition to promoting effector activity, IL-18 supports memory formation and reduces exhaustion. Increased expression of CD62L and enhanced protection upon secondary tumor challenge indicate durable immunity [68]. Reduced PD-1 expression and improved outcomes in combination with PD-L1 blockade further support a role for IL-18 in limiting exhaustion. At the transcriptional level, IL-18 drives a T-bet^{high}FoxO1^{low} state associated with potent cytotoxicity and sustained inflammatory function [65]. Collectively, these findings position IL-18 as a multifaceted enhancer of CAR-T cell therapy.

TME-modulating cytokine: IL-24

IL-24, a member of the IL-10 cytokine family, has been investigated as a cytokine payload to enhance CAR-T cell efficacy, particularly in solid tumors where cancer stem cells (CSCs) contribute to therapeutic resistance and relapse [14,70]. Preclinical studies have evaluated CAR-T cells engineered via lentiviral transduction to coexpress a tumor-specific CAR and IL-24, with confirmed CAR surface expression and cytokine secretion. These IL-24-armored CAR-T cells were assessed in comparison with conventional CAR-T cells across *in vitro* and *in vivo* tumor models [71].

A principal advantage of IL-24 incorporation is its capacity to disrupt tumor stemness. In sphere formation assays, an established functional measure of CSC self-renewal, tumor cells exposed to IL-24-secreting CAR-T cells exhibited significantly reduced sphere number and size, indicating impaired self-renewal capacity [71]. Correspondingly, expression of stemness-associated transcription factors, including SRY-box transcription factor 2 (SOX2), Octamer-binding transcription factor 4 (OCT4), and Nanog homeobox (NANOG), was downregulated. Mechanistic analyses further demonstrated suppression of STAT3 signaling, a pathway critical for CSC maintenance, survival, and tumor progression [71]. These findings suggest that IL-24 directly targets CSC populations, thereby addressing a key driver of recurrence.

In addition to suppressing tumor stemness, IL-24 enhances CAR-T cell effector function. IL-24-engineered CAR-T cells exhibit increased *in vitro* cytotoxicity, augmented production of effector cytokines such as IFN-γ, and reduced expression of exhaustion markers compared with conventional CAR-T cells, indicating a more sustained and functionally robust phenotype [71]. In human lung cancer xenograft models, these cells demonstrate superior tumor regression and delayed progression, with reduced relapse associated with impaired CSC-driven tumor repopulation [71]. Collectively, these findings support IL-24 as a multifunctional cytokine payload capable of enhancing both CAR-T cell activity and long-term tumor control in solid malignancies.

TME-modulating cytokine: IL-33

IL-33 has emerged as a promising cytokine payload for enhancing CAR-T cell efficacy, particularly in solid tumors characterized by poor immune infiltration and a highly immunosuppressive TME [72]. In a recent study, CAR-T cells were engineered to coexpress IL-33 in combination with Super2, an IL-2 variant designed to preferentially expand effector T cells while limiting activation of immunosuppressive populations [26]. The engineered cells demonstrated stable CAR expression and cytokine secretion and were evaluated in both *in vitro* and *in vivo* solid tumor models [26].

A key advantage of IL-33 incorporation is its capacity to remodel the TME. As an alarmin released during cellular stress, IL-33 promotes immune cell recruitment and activation. When coexpressed

with Super2, IL-33 synergistically reshapes intratumoral leukocyte composition, reducing suppressive immune subsets while increasing cytotoxic $CD8^+$ T cells and NK cells. Notably, IL-33 enhances recruitment and activation of endogenous tumor-reactive T cells, indicating amplification of host adaptive immunity beyond the direct effects of engineered CAR-T cells [26].

Functionally, IL-33-armored CAR-T cells exhibited improved tumor infiltration, persistence, and proinflammatory cytokine production compared with conventional CAR-T cells. In multiple solid tumor models, this strategy resulted in delayed tumor growth and superior tumor control, with evidence of reduced antigen escape [26].

TME-modulating cytokine: IL-36γ

IL-36γ, a proinflammatory member of the IL-1 cytokine family, has recently emerged as a promising armoring strategy to enhance CAR-T cell efficacy in solid tumors [73]. Unlike approaches that primarily reinforce intrinsic T cell fitness, IL-36γ exerts its effects largely through remodeling the TME and mobilizing endogenous immunity. Preclinical studies in immunocompetent tumor-bearing mouse models demonstrate that IL-36γ-armored CAR-T cells significantly improve tumor control compared with conventional CAR-T cells [74], highlighting their capacity to overcome key barriers associated with solid tumor immunotherapy.

Single-cell transcriptomic analyses reveal that IL-36γ armoring selectively expands and reprograms tumor-infiltrating neutrophils toward proinflammatory, tumoricidal, and antigen-presenting phenotypes [74]. Functional studies further indicate that these reprogrammed neutrophils are indispensable for therapeutic efficacy, as neutrophil depletion abrogates tumor rejection and T cell activation. Beyond innate immune modulation, IL-36γ also promotes activation and expansion of endogenous $CD8^+$ T cells with antigen diversities extending beyond the CAR target, accompanied by increased effector-memory and central-memory subsets, as well as robust IFN-γ production upon tumor restimulation [74].

Importantly, IL-36γ-armored CAR-T cells can eradicate established solid tumors in the absence of prior lymphodepletion, inducing durable regression and protective immunity upon tumor rechallenge. In contrast, conventional CAR-T cells require lymphodepletion for efficacy and fail to generate sustained host immune memory [74]. Collectively, these findings indicate that IL-36γ enhances CAR-T therapy not only by augmenting direct cytotoxicity but also by orchestrating a coordinated innate and adaptive immune response, thereby enabling durable, host-engaged antitumor immunity.

Clinical trials evaluating cytokine-augmented CAR-T cells

IL-15-boosted CAR-T cells in treating patients with HCC

A recent clinical investigation of GPC3-targeted CAR-T therapy in advanced HCC evaluated conventional CAR-T cells alongside an IL-15-engineered construct. In the initial cohorts (NCT02905188[i] and NCT02932956[ii]), patients received standard GPC3 CAR-T cells, which demonstrated an acceptable safety profile but minimal clinical benefit, with no objective tumor responses and only transient *in vivo* expansion peaking at approximately 2 weeks after infusion [49]. These findings highlighted the limited persistence and intratumoral activity of conventional CAR-T cells in the immunosuppressive HCC microenvironment.

In subsequent cohorts (NCT05103631[iii] and NCT04377932[iv]), patients were treated with GPC3 CAR-T cells coexpressing IL-15. IL-15 engineering significantly enhanced CAR-T expansion, persistence, and tumor infiltration, resulting in a disease control rate of 66% and an objective response rate of 33% in a heavily pretreated population [49]. Molecular profiling of tumor-

infiltrating CAR-T cells from responders showed reduced expression of Switch/Sucrose Non-Fermentable complex (SWI/SNF) chromatin-remodeling components, which regulate transcriptional accessibility, and increased expression of AP-1 transcription factors FOS and JUN, which drive effector activation programs [49]. Genes associated with type I interferon signaling were also upregulated, indicating a more inflammatory and cytotoxic intratumoral phenotype.

However, IL-15 augmentation was associated with increased immune-mediated toxicity, including higher rates of CRS [49]. Although an inducible caspase-9 safety switch allowed rapid control of severe events, these observations underscore dose-dependent safety concerns. Careful dose optimization, step-up infusion strategies, and refined control mechanisms, such as inducible or spatially restricted IL-15 expression, will be necessary to balance enhanced potency with acceptable safety in future development [53].

IL-18-boosted CAR-T cells in treating patients with lymphoma

Relapse after CD19-directed CAR-T cell therapy represents a major clinical challenge, with limited effective treatment options and generally poor outcomes [75,76]. In this context, a Phase 1 study investigated huCART19-IL18, a next-generation CD19-targeted CAR-T cell product engineered to constitutively secrete IL-18, in patients with relapsed or refractory B cell lymphoma who had progressed after prior conventional CAR-T cell therapy (NCT04684563[v]) [77]. Unlike standard constructs, this IL-18-boosted CAR-T design aims to counteract T cell exhaustion and remodel the immunosuppressive TME through local cytokine release.

Twenty-one heavily pretreated patients received huCART19-IL18 following lymphodepleting chemotherapy. The safety profile was consistent with established CAR-T therapies. CRS occurred in 62% of patients, predominantly low grade, and neurotoxicity was observed in 14%, with all cases limited to grade 1–2 severity [77]. No unexpected or delayed immune-related toxicities emerged, suggesting that IL-18 secretion did not exacerbate systemic inflammatory complications.

Preliminary evidence of biological activity was observed. Robust *in vivo* expansion occurred across dose levels, including at relatively low cell doses compared with commercially available CAR-T products. At 3 months postinfusion, the overall response rate reached 81%, with more than half of patients achieving complete remission [77]. Responses were seen even in individuals whose disease had proven resistant to prior CD19 CAR-T cell therapy, indicating that antigen loss was not the dominant mechanism of relapse in most cases. With a median follow-up approaching 18 months, the median duration of response was 9.6 months, and several remissions remained ongoing beyond 2 years [77]. The relatively modest median duration of response highlights the need for longer-term follow-up to fully assess durability.

These findings suggest that IL-18 armoring may reinvigorate antitumor immunity by enhancing CAR-T cell expansion and potentially modifying the TME, rather than simply increasing cytotoxic potency [78]. Although limited by a small sample size and Phase 1 design, this study provides evidence that cytokine-engineered CAR-T cells may overcome functional resistance mechanisms following prior CAR-T cell failure, representing a promising direction for next-generation cellular immunotherapy.

IL-10-boosted CAR-T cells in treating patients with lymphoma and leukemia

A recent Phase 1 clinical trial (NCT06277011[vi]) evaluated IL-10-engineered CD19-targeted CAR-T cells in patients with relapsed or refractory B cell hematologic malignancies. Notably, the administered cell doses (2.0×10^4 to 2.0×10^5 cells/kg) were substantially lower than those typically

used in conventional CD19 CAR-T therapies [79]. Despite the reduced dosing, the trial reported an objective complete response (CR) rate of 100% (20/20 patients), which was sustained at 100% at 3 months (19/19 evaluable patients) and remained 94.1% at 6 months (16/17 evaluable patients) [79]. Five patients have maintained remission beyond 12 months, with the longest observed duration reaching 17 months at the time of reporting.

The safety profile was manageable, with treatment-related adverse events primarily consisting of hematologic toxicities such as **neutropenia**, thrombocytopenia, and **anemia**, all of which resolved with standard supportive care [79]. The use of comparatively low cell doses may reflect a cautious dose-escalation strategy given the cytokine-engineered design, while also underscoring the enhanced *in vivo* potency of IL-10-expressing CAR-T cells. While these early findings suggest potential enhanced *in vivo* activity of IL-10-engineered CAR-T cells, they remain preliminary and should be interpreted with caution given the small sample size, lack of a control group, and limited follow-up duration. Further validation in larger, controlled studies with extended follow-up will be necessary to confirm durability and assess comparative benefit.

Concluding remarks and future perspectives

Cytokine engineering has rapidly emerged as a transformative strategy to enhance the efficacy and durability of CAR-T cell therapy [80,81]. Future efforts are likely to expand beyond currently studied cytokines to identify novel immunoregulatory pathways, including metabolic and neuroimmune axes, that can be harnessed to further refine CAR-T function. In addition, cytokine engineering principles are increasingly being applied to other cellular platforms, such as NK cells, macrophages, and neutrophils, broadening the scope of cytokine-based immunomodulation across diverse adoptive cell therapies (Box 1).

Box 1. Cytokine engineering for CAR-NK cells, macrophages, and neutrophils

Beyond CAR-T cells, cytokine engineering strategies have been extended to other immune effector platforms, including CAR-engineered NK cells, macrophages, neutrophils, and iNKT cells. For these cell types, limited *in vivo* persistence and expansion remain major challenges, making cytokine support a critical component of therapeutic optimization.

In CAR-NK cells, IL-15 is the most extensively studied cytokine payload. IL-15, delivered either as a secreted factor or in a membrane-bound form, enhances NK cell survival, proliferation, and persistence without the need for continuous exogenous cytokine administration [86–90]. Membrane-tethered IL-15 restricts signaling to the engineered cells and their immediate microenvironment, potentially reducing systemic toxicity. Clinical studies of IL-15-engineered, cord blood- and induced pluripotent stem cell (iPSC)-derived allogeneic CAR-NK cells have demonstrated encouraging safety profiles and preliminary antitumor activity, supporting the feasibility of this approach [91–93].

For CAR-macrophages (CAR-Ms), cytokine engineering aims to promote and sustain a proinflammatory (M1-like) phenotype within the TME [94–96]. Payloads, such as IL-12 and IL-1 family cytokines, enhance antigen presentation, stimulate T cell recruitment, and counteract tumor-associated M2 polarization. By reinforcing inflammatory signaling, these cytokines enable CAR-M cells not only to mediate direct phagocytosis but also to remodel the TME and amplify adaptive immune responses.

Cytokine engineering in CAR-neutrophils remains at an early stage. Given the short lifespan and potent inflammatory capacity of neutrophils, localized expression of proinflammatory cytokines (e.g., IL-6 and IL-1 family members) or chemokines may improve tumor recruitment and cooperation with other innate and adaptive immune cells [97]. However, careful regulation is essential, as dysregulated neutrophil activation can exacerbate tissue damage and systemic inflammation.

In CAR-iNKT cells, IL-15 has shown promise in enhancing *in vivo* persistence and antitumor efficacy [13,98–100]. Additional cytokines, including IL-12 and IL-18, have also been investigated. IL-18 induces broad metabolic reprogramming in CAR-iNKT cells, enhancing oxidative phosphorylation, glycolysis, glutaminolysis, and purine metabolism, thereby supporting sustained effector function [101]. IL-12 promotes *de novo* programming of memory-like NKTs, particularly from CD62L-negative subsets, and facilitates differentiation toward long-lived, Th1-polarized memory cells [102]. Collectively, cytokine engineering across these alternative CAR platforms represents a versatile strategy to improve persistence, functional fitness, and immunomodulatory capacity while necessitating careful control to balance efficacy and safety.

Outstanding questions

What are the optimal cytokine combinations and dosing strategies to maximize chimeric antigen receptor-engineered T cell efficacy while minimizing systemic toxicity?

How can spatial and temporal control of cytokine expression be further refined to prevent off-tumor effects and cytokine release-associated toxicities?

Which tumor-intrinsic and microenvironmental factors determine responsiveness to specific cytokine-engineered chimeric antigen receptor-engineered T cell platforms, particularly in solid tumors?

Can cytokine engineering consistently overcome antigen heterogeneity and prevent tumor escape through epitope spreading and endogenous immune recruitment?

What are the long-term consequences of sustained cytokine signaling on chimeric antigen receptor-engineered T cell differentiation, exhaustion, genomic stability, and transformation risk?

How do different cytokines reshape chimeric antigen receptor-engineered T cell metabolism, and can metabolic profiling guide personalized cytokine programming?

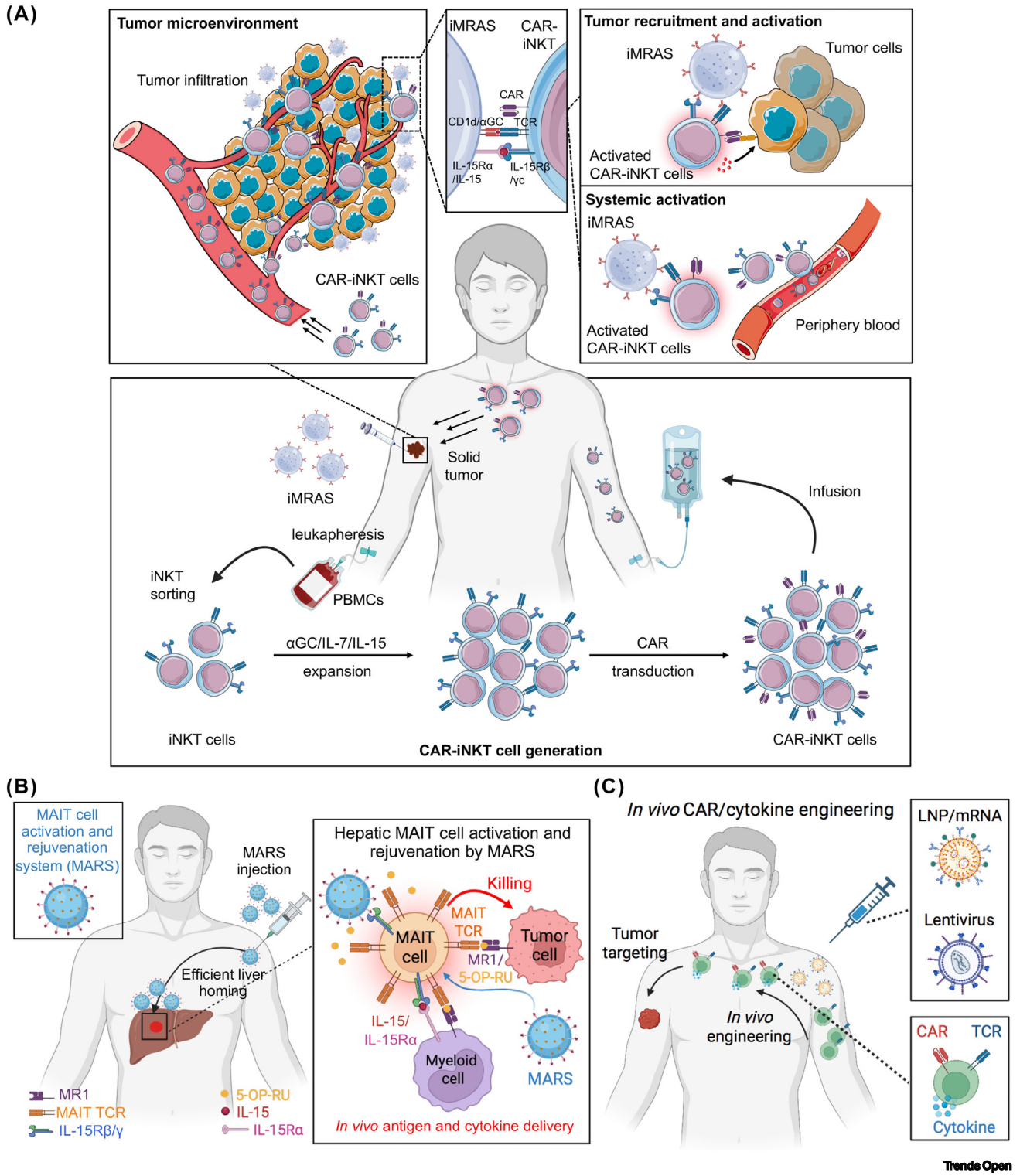
To what extent can cytokine engineering be successfully translated to other immune cell platforms, such as natural killer cells, macrophages, and neutrophils?

What are the comparative advantages of *ex vivo* cytokine armoring versus *in vivo* cytokine or cytokine-mRNA delivery approaches?

Can synthetic cytokine circuits and logic-gated systems reliably function in heterogeneous human tumors with complex antigen landscapes?

How will cytokine-engineered chimeric antigen receptor-engineered T cell therapies perform in allogeneic settings, and what additional safety safeguards will be required?

What biomarkers can predict efficacy, toxicity, and durability of cytokine-augmented chimeric antigen receptor-engineered T cell therapies in patients?



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Box 2. *In vivo* CAR and cytokine delivery

In vivo CAR engineering refers to the direct delivery of CAR-encoding genetic material into T cells within the patient, thereby eliminating the need for *ex vivo* cell manufacturing [5,85,103]. Preclinical studies and early clinical trials have demonstrated the feasibility of this approach, particularly using LNP-based mRNA delivery systems and lentiviral vectors. To date, successful applications have primarily been limited to hematologic malignancies and B cell-mediated autoimmune diseases, where circulating target cells are readily accessible [84,104–106]. The lack of efficacy in solid tumors likely reflects barriers, such as inefficient *in situ* transduction, limited trafficking, and the immunosuppressive TME. Incorporating cytokine engineering into *in vivo* CAR platforms may enhance therapeutic performance, for example, by codelivering cytokine-encoding mRNA within the same LNP formulation or vector system to provide localized immune support.

To enhance persistence and functionality in solid tumors, *in vivo*-engineered CAR-T cells can be further armored with immunostimulatory cytokines, such as IL-12, IL-15, and IL-18. IL-12 promotes local TME remodeling and recruitment of innate immunity, typically through inducible or synapse-restricted expression to minimize systemic toxicity. IL-15 supports T cell survival and memory formation via autocrine signaling, while IL-18 enhances effector function and metabolic fitness and can synergize with IL-12 to amplify antitumor immunity [82]. Together, these cytokines provide complementary support for sustaining CAR-T activity in hostile tumor environments while reducing the risks associated with systemic cytokine administration.

Beyond *ex vivo* engineering, *in vivo* cytokine delivery strategies, including targeted protein administration, mRNA-based delivery systems, and *in situ* gene transfer, represent an important emerging frontier (Figure 2). Biomaterial-based platforms, such as microparticles and nanoparticles, can be engineered to recruit and stimulate specific innate-like T cell populations within the TME. For example, systems targeting invariant natural killer T (iNKT) or mucosal-associated invariant T (MAIT) cells combine antigen-specific activation signals (e.g., α-galactosylceramide [αGC] or 5-(2-oxopropylideneamino)-6-D-ribitylamino-uracil [5-OP-RU], respectively) with cytokines such as IL-15 to promote local expansion and effector function [82,83]. Spatially restricted or inducible cytokine expression may also reduce systemic toxicity, such as CRS, while preserving potent local immune activation. In parallel, advances in gene delivery technologies, including lipid nanoparticle (LNP)-encapsulated mRNA and viral vectors, enable direct *in vivo* engineering of T cells to express CARs with or without cytokine support [84,85]. This strategy allows coordinated CAR expression and cytokine signaling *in situ*, potentially overcoming limitations associated with *ex vivo* manufacturing while enhancing therapeutic potency and durability (Box 2).

Moving forward, the central challenge will be achieving precise temporal and spatial control of cytokine signaling to balance efficacy and safety (Box 3) (see Outstanding questions). Advanced synthetic biology tools, feedback-regulated circuits, and combinatorial cytokine programming are poised to play critical roles in this next phase of development. With continued mechanistic insight and clinical validation, controlled cytokine engineering is likely to become a cornerstone of next-generation cancer immunotherapy.

Figure 2. *In vivo* cytokine delivery strategies to enhance CAR-based cell therapy. (A) Invariant natural killer T (iNKT) cell-targeted microparticle recruitment and activation system (iMRAS). A biomimetic, *in vivo* immunostimulatory platform designed to locally recruit and activate iNKT cells within the TME. iMRAS particles present CD1d loaded with α-galactosylceramide (αGC) to engage the invariant NKT TCR while simultaneously delivering IL-15 to support CAR-iNKT cell survival, expansion, and persistence. Acting as an *in situ* 'charging station', this system enhances intratumoral activation, systemic expansion, and durable antitumor responses. (B) Mucosal-associated invariant T (MAIT) cell activation and rejuvenation system (MARS). A nanoparticle-based platform containing the MR1 ligand 5-OP-RU to stimulate MAIT TCR signaling together with IL-15 to promote *in vivo* persistence and effector function. Following systemic administration, MARS preferentially accumulates in the liver, where it activates resident MAIT cells and augments their cytotoxic and cytokine-producing capacities against hepatic tumors. (C) *In vivo* CAR and cytokine coengineering. Genetic delivery platforms, such as lipid nanoparticle (LNP)-encapsulated mRNA or lentiviral vectors, enable direct *in vivo* transduction of T cells to express CAR constructs alone or together with cytokines. This approach allows simultaneous CAR targeting and cytokine support within the patient, enhancing tumor-directed potency while potentially reducing the need for *ex vivo* cell manufacturing. αGC: α-galactosylceramide; CAR: chimeric antigen receptor; IL: interleukin; iNKT: invariant natural killer T; MR1: MHC class I-related protein 1; PBMCs: peripheral blood mononuclear cells; TCR: T cell receptor; TME: tumor microenvironment. This figure was created using BioRender (www.biorender.com).

Box 3. Safety and manufacturing considerations of cytokine engineering in CAR-T cell therapy

While cytokine engineering enhances CAR-T cell efficacy, it also introduces safety challenges that require careful evaluation. Across cytokines, the most common toxicities include CRS, ICANS, off-target immune activation, and, in some cases, prolonged immune dysregulation [6,107–109]. These effects are often driven by excessive systemic cytokine exposure, particularly with potent proinflammatory mediators such as IL-2 and IL-12, which can amplify IFN-γ-dependent toxicity.

Strategies to mitigate these risks increasingly focus on restricting cytokine activity to the TME or CAR-T cells themselves. Approaches, such as membrane-bound cytokines (e.g., IL-15), CAR activation-dependent expression (e.g., IL-23), orthogonal cytokine-receptor systems, and tumor-targeted fusion proteins (e.g., IL-12 immunocytokines), reduce systemic exposure while preserving therapeutic benefit. Engineered cytokine variants with altered receptor affinity (e.g., IL-2 mutants) further improve selectivity by limiting expansion of immunosuppressive or nontarget cell populations. Clinically, cytokines such as IL-15 have demonstrated a more favorable safety profile compared with IL-2, although dose-dependent toxicities remain a concern [49,53]. Early-phase trials incorporating cytokine-armored CAR-T cells report manageable safety signals, often using established interventions such as tocilizumab and corticosteroids for CRS and ICANS. Additionally, incorporation of safety switches, including inducible caspase-9 systems, provides a mechanism for rapid abrogation of CAR-T activity in severe cases [52,91,110–112].

In addition to cytokine-intrinsic effects, the cellular state established during *ex vivo* manufacturing represents a critical determinant of both therapeutic efficacy and safety. Factors such as differentiation trajectory, metabolic fitness, and culture conditions shape CAR-T cell behavior, influencing expansion, persistence, and susceptibility to toxicity following infusion [113]. High-dimensional analyses of CAR-T products across manufacturing time points have demonstrated that early- to mid-expansion cells are enriched for stemlike and memory-associated subsets, including T-memory stem and central-memory populations, which are associated with enhanced proliferative capacity and long-term persistence [113]. In contrast, prolonged culture duration promotes terminal differentiation toward effector phenotypes, including $CD8^+$ T and NK-like cells, accompanied by reduced proliferative capacity, diminished metabolic fitness, and increased expression of exhaustion-associated markers [113].

Cytokine supplementation during manufacturing further influences these outcomes by promoting expansion but also driving progressive differentiation and checkpoint expression, particularly under prolonged culture or repeated antigen stimulation. Collectively, these findings highlight manufacturing as a critical and tunable layer of control that intersects with cytokine engineering to determine both the safety profile and functional durability of CAR-T cell therapies.

Author contributions

Y-R.L. developed the conceptualization for this review. Y-R.L., T.Y., S.Y., C.L.C., and P.D.-V. wrote the manuscript. L.Y. edited the manuscript. All authors have read and approved the final manuscript.

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Declaration of interests

L.Y. is a scientific advisor to AlzChem and Amberstone Biosciences, and a cofounder, stockholder, and advisory board member of Appia Bio. None of the declared companies contributed to this study. The other authors declare no competing interests.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to assist with improving the clarity, organization, and scientific language of the text. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Resources

[i]NCT02905188 https://clinicaltrials.gov/study/NCT02905188

[ii]NCT02932956 https://clinicaltrials.gov/study/NCT02932956

[iii]NCT05103631 https://clinicaltrials.gov/study/NCT05103631

Key references

1. Zhao, Y. *et al.* (2024) IL-10-expressing CAR T cells resist dysfunction and mediate durable clearance of solid tumors and metastases. *Nat. Biotechnol.* 42, 1693–1704

This study reveals that IL-10 engineering metabolically reprograms CAR-T cells to resist exhaustion and sustain antitumor activity in solid tumors and metastatic models. It provides mechanistic insight into how cytokine-driven metabolic support can durably enhance CAR-T persistence and therapeutic efficacy.

2. Li, Y-R. *et al.* (2025) Generation of allogeneic CAR-NKT cells from hematopoietic stem and progenitor cells using a clinically guided culture method. *Nat. Biotechnol.* 43, 329-344

This study establishes a clinically guided platform for generating allogeneic IL-15-boosted CAR-NKT cells from stem cells, demonstrating scalable production and potent antitumor activity. It expands the cytokine engineering paradigm beyond conventional CAR-T cells, supporting the development of off-the-shelf unconventional T cell therapies with enhanced persistence and safety.

3. Ma, X. *et al.* (2020) Interleukin-23 engineering improves CAR T cell function in solid tumors. *Nat. Biotechnol.* 38, 448–459

This study exemplifies how antigen-dependent cytokine engineering can enhance CAR-T efficacy while mitigating systemic toxicity. By designing an autocrine IL-23 circuit activated only upon T cell stimulation, the work directly supports the concept that precisely rewiring CAR-T cell fate through controlled IL-23 cytokine signaling to improve function in solid tumors.

4. Steffin, D. *et al.* (2024) Interleukin-15-armoured GPC3 CAR T cells for patients with solid cancers. *Nature* 637, 940–946

This study provides clinical proof-of-concept that IL-15 armoring enhances CAR-T cell expansion, persistence, and antitumor activity in solid tumors,

[iv]NCT04377932 https://clinicaltrials.gov/study/NCT04377932
[v]NCT04684563 https://clinicaltrials.gov/study/NCT04684563
[vi]NCT06277011 https://clinicaltrials.gov/study/NCT06277011

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