Immune Netw. 2025 Dec;25(6):e43
https://doi.org/10.4110/in.2025.25.e43
pISSN 1598-2629·eISSN 2092-6685

IMMUNE NETWORK

Review Article

Check for updates

Cytokine Engineering in CAR-T Cell Therapy: Next-Generation Strategies

Sunggu Kim [1,2,†], **So Hyeon Heo** [1,2,†], **Hyojin Baek** [1,2,†], **Soo Seok Hwang** [1,2,*]

[1]School of Biological Sciences, Seoul National University, Seoul 08826, Korea
[2]Institute of Molecular Biology and Genetics, Seoul National University, Seoul 08826, Korea

OPEN ACCESS

Received: Oct 3, 2025
Revised: Dec 4, 2025
Accepted: Dec 4, 2025
Published online: Dec 18, 2025

***Correspondence to**
Soo Seok Hwang
Institute of Molecular Biology and Genetics, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Korea.
Email: sshwang@snu.ac.kr

[†]Sunggu Kim, So Hyeon Heo, and Hyojin Baek contributed equally to this work.

ORCID iDs
Sunggu Kim
https://orcid.org/0009-0004-7546-2418
So Hyeon Heo
https://orcid.org/0009-0005-4068-3226
Hyojin Baek
https://orcid.org/0009-0004-9296-8824
Soo Seok Hwang
https://orcid.org/0000-0002-1635-4191

Abbreviations
AcTakines, activity-on-target cytokines; CAR, chimeric Ag receptor; CEA, carcinoembryonic

ABSTRACT

Chimeric Ag receptor (CAR)-T cell therapy has demonstrated success primarily in B-cell malignancies, but efficacy in solid tumors remains limited by Ag heterogeneity, immunosuppressive tumor microenvironments, and restricted infiltration. Cytokine engineering has emerged as a promising strategy to overcome these barriers. Fourth-generation CAR-T cells, known as T cells redirected for universal cytokine-mediated killing, demonstrated the feasibility of localized immune modulation through activation-induced IL-12 release, and this concept has been extended to various cytokines. Receptor engineering strategies, including switch/inverted and orthogonal designs, restrict cytokine signaling to CAR-T cells, thereby enhancing both specificity and safety. Beyond CAR-T engineering, external cell-based 'cytokine factories' and immune-cytokines further underscore the versatility of localized cytokine delivery strategies. In addition, fifth-generation CAR-T cells, incorporating approaches that enhance or mimic cytokine-mediated JAK-STAT signaling pathways, highlight a new direction toward programmable intracellular signaling. These strategies remain in the early stages of clinical application due to substantial limitations related to safety and clinical translation, including risks of uncontrolled cytokine activation and complexities in manufacturing. Nevertheless, they offer significant potential to improve therapeutic outcomes not only in hematologic malignancies but also across a broad range of solid tumors.

Keywords: CAR T-cell therapy; Cytokine; Cancer; Immunotherapy

INTRODUCTION

Chimeric Ag receptor (CAR)-T cell therapy, first proposed by Kuwana et al. (1) in 1984, has achieved groundbreaking success in the field of hematologic malignancies over the past decade, establishing a new paradigm in immunotherapy. After CD19 CAR-T achieved complete remission in pediatric Acute lymphoblastic leukemia patients in 2012, the first Food and Drug Administration (FDA) approval in 2017 firmly established it as a therapeutic strategy for blood cancers (2,3). However, in various types of cancer including solid tumors, the outcomes have shown limited efficacy in solid tumors. One major challenge is the

Ag; CRISPR, clustered regularly interspaced short palindromic repeats; CRS, cytokine release syndrome; CUL5, cullin-5; dnTGFβRII, dominant-negative TGF-β receptor II; EV, extracellular vesicle; FDA, Food and Drug Administration; 15.CAR, IL-15–constitutively expressed GPC3-CAR-T; GzB, granzyme B; iIL-12, inducible IL-12; IL-2Rβ, IL-2 receptor β-chain; MS, multiple sclerosis; MSC, mesenchymal stem cell; NB, neuroblastoma; NR4A2, nuclear receptor subfamily 4 group A member 2; OV, oncolytic virus; PEG, polyethylene glycol; RPE, retinal pigment epithelial; SynNotch, synthetic notch; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment; TRUCKs, T cells redirected for universal cytokine-mediated killing; YXXQ, Tyrosine-X-X-Glutamine motif.

Conflict of Interest

The authors declare no potential conflicts of interest.

Author Contributions

Conceptualization: Hwang SS; Funding acquisition: Hwang SS; Project administration: Hwang SS; Supervision: Hwang SS; Visualization: Heo SH; Writing - original draft: Kim S, Heo SH, Baek H, Hwang SS; Writing - review & editing: Kim S, Hwang SS.

marked Ag heterogeneity of solid tumors relative to hematologic malignancies. High levels of Ag heterogeneity can reduce the initial efficacy of CAR-T cells and, under selective pressure, frequently drive Ag escape, thereby contributing to variable therapeutic responses across patients (4). Another key barrier is the secretion of immunosuppressive cytokines within the tumor microenvironment (TME). Tumor cells, either directly or through the recruitment of immunoregulatory cell populations, produce factors such as TGF-β and IL-10. These cytokines suppress the release of pro-inflammatory mediators and dampen the activation and cytotoxic activity of both NK cells and T cells (5). A further obstacle is the physical barrier imposed by the architecture of solid tumors. Aberrant angiogenesis, driven by tumor-derived angiogenic factors, leads to structurally and functionally abnormal vasculature characterized by downregulation of adhesion molecules, which restricts immune cell extravasation. In parallel, the extracellular matrix is excessively deposited and undergoes pathological remodeling, resulting in increased stiffness. Together, these changes limit the penetration of therapeutic agents and effector immune cells into the tumor core (6). Accordingly, emerging next-generation CAR-T cell designs extend beyond mere Ag recognition, integrating additional molecular strategies to ensure sustained survival and functional persistence within the TME (7).

The evolution of CAR-T cells has reflected the need to overcome therapeutic limitations through successive generations (**Fig. 1**, **Table 1**). First-generation CAR-T cells expressed a chimeric receptor composed of a single-chain variable fragment and the CD3ζ signaling domain, enabling MHC-independent Ag recognition and cytotoxicity; however, the absence of co-stimulatory signaling restricted their proliferation and persistence *in vivo* (8). To address this limitation, second-generation CAR-T cells incorporated an additional co-stimulatory domain, such as CD28 or 4-1BB, into the original CAR construct. This modification markedly enhanced T-cell expansion, survival, and persistence, thereby enabling prolonged antitumor activity *in vivo* (14). Nevertheless, their efficacy against solid tumors remained limited. Third-generation CAR-T cells sought to further augment signaling strength by combining multiple co-stimulatory domains, but clinical studies failed to demonstrate clear superiority over second-generation CAR-T cells (15). To overcome these barriers, fourth-generation CAR-T cells, known as T cells redirected for universal cytokine-mediated killing (TRUCKs), were developed. These are engineered to release cytokines selectively upon Ag engagement, thereby reprogramming the TME and enhancing both the persistence and functionality of CAR-T cells (16).

Table 1. Evolution of CAR-T generations

Generation	Structural features	Example signal domains	Advantages	Limitations	References
1st	scFv + CD3ζ	CD3ζ (signal 1)	MHC-independent Ag recognition	Restricted proliferation and persistence	(8)
2nd	scFv + CD3ζ + costimulatory domain	CD3ζ + CD28/ 4-1BB (signal 1/2)	Improved T cell proliferation, persistence and cytotoxicity	Limited efficacy against solid tumors	(9,10)
3rd	scFv + CD3ζ + two costimulatory domain	CD3ζ + CD28 + 4-1BB (signal 1/2)	Enhanced co-stimulatory signal	No improvement over 2nd gen	(11)
4th (TRUCKs)	Typically 2nd generation backbone + inducible cytokine cassette	CD3ζ + CD28 + NFAT-driven IL-12 (signal 1/2/3)	TME specific Cytokine secretion, TME remodeling	Systemic toxicity by secreted cytokines	(12)
5th	2nd generation backbone + Cytokine receptor signaling domain	CD3ζ + CD28 + IL-2Rβ-YXXQ (STAT3/5 signaling) (signal 1/2/3)	Cytokine-independent cytokine signaling	Limited experimental/clinical evidence; long-term safety remains to be validated	(13)

ScFv, single-chain variable fragment.

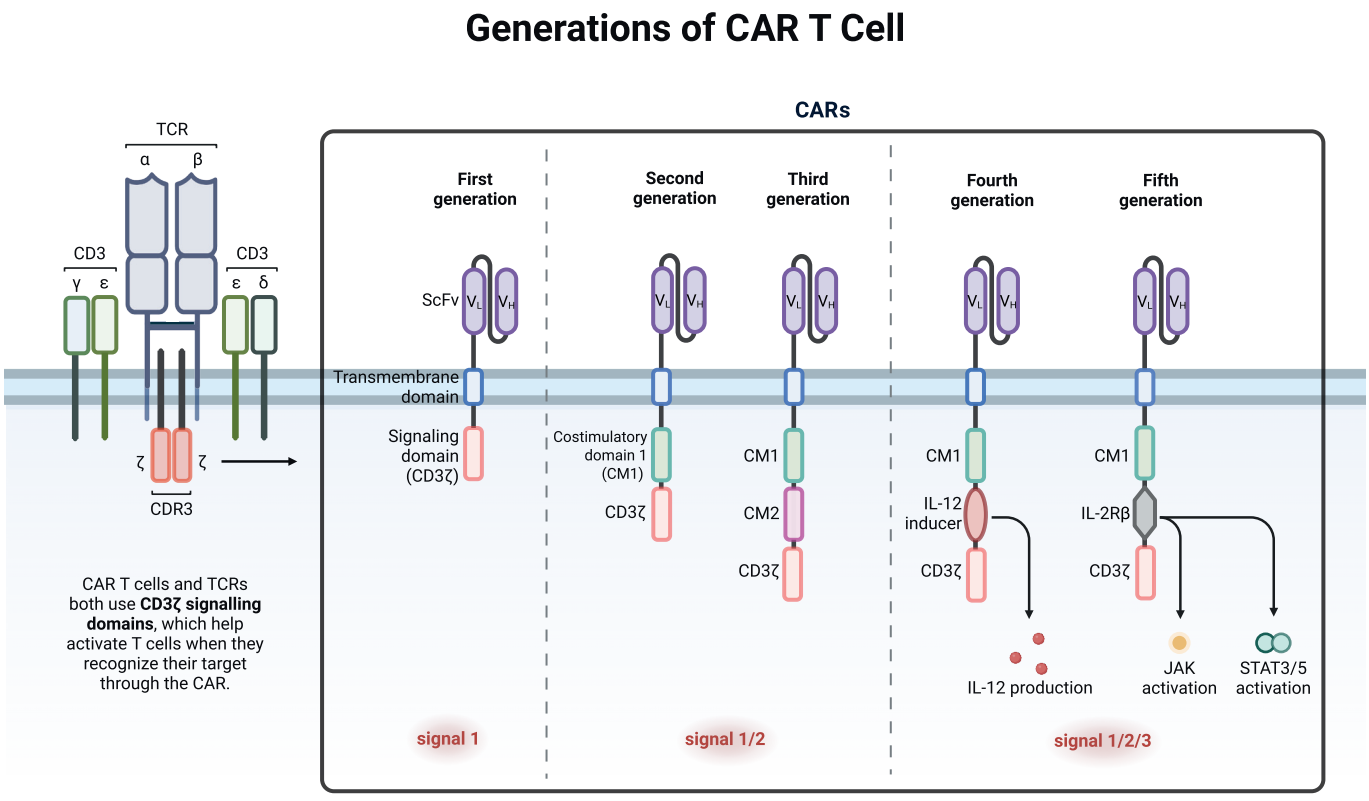

Figure 1. Generations of CAR T cell.
CARs are synthetic receptors that couple an Ag-binding domain (scFv) with intracellular signaling modules. First-generation CARs contain only the CD3ζ signaling domain. Second-generation CARs add a single costimulatory domain (e.g., CD28 or 4-1BB), whereas third-generation CARs incorporate two costimulatory domains. Fourth-generation CARs (TRUCKs) introduce activation-inducible cytokine cassettes to modulate the tumor microenvironment. Fifth-generation CARs fuse intracellular cytokine receptor domains (e.g., IL-2Rβ with a STAT3/5-activating motif), enabling Ag recognition to directly trigger cytokine-associated signaling pathways.
ScFv, single-chain variable fragment.

Cytokines were originally employed in cancer immunotherapy to enhance the activity of T cells and NK cells; however, systemic administration strategies using agents such as IL-2 or IL-12, despite their efficacy, were limited in clinical application due to severe systemic toxicities (17,18). With the rise of CAR-T cell therapy, renewed interest has emerged in strategies that combine cytokines with CAR-T cells or integrate cytokine expression directly into the CAR-T framework. The introduction of the TRUCKs concept opened the possibility of utilizing cytokines in a localized and conditional manner, thereby inspiring broader technological innovations, including synthetic receptor–based conditional signaling, delivery platforms using auxiliary cells, viral vectors, or exosomes, and inducible circuits designed to enhance safety. These approaches not only mitigate toxicity but also hold the potential to improve CAR-T cell persistence, tumor infiltration, and functional maintenance. Accordingly, this review aims to categorize and compare the diverse cytokine-based strategies developed to augment CAR-T cell therapy from a technological perspective, while discussing their translational potential and remaining challenges, as summarized in **Fig. 2**, which provides an overview of cytokine-based strategies to enhance CAR-T therapy in the TME.

https://doi.org/10.4110/in.2025.25.e43

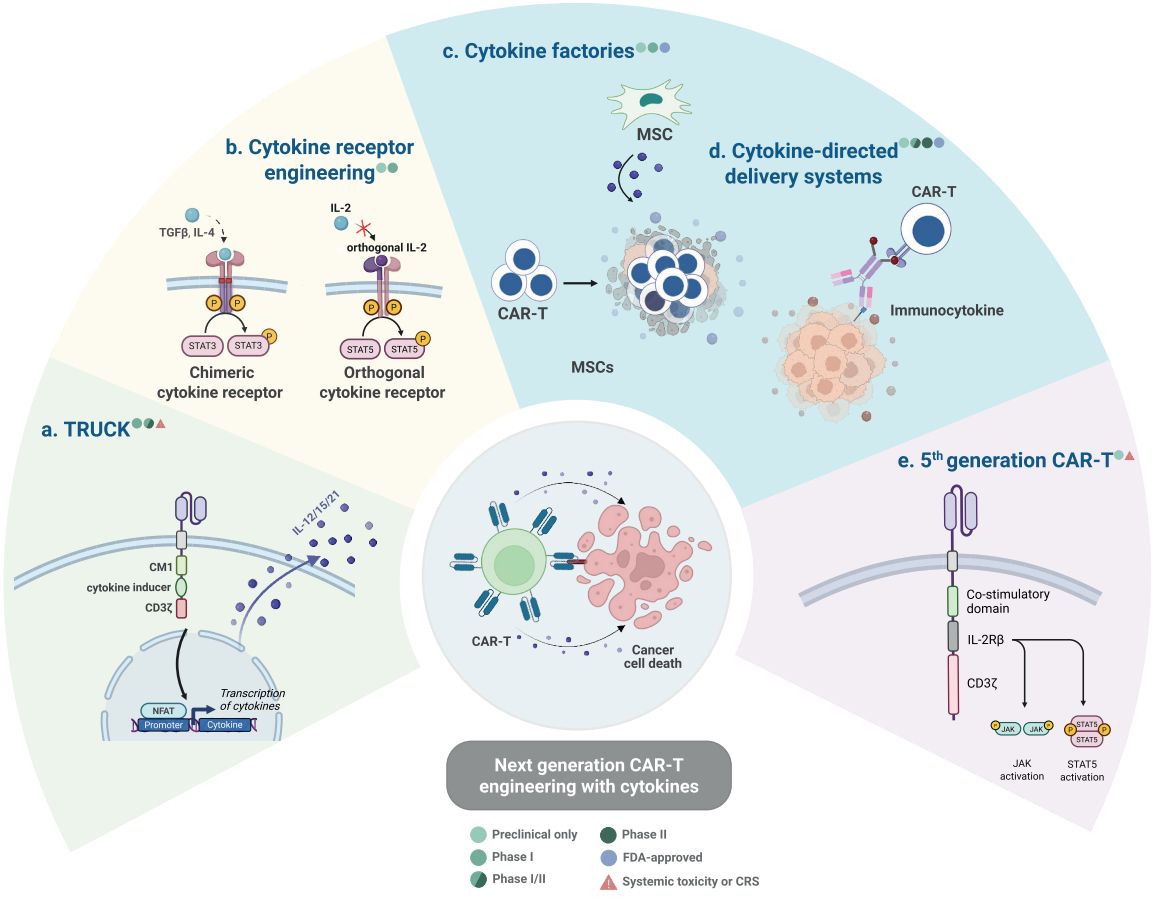

Figure 2. Approaches for TME-restricted cytokine signaling in CAR-T cell therapy.
a. TRUCKs (4th generation): NFAT-inducible promoters drive cytokine (e.g. IL-12, 18, 15/21) secretion upon Ag recognition. b. Cytokine receptor engineering: left: chimeric cytokine receptors convert inhibitory cytokine signals (e.g., IL-4, TGF-β) into stimulatory pathways. Right: orthogonal cytokine receptors selectively respond only to engineered orthogonal cytokines. c. Cytokine factories: engineered MSCs or other auxiliary cells home to the TME and deliver localized cytokine support. d. Cytokine-directed delivery systems: Modified cytokines or immunocytokine conjugates enhance tumor specificity and limit systemic toxicity. e. Fifth-generation CAR-T cells: Incorporation of cytokine receptor signaling domains enables CAR engagement to directly trigger JAK-STAT activation.

TRUCKs AND BEYOND: CYTOKINE-ARMING STRATEGIES FOR CAR-T CELLS

TRUCKs

TRUCKs refer to fourth-generation CAR-T cells engineered to secrete cytokines locally and conditionally within the TME upon CAR-mediated Ag recognition (19,20). The first TRUCKs, developed in 2011 by the Abken group, were composed of a first-generation CAR cassette combined with an inducible IL-12 (iIL-12) cassette driven by an NFAT-responsive promoter. In this design, CAR stimulation activates NFAT signaling downstream, thereby inducing iIL-12 expression. By restricting IL-12 production—otherwise highly toxic when administered systemically—to the tumor site, this approach minimized systemic toxicity while eliciting potent local immune stimulation. Notably, IL-12 TRUCKs not only enhanced the direct cytotoxicity of CAR-T cells but also reactivated macrophages within the TME, enabling the elimination of Ag-loss tumor variants (12). These findings demonstrated that the TRUCKs concept could extend beyond merely augmenting CAR-T effector functions, offering a strategy to reprogram the TME itself.

https://doi.org/10.4110/in.2025.25.e43

Originating from the initial design in which an NFAT promoter was used to drive IL-12 expression, TRUCKs have since evolved into a versatile platform in which the payload has been expanded to include a variety of cytokines such as IL-18, IL-15, and IL-21. This diversification provides the flexibility to selectively harness different cytokines according to the distinct immunosuppressive mechanisms and microenvironmental features of individual tumors. For example, the Abken group, which first pioneered TRUCKs, considered it important to maintain an effector-like state in order to prevent CAR-T cell exhaustion. The researchers searched for cytokines capable of inducing such a state, and as a result, identified IL-18 as a promising candidate. This discovery led to the development of IL-18 TRUCKs, and IL-18 was found to create an acute inflammatory environment while simultaneously reducing the frequencies of M2 macrophages and Treg cells, thereby inducing stronger antitumor activity than conventional CAR-T cells (21). In addition, the Rosa Nguyen group developed NFAT promoter–driven TRUCKs engineered to express membrane-bound IL-15 and IL-21, both of which had been reported to exert antitumor activity in neuroblastoma (NB). These TRUCKs enhanced CAR-T efficacy against NB while significantly reducing systemic toxicity, showing comparable tumor growth inhibition to CAR-T cells with constitutive cytokine secretion but with markedly improved safety profiles (22).

Advanced inducible cytokine control platforms

NFAT-based promoters enable cytokine expression in response to CAR signaling; however, concerns have been raised regarding background expression (leakage) as well as the potential for unintended activation by physiological TCR stimulation. As a result, IL-12 expression at inappropriate sites may lead to systemic toxicity. Indeed, in an early-phase trial of iIL-12 tumor-infiltrating lymphocyte (TIL) therapy (NCT01236573), systemic toxicity was reported due to Ag-independent IL-12 secretion (19). Consequently, alternative systems have recently been developed to achieve more precise regulation of expression strength and sensitivity. A representative example is a cytokine expression system driven by the endogenous nuclear receptor subfamily 4 group a member 2 (NR4A2) promoter. Compared with the NFAT promoter, the NR4A2 promoter exhibited more tumor-specific activity, thereby further reducing nonspecific cytokine expression, while also achieving higher levels of IL-12 expression (23,24). Additionally, studies have applied the synthetic Notch (synNotch) system to achieve inducible cytokine expression in CAR-T cells. The synNotch receptor consists of an extracellular sensing domain that recognizes Ag, a core Notch regulatory region that is cleaved upon signal transmission, and an intracellular transcriptional regulator that is released and translocates into the nucleus to function as a transcription factor. Upon Ag recognition, activation of synNotch signaling induces the expression of the target cytokine. The promoter used in this system is a synthetic promoter that is not recognized by endogenous transcription factors, resulting in minimal basal leakage and enabling cytokine secretion only when the CAR-T cells encounter Ag (25,26). There have also been studies aiming to control inducible cytokine expression in T cells through small-molecule administration. For example, Alsaieedi et al. (27) reported that when IL-12 was inserted into T cells expressing a melanoma-specific TCR and placed under a Tet-On inducible promoter, IL-12 was expressed only upon doxycycline administration, leading to enhanced intratumoral T-cell infiltration and antitumor activity. In contrast, when doxycycline was not administered, basal expression remained minimal and no systemic toxicity was observed (27).

Constitutive-armored CAR-T

Various approaches have been developed to integrate cytokine-expression capability into CAR-T cells in addition to inducible platforms. One of the simplest strategies is constitutive

armoring, in which CAR-T cells are engineered to continuously express a given cytokine regardless of their activation status. A representative clinical example of this approach is the low-level constitutive IL-12-armored CAR-T reported by Koneru et al. (28). As mentioned earlier, IL-12 is difficult to apply in immunotherapy due to its systemic toxicity. In contrast to IL-12 TRUCKs—which control IL-12 release locally—the authors sought to regulate toxicity by designing a retroviral tricistronic vector that co-expresses the CAR, a limited amount of IL-12, and EGFRt, thereby keeping IL-12 expression at a low level while incorporating a safety switch (28).

IL-15 is a cytokine that, while not expanding Treg cells, supports the survival and stem-like state of CD8 T cells and NK cells, and has acceptable safety due to causing relatively less systemic inflammatory cytokine release compared to IL-12 (29). Steffin et al. (30) clinically evaluated an IL-15–constitutively expressed GPC3-CAR-T (15.CAR) to address the issue of insufficient expansion and persistence of CAR-T cells in the solid tumor TME. In this study, 15.CAR-T cells showed superior *in vivo* expansion and higher tumor infiltration compared to non-armored CAR-T cells, and demonstrated approximately a 33% objective response rate and a 66% disease control rate in patients. In addition, single-cell analysis of tumor-infiltrating CAR-T cells revealed increased expression of FOS/JUN family genes and type I IFN signaling–related genes, along with suppression of SWI/SNF epigenetic regulators, suggesting that IL-15 armoring enhances both the effector function and the persistence of CAR-T cells (30).

There are also cases in which IL-18 has been incorporated into CAR-T cells to enable continuous secretion. Jaspers et al. (31) inserted the IL-18 gene into DLL3-targeting CAR-T cells to treat small cell lung cancer, presenting a preclinical model that can broadly activate surrounding immune cells in addition to Ag-specific responses. In the group treated with IL-18 CAR-T cells, not only the CAR-T cells themselves but also endogenous TILs and APCs showed increased activation and infiltration, demonstrating that converting the entire TME into an inflammatory immune environment can result in a more potent antitumor effect (31). Meanwhile, Hull et al. (32) noted that this constitutive IL-18 armoring strategy carries potential safety concerns, and therefore sought to develop a system that could more precisely regulate IL-18 signaling. To this end, the researchers developed a latent form of IL-18 (granzyme B [GzB]-IL18) that becomes activated by GzB. In this system, when CAR-T cells are in an inactive state, IL-18 is secreted in a functionally latent form, and active IL-18 is generated only at the time GzB is induced upon recognition of the tumor Ag. This regulatory mechanism is evaluated as an approach that preserves the antitumor effects of IL-18 while minimizing the safety concerns associated with its constitutive expression (32).

Although IL-12 and IL-18 currently represent the most advanced and clinically validated cytokine-armoring strategies in CAR-T therapy, emerging cytokine-based designs such as IL-23 (33), IL-27 (34), IL-10 (35), and IL-36 (36) have recently gained increasing attention. These novel approaches highlight the potential for expanding CAR-T cytokine engineering through diverse immunomodulatory mechanisms and suggest opportunities for developing more refined design strategies in the future.

CYTOKINE RECEPTOR ENGINEERING

While TRUCKs strategies mitigate systemic toxicity through localized cytokine release, they still carry the limitation that secreted cytokines may affect neighboring normal cells.

https://doi.org/10.4110/in.2025.25.e43

By contrast, cytokine receptor modulation strategies restrict responsiveness to engineered T cells, thereby reducing unwanted off-target toxicity. However, approaches that directly exploit native cytokine receptors face several constraints. First, overexpression of certain receptors has been reported in various hematologic malignancies, raising concerns regarding safety (37). Second, overexpression of receptors associated with IL-2 signaling—critical for T-cell proliferation and function—may paradoxically induce activation-induced cell death (38), thereby compromising the long-term persistence of CAR-T cells. Third, the TME is often enriched in immunosuppressive cytokines but depleted of immunostimulatory ones (39), limiting the efficacy of simply augmenting native receptor expression. For example, CAR-T cells engineered to overexpress IL-7R exhibited enhanced antitumor activity only when exogenous IL-7 was provided, whereas no benefit was observed in its absence (40). These limitations have shifted attention from merely reinforcing native receptors toward synthetic receptor strategies that allow fine-tuned control of cytokine signaling or confer entirely novel functionalities. Prominent approaches include decoy receptors, chimeric cytokine receptors, and orthogonal receptor systems, all of which are being actively investigated for their ability to reprogram or expand cytokine responsiveness in a TME-tailored manner.

Decoy receptor

Excessive secretion of immunosuppressive cytokines within the TME severely restricts the antitumor activity of T cells. Thus, even selectively blocking inhibitory cytokine signaling can be expected to improve CAR-T cell function. A representative approach in this context is the use of decoy receptors. Decoy receptors can bind cytokines but lack intracellular signaling domains, thereby preventing downstream signal transduction. By competitively sequestering ligands or forming nonfunctional dimers with native receptors, they effectively abrogate inhibitory signaling. The most extensively studied example is the dominant-negative TGF-β receptor II (dnTGFβRII), which retains a truncated intracellular kinase domain and binds all three isoforms of TGF-β without transmitting inhibitory signals (41). In a seminal study, Kloss et al. (42) engineered CAR-T cells targeting the prostate-specific membrane Ag to co-express dnTGFβRII, thereby conferring resistance to TGF-β. Compared to control CAR-T cells, dnTGFβRII-expressing CAR-T cells displayed superior intratumoral expansion, and while tumors in the control group continued to progress, complete tumor eradication was achieved in 4 out of 5 mice (42). Based on these promising preclinical findings, a clinical trial was subsequently initiated (NCT03089203). In the clinical setting, 13 out of 18 enrolled patients received cell infusions, during which marked changes in serum cytokine levels associated with cytokine release syndrome (CRS) were observed, indicating that this strategy was capable of eliciting immune responses *in vivo*. Nevertheless, objective clinical responses were limited, with only three patients achieving a PSA30 response, and both the overall response rate and durability remained suboptimal. Moreover, compensatory upregulation of other immunosuppressive signals within the TME was detected (43). Taken together, while the TGF-β decoy receptor approach demonstrated safety and transient antitumor activity, these findings suggest that TGF-β blockade alone is insufficient, highlighting the need for additional enhancements such as combinatorial armoring strategies.

Chimeric cytokine receptor (= switch/inverted cytokine receptor)

Beyond simply blocking immunosuppressive cytokine signals in the TME, as in the case of decoy receptors, attempts have also been made to exploit these signals in reverse. Chimeric cytokine receptors are designed by fusing the extracellular domains of immunosuppressive cytokine receptors—such as IL-4R or TGF-βR—with the intracellular domains of cytokine receptors that promote antitumor activity, including IL-7R (44), IL-15R (45), or IL-21R (46).

https://doi.org/10.4110/in.2025.25.e43

Through this design, the receptor expressed on the T-cell surface converts inhibitory cytokine signals prevalent in the TME into stimulatory ones, thereby enhancing T-cell antitumor function. This strategy offers two key advantages. First is TME specificity: because it utilizes immunosuppressive cytokines enriched in the tumor milieu as ligands, it can operate in a more tumor-restricted manner compared to constitutive receptor expression strategies. Second is design flexibility: extracellular–intracellular domain combinations can be freely tailored to match the immunological characteristics of different tumor types, greatly broadening the range of potential applications. Multiple studies have validated this concept. Mohammed, Mohammed et al. (47) expressed a receptor combining the IL-4R extracellular domain with the IL-7R intracellular domain in CAR-T cells, which resulted in enhanced T-cell proliferation specifically under IL-4–rich conditions in the presence of Ag, as well as improved *in vivo* tumor control. Wang et al. (48) engineered a receptor fusing the IL-4R extracellular domain to the IL-21R intracellular domain, demonstrating reduced T-cell exhaustion and maintenance of a central memory phenotype under IL-4–rich conditions, thereby improving CAR-T persistence. Furthermore, Noh et al. (49) developed a receptor composed of a TGF-β–binding extracellular domain and the IL-7R intracellular domain, which prolonged survival and prevented tumor relapse in a B cell lymphoma mouse model. Recently, an inverted cytokine receptor has been developed to exploit the IL-6–rich TME. Yoshikawa et al. (50) engineered a chimeric receptor composed of the extracellular domain of IL-6R and the intracellular domain of IL-7R, expressed it in CAR-T cells, and demonstrated that, in both hematologic and solid tumor models, these cells were not affected by the immunosuppressive responses driven by IL-6 and instead showed enhanced antitumor activity.

Orthogonal cytokine receptor system

Chimeric receptors provide the advantage of enabling TME-dependent CAR-T activation by converting inhibitory signals into stimulatory ones. However, because they rely on endogenous cytokines, the signaling triggered by these cytokines cannot be externally controlled, and the risk of unintended nonspecific or off-target activation cannot be fully eliminated. In contrast to these limitations, orthogonal cytokine receptor systems allow externally controllable and precisely targeted cytokine signaling. This strategy introduces engineered receptor–ligand pairs into CAR-T cells that do not cross-react with their wild-type counterparts. As a result, signal 3 can be selectively delivered to defined cell populations, while nonspecific activation by endogenous cytokines is effectively avoided. This enables precise control of cytokine signaling, offering a potential solution to both systemic toxicity and reduced therapeutic efficacy observed with conventional approaches. The most representative example is the orthogonal IL-2 cytokine receptor system, designed to selectively transmit IL-2 signaling—critical for T-cell proliferation and survival—while bypassing toxicity associated with endogenous IL-2. In a pioneering study, Sockolosky et al. (51) engineered a synthetic IL-2/IL-2 receptor β-chain (IL-2Rβ) pair (ortho IL-2/ortho IL-2Rβ) that bound exclusively to each other without interacting with their wild-type counterparts. Administration of ortho IL-2, even at high doses, produced no toxicity, and when ortho IL-2 was administered together with ortho IL-2Rβ–expressing T cells in tumor-bearing mice, the outcome was comparable to treatment with native IL-2, resulting in significant tumor growth delay and survival benefit (51). This concept was later extended by Zhang et al. (52) to a human orthogonal IL-2/IL-2Rβ system. The authors generated a mutant IL-2–receptor pair compatible with human T cells, capable of selective signaling without cross-reactivity to wild-type IL-2/IL-2R. When introduced into CAR-T cells and tested in a leukemia mouse model, treatment with ortho IL-2 drove robust expansion—by several hundred-fold—and induced potent antitumor activity even with low initial T-cell doses (52).

https://doi.org/10.4110/in.2025.25.e43

Table 2. Cytokine engineering strategies in CAR-T cells

Strategy	Mechanism	Key cytokine(s)	Advantages	Limitations/risks	Controls/mitigation	Clinical status	Reference
TRUCKs (4th gen)	CAR-inducible promoter drives cytokine secretion upon Ag recognition	IL-12, IL-15, IL-18, IL-21	Local immune activation; TME remodeling	Promoter leakage; systemic toxicity	Promoter optimization, drug-inducible circuits, membrane-tethering, safety switch	iIL-12 TIL (NCT01236573) - phase I/II (terminated) terminated due to toxicity IL-18 TRUCK (NCT04684563) – phase I (ongoing), Early signals: ORR, 81%, CR, 52%, manageable CRS	(12,21,22)
Constitutive-armored CAR-T	Ag-independent constitutive cytokine secretion	IL-15, IL-18	Enhanced persistence; improved antitumor efficacy	Risk of systemic cytokine toxicity; higher CRS incidence; lack of external control	Expression level tuning, low-affinity variants, safety switch	IL-15 (NCT04377932/ NCT05103631) – phase I (completed), ORR, 33%, DCR, 66%, manageable CRS	(30)
Decoy receptor	Bind inhibitory cytokines without signaling	TGF-βRII (dnTGFβRII)	Blocks suppressive signals; restores CAR-T activity	Compensatory immunosuppression; limited durability	Affinity tuning, combinatorial circuits	dnTGFβRII (NCT03089203) - phase I (follow-up), limited durability, low ORR	(42)
Switch/Inverted receptor	Fuse inhibitory receptor ectodomain with stimulatory endodomain	IL-4→IL-7R, IL-4→IL-21R, TGF-β→IL-7R	Convert suppressive cues to stimulatory; TME specificity	Dependent on endogenous cytokines; limited external control	Environment-responsive designs; feedback control undeveloped	Preclinical only	(47-49)
Orthogonal receptor system	Engineered cytokine–receptor pairs non-crossreactive with WT	Ortho-IL-2/ ortho-IL-2Rβ	Precise external control; avoids off-target activation	over-expansion; translational complexity	Titratable dosing, safety switches	Preclinical only	(51)
Fifth-generation CAR	CAR fused with IL-2Rβ and STAT3-binding motifs (YXXQ)	Endogenous IL-2Rβ-STAT3	Signal 3 delivery without exogenous cytokines; improved persistence	Over-expansion, CRS (not yet empirically demonstrated)	Currently unclear; requires systematic safety evaluation and long-term *in vivo* data	Preclinical only	(13)

ORR, objective response rate; CR, complete response; DCR, disease control rate.

In summary, decoy receptors block inhibitory signals in the TME to prevent infused cells from being suppressed, whereas switch receptors go further by converting inhibitory signals into stimulatory ones, thereby enabling TME-dependent CAR-T activation. However, both strategies depend on endogenous cytokines, which makes precise control difficult. In contrast, orthogonal cytokine receptor systems allow more precise control, since cytokines that respond only to the infused cells can be administered externally at desired time points, making it possible to deliver signals independently of the internal environment. The current clinical progress remains very limited. In the case of decoy receptors, only one clinical trial (NCT03089203) has been conducted in actual patients, and although proof of concept was achieved, low response rates and insufficient durability were reported as limitations. Switch/inverted receptors have shown increased TME-dependent activity in several preclinical studies, but no cases have yet advanced to clinical trials. Orthogonal cytokine receptors have likewise demonstrated low toxicity and strong antitumor activity at the preclinical stage but have not yet been validated in humans. Therefore, in order to evaluate the actual clinical efficacy and safety of these strategies, more systematic and active clinical trials will be required in the future. Representative cytokine engineering approaches—including TRUCKs, decoy receptors, switch/inverted receptors, and orthogonal receptor systems—are compared in **Table 2**.

EXTERNAL CELL- AND PLATFORM-BASED CYTOKINE DELIVERY

Cytokine delivery systems

Cytokines have a short half-life, necessitating frequent high-dose administrations to achieve therapeutic efficacy. However, excessive cytokine infusion often causes extensive distribution

beyond the target site, leading to damage in non-target tissues and considerable systemic toxicity (53,54). In immuno-oncology, precise and localized activation of target cells is essential for achieving effective and safe therapeutic outcomes. To address the challenges associated with systemic cytokine infusion, various cytokine-directed delivery systems have been developed. More recently, delivery methods employing engineered living cytokine factories, such as mesenchymal stem cells (MSCs) or retinal pigment epithelial (RPE) cells, have attracted attention. This strategy takes advantage of the unique biological properties of different cell types to achieve sustained and localized cytokine delivery within the TME, while offering the potential to incorporate more diverse and complex mechanisms compared with other approaches. This section highlights these two major strategies for enhancing cytokine-based cancer immunotherapy.

Cytokine factory

MSCs offer several therapeutic advantages: they are easily sourced, proliferate efficiently in culture, and are amenable to genetic engineering. Most notably, MSCs exhibit tumor tropism, which allows them to selectively migrate toward tumor sites and deliver cytokines or chemokines in a targeted manner. MSC-derived extracellular vesicles (EVs) retain many of these beneficial properties as well.

MSC-based platforms have been engineered to deliver various cancer-inhibitory cytokines, including type I IFNs (IFN-α and IFN-β), IL-2, and IL-12, directly into the TME. These cytokines exert multiple antitumor effects such as suppressing tumor cell proliferation, inhibiting angiogenesis, promoting apoptosis, and enhancing the activation of host immune responses (55). However, their clinical application is hampered by limited expression within the TME, short biological half-life, and associated toxicity (56). MSCs effectively address key clinical limitations of conventional cytokine therapies, including short half-life and systemic toxicity, by enabling localized, sustained cytokine delivery that increases therapeutic concentrations while minimizing off-target effects.

For example, IFN-α-expressing MSCs induce apoptosis in both tumor and tumor-associated endothelial cells and boost CXCL10 production to promote CD8+ T cell cytotoxicity and infiltration (55,56). Likewise, IFN-β-MSCs encapsulated within synthetic extracellular matrix have been shown to induce tumor cell cycle arrest and enhance CD8+ T cell infiltration in the peritumoral microenvironment (57).

Insufficient IL-2 signaling can lead to reduced TIL expansion, limited antitumor CD8+ T cell persistence, and accumulation of terminally exhausted TILs. To effectively deliver IL-2 within tumors, research utilizing IL-2-engineered MSCs has gained momentum. Recent studies demonstrated that peri-tumoral injection of IL-2-expressing MSCs can expand pre-existing CD8+ T cells and reinvigorate exhausted TILs, thereby remodeling the TME. Notably, the incorporation of hypoxia-responsive expression systems and on/off switches enables tumor-specific activation, maximizing antitumor immunity while minimizing systemic side effects (58). Similarly, MSCs delivering IL-12 have demonstrated potent antitumor and anti-angiogenic activity in preclinical models by reducing vascular density and enhancing the infiltration of antitumor M1 macrophages and CD8+ T cells within the TME (59).

Despite these promising features, there are critical caveats. The efficiency of MSC homing and long-term retention within heterogeneous tumor niches can be highly variable, as factors like chemokine gradients and local stromal architecture strongly influence MSC trafficking

https://doi.org/10.4110/in.2025.25.e43

and persistence at the target site. This unpredictability can result in subtherapeutic cytokine concentrations or off-target cell accumulation. Moreover, MSCs themselves are capable of immunosuppressive activity or, in certain contexts, may inadvertently support tumor growth, therefore raising safety concerns that require careful preclinical validation and clinical monitoring (60).

MSC-derived EVs provide a cell-free delivery method capable of transporting cytokines and other therapeutic agents to the TME. This EV-based strategy avoids some risks associated with cell transplantation but faces challenges such as donor-dependent heterogeneity in cargo, batch-to-batch variability, and rapid clearance *in vivo*, which currently limit reproducibility and efficacy compared to live-cell therapies (60).

In contrast, RPE cells exhibit strict contact inhibition, minimizing the risk of tumorigenicity. They are amenable to genetic modification and have demonstrated safety in clinical trials as delivery vehicles for therapeutics. Recent studies have engineered RPE cells to constitutively secrete cytokines and encapsulated them within alginate-based capsules for localized implantation near tumors, minimizing systemic toxicity. Importantly, the capsules undergo fibrosis mediated by the host immune response over time, leading to natural elimination, thereby confirming safety. Specifically, IL-2–secreting RPE cells demonstrated the capacity to slow progression and induce robust T cell responses in ovarian and colorectal peritoneal tumor models without observable toxicity. Despite these encouraging results, capsule fibrosis and immune-mediated elimination post-implantation potentially limit the long-term therapeutic durability of this platform. Moreover, challenges remain in sustaining stable engraftment and high-level cytokine production over extended time periods (61).

In addition to externally supplied platforms, approaches have also been developed to convert endogenous cells into cytokine factories within the body. virus-based oncolytic platforms employ engineered viruses that selectively infect and lyse tumor cells while simultaneously producing immunostimulatory cytokines such as GM-CSF or IL-12 within the TME (62). These oncolytic viruses (OVs) function through a dual mechanism: direct oncolysis and potent immune activation (63). By converting immunologically “cold” tumors lacking substantial immune infiltration into “hot” tumors rich in activated immune cells, OVs enhance susceptibility to immune-mediated tumor clearance (64). A prominent example is talimogene laherparepvec (IMLYGIC or TVEC), a herpes simplex virus-1–derived oncolytic virus genetically modified to express GM-CSF, which received FDA approval for advanced melanoma treatment (65). Multiple oncolytic viral platforms based on adenoviruses, poxviruses, and vesicular stomatitis virus have incorporated cytokine expression cassettes to improve *in vivo* immunostimulation, with ongoing clinical trials evaluating their efficacy across a range of malignancies, including brain tumors, pancreatic cancer, and ovarian cancer. While highly promising, challenges such as antiviral immunity, vector delivery, and tumor heterogeneity must be carefully managed to maximize therapeutic potential. However, the clinical efficacy of OVs can be hampered by pre-existing antiviral immunity, unfavorable vector biodistribution, and tumor heterogeneity, complicating repeat dosing strategies. Risks of neutralizing antibodies and inflammatory toxicities further challenge therapeutic planning, particularly in patients with prior viral exposures (66).

Recent advances have demonstrated that external cytokine platforms can be integrated with CAR-T cell therapy through either concurrent combination strategies or temporally/sequentially distinct approaches. In the combination setting, engineered MSCs or oncolytic

adenoviruses are administered at or near the time of CAR-T cell infusion to remodel the TME and provide localized, sustained cytokine cues that enhance CAR-T cell persistence and antitumor potency. For example, peritumoral administration of IL7/IL12-engineered MSCs immediately before or alongside CAR-T cell transfer shifts the TME toward a Th1-dominant, pro-inflammatory state, leading to increased CAR-T expansion, enhanced target cell killing, and improved tumor control in preclinical colorectal cancer models (67). Similarly, local or regional delivery of oncolytic adenoviruses expressing immunostimulatory cytokines such as IL-7, IL-15, or CCL5 in coordination with CAR-T infusion has produced strong synergistic antitumor activity, sustained T-cell functionality, and prolonged survival in solid tumor models (67-69). Optimizing the route of administration—intratumoral, intraperitoneal, or systemic—and the dosing schedule is therefore critical for maximizing cytokine bioavailability, facilitating CAR-T recruitment, and minimizing off-target toxicity.

By contrast, independent or sequential approaches deploy external cytokine platforms as standalone interventions, either to precondition the tumor bed before adoptive T-cell infusion or to periodically reactivate transferred lymphocytes after therapy. MSCs or OVs can be applied prior to CAR-T treatment to induce local immune infiltration and TME inflammation, thereby reducing immune-exclusion barriers and improving subsequent CAR-T trafficking and function (68). Descriptive summaries from preclinical studies also support the role of TME preconditioning in enhancing adoptive cell therapy performance. Alternatively, periodic post-infusion administration of cytokine-secreting MSCs can sustain memory differentiation and survival of CAR-T cells, helping to prevent functional exhaustion and extend the duration of antitumor responses (70). This independent axis provides additional flexibility in tuning the timing and magnitude of immunostimulatory signals and may be especially useful when concurrent combination strategies pose heightened toxicity risks or logistical constraints.

Cytokine-directed delivery systems

Pegylation technology involves the covalent attachment of cytokines or growth factor proteins to polyethylene glycol (PEG) polymers. This modification substantially increases the circulating half-life of the therapeutic proteins by shielding them from proteolytic enzymes and reducing renal clearance. Pegylated cytokines—such as pegfilgrastim (pegylated granulocyte colony-stimulating factor) (71), pegylated IFNs (e.g., peg-IFN-α) (72), and pegylated forms of IL-2 (peg-IL-2) (73) and IL-10 (peg-IL-10) (74)— have demonstrated enhanced pharmacokinetic profiles that translate into prolonged *in vivo* activity. This allows dosing regimens to be spaced out, reduces systemic toxicity, and improves patient adherence.

Despite these advantages, pegylation may induce conformational changes in the protein structure that can alter receptor binding or reduce target tissue penetration. This underscores the need for ongoing optimization of linker chemistry and conjugation strategies to balance stability with biologic activity (75).

In contrast, immuno-cytokine drug conjugates harness the specificity of monoclonal antibodies by covalently linking them to cytokines, thereby directing cytokine activity specifically to tumor-associated Ags. This approach enhances local cytokine concentration within the TME while sparing healthy tissues, reducing the risk of systemic toxicities commonly observed with recombinant cytokine therapies. Tumor-restricted delivery achieved through these conjugates increases antitumor efficacy by concentrating immune activation directly where it is most needed. Cergutuzumab amunaleukin is an immunocytokine that

fuses a high-affinity monoclonal antibody targeting carcinoembryonic Ag (CEA) with an engineered IL-2 variant designed to avoid regulatory T cell activation. Clinical trials in patients with solid tumors expressing CEA have demonstrated promising response rates and a favorable safety profile. Importantly, these conjugates also exhibit synergistic potential when combined with immune checkpoint inhibitors, amplifying the overall antitumor immune response (76). Challenges remain, including the need for tumor-specific Ag expression, antibody conjugate stability, and minimizing immunogenicity. Molecular strategies such as pegylation and antibody-cytokine conjugation have matured considerably, offering improved pharmacokinetics and tumor targeting. Yet, chemical modifications can unpredictably impact receptor specificity and function, demanding continued refinement in conjugation chemistry and linker design to optimize therapeutic efficacy.

Complementing classical protein conjugation approaches, cytokine-expressing plasmid nanoparticles represent a gene delivery-based approach wherein cytokine-encoding plasmids are introduced directly into tumors to enable endogenous expression of cytokines within the TME. This technology liberates the therapeutic effect from dependency on exogenous recombinant protein administration, allowing cancer cells or local stromal cells to produce cytokines in situ. Various nanoparticle platforms have been developed to facilitate safe and efficient plasmid delivery, including chitosan-based nanoparticles, liposomes, and poly(lactic-co-glycolic acid) nanoparticles (77). GEN-1 serves as a prominent example of a clinically evaluated DNA plasmid encoding IL-12 encapsulated within a PEG-PEI-cholesterol lipopolymer nanoparticle, demonstrating both safety and immunostimulatory activity in ovarian cancer patients (78). Electroporation-assisted delivery of IL-12 plasmids has further enhanced intratumoral uptake and expression, leading to significant antitumor effects in preclinical models and preliminary clinical studies (79). These nonviral systems improve stability and reduce systemic side effects but face challenges related to efficient tumor targeting, transgene expression longevity, and potential host immune responses against the delivery vehicle (80).

Activity-on-target cytokines (AcTakines) represent an emerging class of engineered cytokines designed to overcome systemic toxicities by modulating receptor-binding properties. AcTakines are modified cytokine molecules with reduced affinity for their native receptor complexes in circulation but enhanced binding to tumor cell–specific surface markers or immune cell subsets. This selective activation strategy ensures that cytokines remain inactive systemically, only becoming potent immunomodulators upon engagement with target cells. Preclinical animal models have shown that AcTakines can elicit localized antitumor immune responses with minimal off-target effects, marking them as a promising direction for safe and effective cytokine therapy. Although still in early developmental stages, AcTakines exemplify the next frontier of cytokine engineering aimed at precision immunotherapy (81).

Together, gene-based platforms such as cytokine-encoding plasmid nanoparticles and next-generation engineered molecules including AcTakines embody the cutting edge of cytokine delivery technology. They promise endogenous cytokine production and ultra-localized immune activation while minimizing systemic toxicity. However, they are still largely confined to preclinical or early clinical development due to persistent obstacles: maintaining prolonged gene expression, avoiding unwanted immune recognition of vector components, and achieving uniform tumor penetration (82).

Given the multifaceted immune-evasion mechanisms employed by the TME (5,83), increasingly sophisticated and integrative cytokine delivery strategies are essential.

https://doi.org/10.4110/in.2025.25.e43

Table 3. Cytokine delivery platform beyond CAR-T

Strategy	Platform/cell type/ technology	Delivery method	Advantages	Limitations/risk	Control/mitigation	Preclinical/ clinical status	Reference
Cytokine factory	MSCs	Systemic/local injection, *ex vivo* engineering	Tumor tropism; cytokine/chemokine delivery; genetic tractability	Limited cytokine expression (TME); short cytokine half-life; risk of immunosuppression or unintended tumor support	Payloads with kill switches and tumor-specific promoters for limiting activity and persistence	Early clinical studies, advanced preclinical	(55-59)
	MSC-derived EVs	IV/subQ injection, cell-free cargo	Cell-free delivery, safety, scalable manufacturing	Cargo loading variability; short persistence	Standardized cargo loading; surface engineering (e.g., CD47, targeting ligands) or hydrogel depots	Preclinical studies	(60)
	RPE cells + alginate capsules	Local encapsulated implantation	Controlled release; non-tumorigenic; proven safety in trials	Capsule fibrosis/ elimination; limited long-term function	Optimization of capsule composition (alginate purity, pore size, immuno-inert coatings)	Early clinical trials	(61)
	Virus-based Oncolytic Platforms (e.g. T-VEC)	Intratumoral/ intravenous injection	Dual action: direct tumor lysis and immune activation; gene delivery	Antiviral immunity; dose limitations; tumor heterogeneity	Viral vector engineering to evade antibodies; combined with immune modulators	FDA-approved (T-VEC for melanoma), multi-arm trials	(62-66)
Cytokine directed delivery systems	Pegylated cytokines (e.g. peg-IFN, peg-IL2)	Systemic injection	Prolonged half-life; reduced frequency and toxicity	Potential altered targeting/receptor binding; complex conjugation chemistry	Site-specific PEGylation and linker optimization to preserve receptor binding	Multiple FDA approvals; late clinical	(71-75)
	Immunocytokine drug conjugates (e.g. CEA-IL2v)	Systemic or targeted injection	Tumor-specific immune activation; synergy with CPIs	Requires target Ag; immunogenicity; conjugate stability	Selection of tumor-specific Ags; controlled conjugation sites and linker designs	Advanced clinical trials	(76)
	Cytokine-expressing plasmid nanoparticles (GEN-1)	Intratumoral injection	Endogenous cytokine synthesis; flexible gene delivery	Tumor targeting efficiency; expression persistence; immune response to vehicle	Use of less-immunogenic delivery vehicles; promoter engineering for controlled and durable expression	Phase I/II trials (GEN-1)	(77-80)
	AcTakines	Systemic injection, engineered ligand	Reduced off-target toxicity; ultra-specific activation	Early-stage technology; limited clinical safety/ efficacy data	Structure-guided tuning of receptor affinity; incorporation of reversible or protease-activatable designs	Preclinical, early clinical investigation	(81)

CPI, checkpoint inhibitor.

Leveraging cell- or virus-based platforms can reduce the engineering burden on effector T cells, facilitating more complex and effective antitumor immunotherapeutic regimens. Ultimately, clinical translation will likely depend on combinatorial and temporally programmable cytokine release systems, potentially integrated with modulatory agents and multi-cytokine cocktails, to achieve robust and durable antitumor immunity across heterogeneous patient populations. Key features of cell- and platform-based cytokine delivery methods are outlined in **Table 3**, which highlights their mechanisms, advantages, and clinical status

NEXT GENERATION CAR-T STRATEGIES

Several strategies have been developed to mimic the three signals required for TCR activation. A key feature of this approach is the induction of the JAK-STAT signaling pathway in the absence of exogenous cytokines. The principal advantage is that it fulfills the requirement for signal 3-the induction of JAK-STAT signaling through the common γ-chain—without the need for continuous external cytokine supplementation (84,85).

https://doi.org/10.4110/in.2025.25.e43

This concept was attempted in several studies. In practice, the combination of IL-2Rβ, STAT3-binding tyrosine-X-X-glutamine (Tyrosine-X-X-Glutamine motif [YXXQ]) motif, TCR signaling (CD3z) and co-stimulatory (CD28) domains, referred to as 28-ΔIL2RB-z(YXXQ), demonstrated improved antitumor activity. This combination induces JAK-STAT signaling in an Ag-dependent manner. In practice, enhanced proliferation and decreased terminal differentiation support the efficacy of this strategy. Furthermore, application of this strategy *in vivo* produced strong antitumor effects. The B cell Acute lymphoblastic leukemia xenograft model demonstrated improved overall survival and enhanced persistence in peripheral blood (13). This design has been described in several reviews as a representative example of so-called "fifth-generation CAR-T cells," where the incorporation of JAK-STAT signaling modules is considered a hallmark of this generation (86,87).

Based on this strategy, employing the induction of JAK-STAT signaling pathway, another modified form of CAR-T therapy demonstrated efficacy in Acute Myeloid Leukemia and solid tumors. This design, defined by the structure with G6/7R-expressing *TNF*-KO TNF-NTF, successfully targeted Acute Myeloid Leukemia and showed improved persistency and *in vivo*. The benefit of this approach is that, targeting TNF-NTF did not exhibit off-target cytotoxicity against normal haematopoietic stem and progenitor populations. In other words, reduced cytotoxicity against normal haematopoietic stem and progenitor cell within patients indicates the "resistance to fratricide." When administered to ovarian tumor *in vivo*, it successfully eradicated TNF-expressing ovarian tumor cells with enhanced proliferation and infiltration into tumor mass (88). Although this study is limited to cancers that express TNF, such as ovarian and uterine cancers, it is still regarded as a milestone for the next generation of CAR-T therapy. The potential of CAR-T therapy lies in its limitless capacity for modification and design. As our understanding of cancer advances, more targets and strategies to be applied in CAR-T therapy will emerge.

Furthermore, finding a gene that regulates the fitness of CAR-T cell is critical. A genome-wide clustered regularly interspaced short palindromic repeats (CRISPR) screening was conducted to identify genes that enhance the proliferation and survival of CAR-T cells. Given that, repetitive Ag stimulation from the tumor is the leading cause of exhaustion and death of CAR-T cells, elucidating the set of genes that regulate CAR-T cell function can be a breakthrough. The screen identified cullin-5 (CUL5), one of the core elements that composes the multi-protein E3 ubiquitin-protein ligase complex, as a negative regulator of proliferation and cytokine signaling in CAR-T cells. Indeed, genetic ablation of CUL5 enhanced the effector function of CAR-T cells with improved proliferation. Mechanistically, CUL5 improves CAR-T cell fitness by regulating STAT3 and STAT5 phosphorylation in JAK-STAT pathway in CAR-T cells. This mechanism is supported by delayed degradation of target protein recognized by suppressor of cytokine signaling family proteins, as CUL5 serves as scaffold protein in CRL5 E3 ubiquitin ligase complex. Accordingly, sustained phosphorylation of JAK-STAT signaling pathway leads to improved potency in antitumor activity *in vivo* tumor experiment. CUL5 KO-CD19 CAR-T cells significantly delayed tumor progression and improved overall survival. Considering the superior antitumor effects of CUL5 KO-CD19 CAR, genome-wide CRISPR screening can provide valuable insights for advancing CAR-T therapy (89).

Finally, multi-omics approaches can provide informative insights. For instance, a study combining single-cell RNA seq and CITE-seq analyzed the differences between healthy donor-derived CAR-T cells and patient-derived CAR-T cells from acute lymphoblastic

leukemia. The observed differences, such as higher activation levels and upregulation of major histocompatibility complex class II genes in donor CAR-T cells, suggest that multi-omics characterization can contribute to advancing CAR-T therapy (90). As advanced techniques such as proteomics and metabolomics are emerging, leveraging readouts from multi-omics may be proposed as potential metrics for the evaluation of CAR-T therapies.

CLINICAL OPERATIONS

Strategies designed to artificially provide cytokine signals to CAR-T cells are not inherently superior to one another; rather, each has its own advantages and limitations, and thus it is important to select the appropriate approach depending on the clinical context. Therefore, this section presents a concise decision-making framework summarizing when and under what circumstances each strategy may be used.

TRUCKs or constitutive cytokine-secreting CAR-T cells can be used when the goal is to enhance not only the transferred CAR-T cells but also the functions of endogenous immune cells that contribute to antitumor immunity through reprogramming of the TME. TRUCKs are employed when it is necessary to temporally and spatially restrict cytokine expression due to concerns such as systemic toxicity, and the degree of basal leakage can be precisely controlled depending on the promoter used. When using a synNotch system instead of TRUCKs, basal leakage can be minimized, and when using a Tet-On system, cytokine expression is induced only upon drug administration; however, CAR-T cells expressing cytokines through either approach have not yet been applied clinically. In the case of constitutive cytokine-secreting CAR-T cells, the surrounding TME can be reprogrammed even if the T cells are not activated through CAR stimulation; however, in this case, additional regulation such as reducing expression levels is required to prevent systemic toxicity, or the strategy should be applied to cytokines with relatively low systemic toxicity risk.

Decoy receptors can be used to prevent the suppression of CAR-T-cell function by immunosuppressive cytokines in the TME. However, based on clinical results to date, blocking immunosuppressive signals alone has not been sufficient to achieve robust antitumor effects. Therefore, this approach appears more appropriate when used in combination rather than as a standalone strategy. In contrast, switch/inverted receptors can convert immunosuppressive signals into activating signals, thereby providing the dual benefit of blocking suppression and delivering activation simultaneously. This is particularly useful in environments where immunosuppressive cytokines such as IL-4, IL-6, and TGF-β are abundant and stimulatory cytokines are limited. However, because signal transmission depends on the concentration of endogenous cytokines, it can be difficult to precisely control signaling. In the case of orthogonal cytokine/receptor systems, the administered cytokine does not bind endogenous receptors, which allows escape from systemic toxicity and enables the use of cytokines that previously caused severe systemic toxicity. Moreover, signaling induced by cytokines can be precisely controlled externally. Nevertheless, a limitation of this approach is that clinical data in humans remain limited.

A practical clinical framework for external cytokine platforms aligns delivery strategies with the clinical context and platform-specific risks. External systems such as MSCs, RPE cells, and OVs are particularly useful when persistent, compartmentalized cytokine delivery is required to enhance CAR-T or endogenous immune responses while minimizing systemic toxicity.

https://doi.org/10.4110/in.2025.25.e43

MSCs are especially advantageous in settings where tumor targeting and TME remodeling are desired. Owing to their intrinsic tumor tropism and in situ persistence, MSCs can reshape immunosuppressive TMEs and promote local immune activation (60,68,70,91). However, their homing efficiency and durability are variable, and their immunoregulatory phenotype may shift toward suppression depending on the surrounding cytokine milieu, which represents a key translational limitation.

RPE-based encapsulation platforms provide stable, localized cytokine release with minimal systemic exposure. These systems are particularly beneficial for anatomically confined compartments—such as the peritoneal cavity—where steady-state cytokine delivery is preferred. Nevertheless, long-term durability can be affected by device-associated fibrosis, which may reduce functional output over time (61).

OVs offer a complementary strategy by converting "cold" tumors into inflamed, immunologically active microenvironments that enhance CAR-T-cell function and promote Ag spreading (62,69). Their clinical utility, however, may be constrained by antiviral immunity and inconsistent intratumoral replication, which can limit sustained cytokine production.

DISCUSSION

Cytokine engineering has emerged as one of the most powerful strategies to broaden the therapeutic horizon of CAR-T cell therapy. From the early TRUCK designs employing NFAT-iIL-12 (12) to the expansion toward diverse payloads such as IL-18, IL-15, and IL-21 (21,22), these approaches have demonstrated that cytokine modulation can remodel the TME, prevent T-cell exhaustion, and extend antitumor immunity. However, concerns regarding promoter leakage and systemic cytokine toxicity have underscored the need for precise control (19), thereby driving the development of novel approaches such as synthetic promoters, orthogonal cytokine–receptor systems, and switch/inverted receptor.

In addition, complementary delivery platforms such as MSC–based (55), or RPE cell–based (61) "cytokine factories," immunocytokine conjugates (76), and OVs (63) illustrate the versatility of cytokine-based strategies. While these methods are not CAR-T strategies per se, they enable localized, sustained, and safe cytokine release, thereby holding significant potential for synergy when combined with adoptive cell therapy.

Beyond exogenous cytokine delivery, CAR-T cells have also been engineered to incorporate intracellular signaling modules. The integration of IL-2Rβ and STAT3-binding motifs into CAR constructs, often referred to as a hallmark of "fifth-generation" CAR-T cells, enables Ag-dependent activation of the JAK-STAT pathway. This design has been associated with enhanced proliferation, reduced terminal differentiation, and improved antitumor efficacy *in vivo* (13,86,87). However, the long-term safety of signal-3–augmented CAR-T cells remains to be clarified. While it is theoretically possible that JAK-STAT enhancement could amplify activation in the context of Ag-independent or tonic signaling, experimental evidence directly supporting this risk is currently lacking. Therefore, further validation is required as these designs move toward clinical translation.

Nevertheless, critical challenges remain before clinical translation can be fully realized. Ensuring long-term safety, minimizing off-target toxicity, enabling large-scale manufacturing,

and overcoming regulatory hurdles will be decisive milestones. Despite these obstacles, the developmental trajectory of cytokine-engineered CAR-T cells clearly points in a promising direction—toward increasingly precise, programmable, and tumor-specific immunotherapies. With continued integration of synthetic biology and systems immunology, cytokine-enhanced CAR-T cells hold great potential to establish themselves as transformative immunotherapies for not only hematologic malignancies but also solid tumors.

Beyond the strategies described above, other multiple approaches have been explored to enhance CAR-T efficacy. One such strategy is the use of logic-gated circuits. By engineering AND-gate T cells, activation of T cells occurs only upon dual Ag recognition. Specifically, a SynNotch receptor recognizes Ag 1, which subsequently induces the expression of CAR targeting Ag 2. This enables selective killing of tumor cells expressing both Ag while sparing single-Ag cells. The system is particularly significant as it can reduce off-target effects, which often arise because the Ags targeted by conventional CAR therapy also expressed on non-tumor cells. This system has been demonstrated *in vivo* using mouse models, showing selective activity only against cells expression both Ags (92). This approach has also been applied to glioblastoma, where the prime-and-kill system using EGFRvIII synNotch-CAR-T cells exhibited superior antitumor efficacy while avoiding off-tumor toxicity (93).

In addition to logic-gated systems, other applications of chimeric Ag receptors exist, including CAR-NK and CAR-Treg cells. CAR-Tregs have been utilized to promote transplant tolerance. CAR-Tregs expressing A2.ζ or A2.28ζ delayed skin rejection in an immunocompetent transplant model. And no significant difference was shown between A2.ζ and A2.28ζ CAR-Tregs in suppressing the generation of anti–HLA-A2 IgG Antibodies. Also, there were no detectable differences in functional markers or number of CAR-Tregs in draining lymph nodes and spleen indicating that costimulatory signal is naturally compensated by Ag presenting cells (94).

CAR-Tregs have also been explored in the context of multiple sclerosis (MS) treatment. MOG-specific CAR-Tregs were applied to MS models to enhance therapeutic efficacy, suggesting that producing CAR-Tregs from MS patient derived blood is a potential strategy for treating (95). In other study, applying CAR-Tregs combined with anti-CD154 therapy facilitated graft acceptance in allogenic heart transplant models suggesting the potency of CAR-Tregs in various indications (96).

Furthermore, introducing CAR into NK cells has been considered as an alternative approach. In studies involving patients with non-Hodgkin's lymphoma or chronic lymphocytic leukemia, CAR-NK therapy demonstrated safety and in terms of CRS and neurotoxicity (97). In addition, recent attempts have been made to apply CAR-NK cells to solid tumors, where they demonstrated Ag-specific and effective cytotoxicity in preclinical studies (98). An advantage of CAR-NK cells over CAR-T cells is the abundance of available NK cell sources, because challenges in CAR-T therapy include reduced T cells numbers due to prior treatments and risks of alloreactivity or graft-versus host disease. Therefore, using NK cells as a source may partially circumvent these limitations (99).

Since the advent of CAR-T therapy, numerous efforts have been made to enhance its therapeutic efficacy. Successive generations of CAR designs, together with advances in high-throughput screening and multi-omics technologies, have expanded our capacity to refine

https://doi.org/10.4110/in.2025.25.e43

T cell function and identify novel therapeutic targets. Furthermore, emerging applications beyond conventional T cells, such as CAR-Treg and CAR-NK cells hold promising avenues that can broaden the therapeutic application of CAR-based platforms across a range of diseases.

SUMMARY CONCLUSION

Cytokine engineering has rapidly expanded the therapeutic possibilities of CAR-T cells by enabling precise control over activation, persistence, and TME remodeling. Across inducible platforms such as TRUCKs, cytokine-armored constructs, receptor-remodeling strategies, and orthogonal cytokine systems, a unifying principle has emerged: cytokine signaling can be modularly rewired to strengthen antitumor immunity while mitigating systemic toxicity. External cytokine platforms—including MSCs, RPE cells, and OVs—further complement these approaches by providing sustained, compartmentalized immune stimulation.

Although most cytokine-engineered strategies remain at the preclinical or early clinical stage, the convergence of synthetic biology, multi-omics profiling, and next-generation CAR designs is rapidly closing the translational gap. Continued refinement of safety controls, manufacturability, and patient-specific tailoring will be essential to fully harness cytokine-based engineering. Collectively, these advances position cytokine-enhanced CAR-T cells as promising candidates for overcoming the long-standing barriers of solid-tumor immunotherapy.

ACKNOWLEDGEMENTS

We are grateful to the members of the S.S. Hwang Laboratory for helpful discussions and constructive comments on this manuscript. We also acknowledge the School of Biological Sciences and the Institute of Molecular Biology and Genetics at Seoul National University for their support and excellent research environment. This work was supported by the New Faculty Startup Fund from Seoul National University (grant No. SNU-20230041), the Suh Kyungbae Foundation (grant No. SUHF-22010039), the National Research Foundation (NRF) funded by the Korean government (MSIT) (grant Nos. RS-2021-NR059640, RS-2024-00403897, RS-2024-00398456, and RS-2025-15373099 to S.S.H.), and the Institute of Information & communications Technology Planning & Evaluation (IITP) grant funded by MSIT (grant No. RS-2025-25463302 to S.S.H).

REFERENCES

1. Kuwana Y, Asakura Y, Utsunomiya N, Nakanishi M, Arata Y, Itoh S, Nagase F, Kurosawa Y. Expression of chimeric receptor composed of immunoglobulin-derived V regions and T-cell receptor-derived C regions. *Biochem Biophys Res Commun* 1987;149:960-968. PUBMED | CROSSREF
2. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, Teachey DT, Chew A, Hauck B, Wright JF, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 2013;368:1509-1518. PUBMED | CROSSREF
3. June CH, Sadelain M. Chimeric antigen receptor therapy. *N Engl J Med* 2018;379:64-73. PUBMED | CROSSREF
4. Martinez M, Moon EK. CAR T cells for solid tumors: new strategies for finding, infiltrating, and surviving in the tumor microenvironment. *Front Immunol* 2019;10:128. PUBMED | CROSSREF

5. Tufail M, Jiang CH, Li N. Immune evasion in cancer: mechanisms and cutting-edge therapeutic approaches. *Signal Transduct Target Ther* 2025;10:227. PUBMED | CROSSREF
6. Chung SW, Xie Y, Suk JS. Overcoming physical stromal barriers to cancer immunotherapy. *Drug Deliv Transl Res* 2021;11:2430-2447. PUBMED | CROSSREF
7. Hupperetz C, Lah S, Kim H, Kim CH. CAR T cell immunotherapy beyond haematological malignancy. *Immune Netw* 2022;22:e6. PUBMED | CROSSREF
8. Eshhar Z, Waks T, Gross G, Schindler DG. 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 U S A* 1993;90:720-724. PUBMED | CROSSREF
9. Finney HM, Lawson AD, Bebbington CR, Weir ANC. Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. *J Immunol* 1998;161:2791-2797. PUBMED | CROSSREF
10. Zhong XS, Matsushita M, Plotkin J, Riviere I, Sadelain M. Chimeric antigen receptors combining 4-1BB and CD28 signaling domains augment PI3kinase/AKT/Bcl-XL activation and CD8+ T cell-mediated tumor eradication. *Mol Ther* 2010;18:413-420. PUBMED | CROSSREF
11. Pulè MA, Straathof KC, Dotti G, Heslop HE, Rooney CM, Brenner MK. A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells. *Mol Ther* 2005;12:933-941. PUBMED | CROSSREF
12. Chmielewski M, Kopecky C, Hombach AA, Abken H. IL-12 release by engineered T cells expressing chimeric antigen receptors can effectively Muster an antigen-independent macrophage response on tumor cells that have shut down tumor antigen expression. *Cancer Res* 2011;71:5697-5706. PUBMED | CROSSREF
13. Kagoya Y, Tanaka S, Guo T, Anczurowski M, Wang CH, Saso K, Butler MO, Minden MD, Hirano N. A novel chimeric antigen receptor containing a JAK-STAT signaling domain mediates superior antitumor effects. *Nat Med* 2018;24:352-359. PUBMED | CROSSREF
14. van der Stegen SJ, Hamieh M, Sadelain M. The pharmacology of second-generation chimeric antigen receptors. *Nat Rev Drug Discov* 2015;14:499-509. PUBMED | CROSSREF
15. Tomasik J, Jasiński M, Basak GW. Next generations of CAR-T cells - new therapeutic opportunities in hematology? *Front Immunol* 2022;13:1034707. PUBMED | CROSSREF
16. Chmielewski M, Abken H. TRUCKS, the fourth-generation CAR T cells: current developments and clinical translation. *Advances In Cell And Gene Therapy.* 2020;3:e84. CROSSREF
17. Conlon KC, Miljkovic MD, Waldmann TA. Cytokines in the treatment of cancer. *J Interferon Cytokine Res* 2019;39:6-21. PUBMED | CROSSREF
18. Sprent J, Boyman O. Optimising IL-2 for cancer immunotherapy. *Immune Netw* 2024;24:e5. PUBMED | CROSSREF
19. Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. *Expert Opin Biol Ther* 2015;15:1145-1154. PUBMED | CROSSREF
20. Chmielewski M, Hombach AA, Abken H. Of CARs and TRUCKs: chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma. *Immunol Rev* 2014;257:83-90. PUBMED | CROSSREF
21. Chmielewski M, Abken H. CAR T cells releasing IL-18 convert to T-Bethigh FoxO1low effectors that exhibit augmented activity against advanced solid tumors. *Cell Reports* 2017;21:3205-3219. PUBMED | CROSSREF
22. Okada R, Reyes-González JM, Rodriguez C, Kondo T, Oh J, Sun M, Kelly MC, Zhang L, Gulley J, Shern JF, et al. GPC2-targeted CAR T cells engineered with NFAT-inducible membrane-tethered IL-15/IL-21 exhibit enhanced activity against neuroblastoma. *Cancer Immunol Res* 2025;13:1363-1373. PUBMED | CROSSREF
23. Guo T, Ma D, Lu TK. Sense-and-respond payload delivery using a novel antigen-inducible promoter improves suboptimal CAR-T activation. *ACS Synth Biol* 2022;11:1440-1453. PUBMED | CROSSREF
24. Chen AXY, Yap KM, Kim JS, Sek K, Huang YK, Dunbar PA, Wiebking V, Armitage JD, Munoz I, Todd KL, et al. Rewiring endogenous genes in CAR T cells for tumour-restricted payload delivery. *Nature* 2025;644:241-251. PUBMED | CROSSREF
25. Allen GM, Frankel NW, Reddy NR, Bhargava HK, Yoshida MA, Stark SR, Purl M, Lee J, Yee JL, Yu W, et al. Synthetic cytokine circuits that drive T cells into immune-excluded tumors. *Science* 2022;378:eaba1624. PUBMED | CROSSREF
26. Roybal KT, Williams JZ, Morsut L, Rupp LJ, Kolinko I, Choe JH, Walker WJ, McNally KA, Lim WA. Engineering T cells with customized therapeutic response programs using synthetic notch receptors. *Cell* 2016;167:419-432.e16. PUBMED | CROSSREF
27. Alsaieedi A, Holler A, Velica P, Bendle G, Stauss HJ. Safety and efficacy of Tet-regulated IL-12 expression in cancer-specific T cells. *OncoImmunology* 2018;8:1542917. PUBMED | CROSSREF

28. Koneru M, O'Cearbhaill R, Pendharkar S, Spriggs DR, Brentjens RJ. 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* 2015;13:102. PUBMED | CROSSREF

29. Li Z, Wrangle J, He K, Sprent J, Rubinstein MP. IL-15: from discovery to FDA approval. *J Hematol Oncol* 2025;18:19. PUBMED | CROSSREF

30. Steffin D, Ghatwai N, Montalbano A, Rathi P, Courtney AN, Arnett AB, Fleurence J, Sweidan R, Wang T, Zhang H, et al. Interleukin-15-armoured GPC3 CAR T cells for patients with solid cancers. *Nature* 2025;637:940-946. PUBMED | CROSSREF

31. Jaspers JE, Khan JF, Godfrey WD, Lopez AV, Ciampricotti M, Rudin CM, Brentjens RJ. IL-18-secreting CAR T cells targeting DLL3 are highly effective in small cell lung cancer models. *J Clin Invest* 2023;133:e166028. PUBMED | CROSSREF

32. Hull CM, Larcombe-Young D, Mazza R, George M, Davies DM, Schurich A, Maher J. Granzyme B-activated IL18 potentiates αβ and γδ CAR T cell immunotherapy in a tumor-dependent manner. *Mol Ther* 2024;32:2373-2392. PUBMED | CROSSREF

33. Ma X, Shou P, Smith C, Chen Y, Du H, Sun C, Porterfield Kren N, Michaud D, Ahn S, Vincent B, et al. Interleukin-23 engineering improves CAR T cell function in solid tumors. *Nat Biotechnol* 2020;38:448-459. PUBMED | CROSSREF

34. Maleki AH, Rajabivahid M, Khosh E, Khanali Z, Tahmasebi S, Ghorbi MD. Harnessing IL-27: challenges and potential in cancer immunotherapy. *Clin Exp Med* 2025;25:34. PUBMED | CROSSREF

35. Zhao Y, Chen J, Andreatta M, Feng B, Xie YQ, Wenes M, Wang Y, Gao M, Hu X, Romero P, et al. IL-10-expressing CAR T cells resist dysfunction and mediate durable clearance of solid tumors and metastases. *Nat Biotechnol* 2024;42:1693-1704. PUBMED | CROSSREF

36. Li X, Daniyan AF, Lopez AV, Purdon TJ, Brentjens RJ. Cytokine IL-36γ improves CAR T-cell functionality and induces endogenous antitumor response. *Leukemia* 2021;35:506-521. PUBMED | CROSSREF

37. Silva A, Almeida ARM, Cachucho A, Neto JL, Demeyer S, de Matos M, Hogan T, Li Y, Meijerink J, Cools J, et al. Overexpression of wild-type IL-7Rα promotes T-cell acute lymphoblastic leukemia/lymphoma. *Blood* 2021;138:1040-1052. PUBMED | CROSSREF

38. Refaeli Y, Van Parijs L, London CA, Tschopp J, Abbas AK. Biochemical mechanisms of IL-2-regulated Fas-mediated T cell apoptosis. *Immunity* 1998;8:615-623. PUBMED | CROSSREF

39. Kureshi CT, Dougan SK. Cytokines in cancer. *Cancer Cell* 2025;43:15-35. PUBMED | CROSSREF

40. Vera JF, Hoyos V, Savoldo B, Quintarelli C, Giordano Attianese GM, Leen AM, Liu H, Foster AE, Heslop HE, Rooney CM, et al. Genetic manipulation of tumor-specific cytotoxic T lymphocytes to restore responsiveness to IL-7. *Mol Ther* 2009;17:880-888. PUBMED | CROSSREF

41. Gorelik L, Flavell RA. Abrogation of TGFbeta signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity* 2000;12:171-181. PUBMED | CROSSREF

42. Kloss CC, Lee J, Zhang A, Chen F, Melenhorst JJ, Lacey SF, Maus MV, Fraietta JA, Zhao Y, June CH. Dominant-negative TGF-β receptor enhances PSMA-targeted human CAR T cell proliferation and augments prostate cancer eradication. *Mol Ther* 2018;26:1855-1866. PUBMED | CROSSREF

43. Narayan V, Barber-Rotenberg JS, Jung IY, Lacey SF, Rech AJ, Davis MM, Hwang WT, Lal P, Carpenter EL, Maude SL, et al. PSMA-targeting TGFβ-insensitive armored CAR T cells in metastatic castration-resistant prostate cancer: a phase 1 trial. *Nat Med* 2022;28:724-734. PUBMED | CROSSREF

44. Park JH, Lee SW, Choi D, Lee C, Sung YC. Harnessing the power of IL-7 to boost T cell immunity in experimental and clinical immunotherapies. *Immune Netw* 2024;24:e9. PUBMED | CROSSREF

45. Lee H, Park SH, Shin EC. IL-15 in T-cell responses and immunopathogenesis. *Immune Netw* 2024;24:e11. PUBMED | CROSSREF

46. Koh CH, Kim BS, Kang CY, Chung Y, Seo H. IL-17 and IL-21: their immunobiology and therapeutic potentials. *Immune Netw* 2024;24:e2. PUBMED | CROSSREF

47. Mohammed S, Sukumaran S, Bajgain P, Watanabe N, Heslop HE, Rooney CM, Brenner MK, Fisher WE, Leen AM, Vera JF. Improving chimeric antigen receptor-modified T cell function by reversing the immunosuppressive tumor microenvironment of pancreatic cancer. *Mol Ther* 2017;25:249-258. PUBMED | CROSSREF

48. Wang Y, Jiang H, Luo H, Sun Y, Shi B, Sun R, Li Z. An IL-4/21 inverted cytokine receptor improving CAR-T cell potency in immunosuppressive solid-tumor microenvironment. *Front Immunol* 2019;10:1691. PUBMED | CROSSREF

49. Noh KE, Lee JH, Choi SY, Jung NC, Nam JH, Oh JS, Song JY, Seo HG, Wang Y, Lee HS, 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* 2021;22:8706. PUBMED | CROSSREF

50. Yoshikawa T, Ito Y, Wu Z, Kasuya H, Nakashima T, Okamoto S, Amaishi Y, Zhang H, Li Y, Matsukawa T, et al. Development of a chimeric cytokine receptor that captures IL-6 and enhances the antitumor response of CAR-T cells. *Cell Rep Med* 2024;5:101526. PUBMED | CROSSREF

51. Sockolosky JT, Trotta E, Parisi G, Picton L, Su LL, Le AC, Chhabra A, Silveria SL, George BM, King IC, et al. Selective targeting of engineered T cells using orthogonal IL-2 cytokine-receptor complexes. *Science* 2018;359:1037-1042. PUBMED | CROSSREF

52. Zhang Q, Hresko ME, Picton LK, Su L, Hollander MJ, Nunez-Cruz S, Zhang Z, Assenmacher CA, Sockolosky JT, Garcia KC, et al. A human orthogonal IL-2 and IL-2Rβ system enhances CAR T cell expansion and antitumor activity in a murine model of leukemia. *Sci Transl Med* 2021;13:eabg6986. PUBMED | CROSSREF

53. Hangasky JA, Waldmann TA, Santi DV. Interleukin 15 pharmacokinetics and consumption by a dynamic cytokine sink. *Front Immunol* 2020;11:1813. PUBMED | CROSSREF

54. Liao W, Lin JX, Leonard WJ. Interleukin-2 at the crossroads of effector responses, tolerance, and immunotherapy. *Immunity* 2013;38:13-25. PUBMED | CROSSREF

55. Ren C, Kumar S, Chanda D, Chen J, Mountz JD, Ponnazhagan S. Therapeutic potential of mesenchymal stem cells producing interferon-α in a mouse melanoma lung metastasis model. *Stem Cells* 2008;26:2332-2338. PUBMED | CROSSREF

56. Zhang T, Wang Y, Li Q, Lin L, Xu C, Xue Y, Hu M, Shi Y, Wang Y. Mesenchymal stromal cells equipped by IFNα empower T cells with potent anti-tumor immunity. *Oncogene* 2022;41:1866-1881. PUBMED | CROSSREF

57. Choi SH, Stuckey DW, Pignatta S, Reinshagen C, Khalsa JK, Roozendaal N, Martinez-Quintanilla J, Tamura K, Keles E, Shah K. Tumor resection recruits effector T cells and boosts therapeutic efficacy of encapsulated stem cells expressing IFNβ in glioblastomas. *Clin Cancer Res* 2017;23:7047-7058. PUBMED | CROSSREF

58. Bae J, Liu L, Moore C, Hsu E, Zhang A, Ren Z, Sun Z, Wang X, Zhu J, Shen J, et al. IL-2 delivery by engineered mesenchymal stem cells re-invigorates $CD8^+$ T cells to overcome immunotherapy resistance in cancer. *Nat Cell Biol* 2022;24:1754-1765. PUBMED | CROSSREF

59. Kułach N, Pilny E, Cichoń T, Czapla J, Jarosz-Biej M, Rusin M, Drzyzga A, Matuszczak S, Szala S, Smolarczyk R. Mesenchymal stromal cells as carriers of IL-12 reduce primary and metastatic tumors of murine melanoma. *Sci Rep* 2021;11:18335. PUBMED | CROSSREF

60. Shi Y, Zhang J, Li Y, Feng C, Shao C, Shi Y, Fang J. Engineered mesenchymal stem/stromal cells against cancer. *Cell Death Dis* 2025;16:113. PUBMED | CROSSREF

61. Nash AM, Jarvis MI, Aghlara-Fotovat S, Mukherjee S, Hernandez A, Hecht AD, Rios PD, Ghani S, Joshi I, Isa D, et al. Clinically translatable cytokine delivery platform for eradication of intraperitoneal tumors. *Sci Adv* 2022;8:eabm1032. PUBMED | CROSSREF

62. Pol JG, Workenhe ST, Konda P, Gujar S, Kroemer G. Cytokines in oncolytic virotherapy. *Cytokine Growth Factor Rev* 2020;56:4-27. PUBMED | CROSSREF

63. Pol JG, Rességuier J, Lichty BD. Oncolytic viruses: a step into cancer immunotherapy. *Virus Adapt Treat* 2011;4:1-21.

64. Kaufman HL, Amatruda T, Reid T, Gonzalez R, Glaspy J, Whitman E, Harrington K, Nemunaitis J, Zloza A, Wolf M, et al. Systemic versus local responses in melanoma patients treated with talimogene laherparepvec from a multi-institutional phase II study. *J Immunother Cancer* 2016;4:12. PUBMED | CROSSREF

65. Greig SL. Talimogene laherparepvec: first global approval. *Drugs* 2016;76:147-154. PUBMED | CROSSREF

66. Nash AM, Aghlara-Fotovat S, Castillio B, Hernandez A, Pugazenthi A, Lee HS, Jang HJ, Nguyen A, Lu A, Burt BM, et al. Activation of adaptive and innate immune cells via localized IL2 cytokine factories eradicates mesothelioma tumors. *Clin Cancer Res* 2022;28:5121-5135. PUBMED | CROSSREF

67. Huang J, Zheng M, Zhang Z, Tang X, Chen Y, Peng A, Peng X, Tong A, Zhou L. Interleukin-7-loaded oncolytic adenovirus improves CAR-T cell therapy for glioblastoma. *Cancer Immunol Immunother* 2021;70:2453-2465. PUBMED | CROSSREF

68. Baig ZA, Shafqat F, Mushtaq I, Aslam U, Faryal A, Maryam A. Therapeutic potential of mesenchymal stem cells and its exosomes in colorectal cancer: paving way from preclinical towards clinical road. *Adv Cancer Biol Metastasis* 2024;11:100123. CROSSREF

69. Porter CE, Rosewell Shaw A, Jung Y, Yip T, Castro PD, Sandulache VC, Sikora A, Gottschalk S, Ittman MM, Brenner MK, et al. Oncolytic adenovirus armed with BiTE, cytokine, and checkpoint inhibitor enables CAR T cells to control the growth of heterogeneous tumors. *Mol Ther* 2020;28:1251-1262. PUBMED | CROSSREF

70. Kolahi Azar H, Imanpour A, Rezaee H, Ezzatifar F, Zarei-Behjani Z, Rostami M, Azami M, Behestizadeh N, Rezaei N. Mesenchymal stromal cells and CAR-T cells in regenerative medicine: the homing procedure and their effective parameters. *Eur J Haematol* 2024;112:153-173. PUBMED | CROSSREF

71. Molineux G. The design and development of pegfilgrastim (PEG-rmetHuG-CSF, Neulasta). *Curr Pharm Des* 2004;10:1235-1244. PUBMED | CROSSREF

72. How J, Hobbs G. Use of interferon alfa in the treatment of myeloproliferative neoplasms: perspectives and review of the literature. *Cancers (Basel)* 2020;12:1954. PUBMED | CROSSREF

73. Charych D, Khalili S, Dixit V, Kirk P, Chang T, Langowski J, Rubas W, Doberstein SK, Eldon M, Hoch U, et al. Modeling the receptor pharmacology, pharmacokinetics, and pharmacodynamics of NKTR-214, a kinetically-controlled interleukin-2 (IL2) receptor agonist for cancer immunotherapy. *PLoS One* 2017;12:e0179431. PUBMED | CROSSREF

74. Naing A, Papadopoulos KP, Autio KA, Ott PA, Patel MR, Wong DJ, Falchook GS, Pant S, Whiteside M, Rasco DR, et al. Safety, antitumor activity, and immune activation of pegylated recombinant human interleukin-10 (AM0010) in patients with advanced solid tumors. *J Clin Oncol* 2016;34:3562-3569. PUBMED | CROSSREF

75. Mishra P, Nayak B, Dey R. PEGylation in anti-cancer therapy: an overview. *Asian J Pharm Sci* 2016;11:337-348.

76. Klein C, Waldhauer I, Nicolini VG, Freimoser-Grundschober A, Nayak T, Vugts DJ, Dunn C, Bolijn M, Benz J, Stihle M, et al. Cergutuzumab amunaleukin (CEA-IL2v), a CEA-targeted IL-2 variant-based immunocytokine for combination cancer immunotherapy: Overcoming limitations of aldesleukin and conventional IL-2-based immunocytokines. *OncoImmunology* 2017;6:e1277306. PUBMED | CROSSREF

77. Fewell JG, Matar M, Slobodkin G, Han SO, Rice J, Hovanes B, Lewis DH, Anwer K. Synthesis and application of a non-viral gene delivery system for immunogene therapy of cancer. *J Control Release* 2005;109:288-298. PUBMED | CROSSREF

78. Thaker PH, Brady WE, Lankes HA, Odunsi K, Bradley WH, Moore KN, Muller CY, Anwer K, Schilder RJ, Alvarez RD, et al. A phase I trial of intraperitoneal GEN-1, an IL-12 plasmid formulated with PEG-PEI-cholesterol lipopolymer, administered with pegylated liposomal doxorubicin in patients with recurrent or persistent epithelial ovarian, fallopian tube or primary peritoneal cancers: an NRG Oncology/Gynecologic Oncology Group study. *Gynecol Oncol* 2017;147:283-290. PUBMED | CROSSREF

79. Lucas ML, Heller L, Coppola D, Heller R. IL-12 plasmid delivery by in vivo electroporation for the successful treatment of established subcutaneous B16.F10 melanoma. *Mol Ther* 2002;5:668-675. PUBMED | CROSSREF

80. Ibe S, Qin Z, Schüler T, Preiss S, Blankenstein T. Tumor rejection by disturbing tumor stroma cell interactions. *J Exp Med* 2001;194:1549-1559. PUBMED | CROSSREF

81. Huyghe L, Van Parys A, Cauwels A, Van Lint S, De Munter S, Bultinck J, Zabeau L, Hostens J, Goethals A, Vanderroost N, et al. Safe eradication of large established tumors using neovasculature-targeted tumor necrosis factor-based therapies. *EMBO Mol Med* 2020;12:e11223. PUBMED | CROSSREF

82. Yi M, Li T, Niu M, Zhang H, Wu Y, Wu K, Dai Z. Targeting cytokine and chemokine signaling pathways for cancer therapy. *Signal Transduct Target Ther* 2024;9:176. PUBMED | CROSSREF

83. Li Z, Li J, Bai X, Huang X, Wang Q. Tumor microenvironment as a complex milieu driving cancer progression: a mini review. *Clin Transl Oncol* 2025;27:1943-1952. PUBMED | CROSSREF

84. Alsaieedi AA, Zaher KA. Tracing the development of CAR-T cell design: from concept to next-generation platforms. *Front Immunol* 2025;16:1615212. PUBMED | CROSSREF

85. Asmamaw Dejenie T, Tiruneh G/Medhin M, Dessie Terefe G, Tadele Admasu F, Wale Tesega W, Chekol Abebe E. Current updates on generations, approvals, and clinical trials of CAR T-cell therapy. *Hum Vaccin Immunother* 2022;18:2114254. PUBMED | CROSSREF

86. Tokarew N, Ogonek J, Endres S, von Bergwelt-Baildon M, Kobold S. Teaching an old dog new tricks: next-generation CAR T cells. *Br J Cancer* 2019;120:26-37. PUBMED | CROSSREF

87. Zhou J, Lei B, Shi F, Luo X, Wu K, Xu Y, Zhang Y, Liu R, Wang H, Zhou J, et al. CAR T-cell therapy for systemic lupus erythematosus: current status and future perspectives. *Front Immunol* 2024;15:1476859. PUBMED | CROSSREF

88. Nakashima T, Ouchida T, Ishikawa Y, Ito Y, Hayakawa T, Yoshikawa T, Zhang H, Kasuya H, Li Y, Matsukawa T, et al. JAK-STAT-activated, fratricide-resistant CAR-T cells targeting membrane-bound TNF effectively treat AML and solid tumors. *J Immunother Cancer* 2025;13:e011067. PUBMED | CROSSREF

89. Adachi Y, Terakura S, Osaki M, Okuno Y, Sato Y, Sagou K, Takeuchi Y, Yokota H, Imai K, Steinberger P, et al. Cullin-5 deficiency promotes chimeric antigen receptor T cell effector functions potentially via the modulation of JAK/STAT signaling pathway. *Nat Commun* 2024;15:10376. PUBMED | CROSSREF

90. Bai Z, Lundh S, Kim D, Woodhouse S, Barrett DM, Myers RM, Grupp SA, Maus MV, June CH, Camara PG, et al. Single-cell multiomics dissection of basal and antigen-specific activation states of CD19-targeted CAR T cells. *J Immunother Cancer* 2021;9:e002328. PUBMED | CROSSREF

91. Hombach AA, Geumann U, Günther C, Hermann FG, Abken H. IL7-IL12 engineered mesenchymal stem cells (MSCs) improve a CAR T cell attack against colorectal cancer cells. *Cells* 2020;9:873. PUBMED | CROSSREF

92. Roybal KT, Rupp LJ, Morsut L, Walker WJ, McNally KA, Park JS, Lim WA. Precision tumor recognition by T cells with combinatorial antigen-sensing circuits. *Cell* 2016;164:770-779. PUBMED | CROSSREF

93. Choe JH, Watchmaker PB, Simic MS, Gilbert RD, Li AW, Krasnow NA, Downey KM, Yu W, Carrera DA, Celli A, et al. SynNotch-CAR T cells overcome challenges of specificity, heterogeneity, and persistence in treating glioblastoma. *Sci Transl Med* 2021;13:eabe7378. PUBMED | CROSSREF

94. Rosado-Sánchez I, Haque M, Salim K, Speck M, Fung VC, Boardman DA, Mojibian M, Raimondi G, Levings MK. Tregs integrate native and CAR-mediated costimulatory signals for control of allograft rejection. *JCI Insight* 2023;8:e167215. PUBMED | CROSSREF

95. Frikeche J, David M, Mouska X, Treguer D, Cui Y, Rouquier S, Lecorgne E, Proics E, Fall PB, Lafon A, et al. MOG-specific CAR Tregs: a novel approach to treat multiple sclerosis. *J Neuroinflammation* 2024;21:268. PUBMED | CROSSREF

96. Durgam SS, Rosado-Sánchez I, Yin D, Speck M, Mojibian M, Sayin I, Hynes GE, Alegre ML, Levings MK, Chong AS. CAR Treg synergy with anti-CD154 promotes infectious tolerance and dictates allogeneic heart transplant acceptance. *JCI Insight* 2025;10:e188624. PUBMED | CROSSREF

97. Liu E, Marin D, Banerjee P, Macapinlac HA, Thompson P, Basar R, Nassif Kerbauy L, Overman B, Thall P, Kaplan M, et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. *N Engl J Med* 2020;382:545-553. PUBMED | CROSSREF

98. Jung D, Choi E, Jeoung YH, Lee J, Gong EY, Jo SG, Noh K, Ha K, Wee G, Kim H, et al. Nanobody-based CAR NK cells for possible immunotherapy of mesothelin^{+} tumors. *Immune Netw* 2025;25:e23. PUBMED | CROSSREF

99. Pan K, Farrukh H, Chittepu VCSR, Xu H, Pan CX, Zhu Z. CAR race to cancer immunotherapy: from CAR T, CAR NK to CAR macrophage therapy. *J Exp Clin Cancer Res* 2022;41:119. PUBMED | CROSSREF