Cell Reports Medicine

CellPress
OPEN ACCESS

Review

CAR-T cells in solid tumors: Challenges and breakthroughs

Giulia Escobar,[1,2,3] Trisha R. Berger,[1,2] and Marcela V. Maus[1,2,3,*]
[1]Krantz Family Center for Cancer Research, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA
[2]Cellular Immunotherapy Program, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA
[3]Harvard Medical School, Boston, MA, USA
*Correspondence: mvmaus@mgh.harvard.edu
https://doi.org/10.1016/j.xcrm.2025.102353

SUMMARY

Chimeric antigen receptor (CAR)-T cell therapy has revolutionized the treatment of hematologic malignancies, but its efficacy in solid tumors is limited by several challenges. Key obstacles include insufficient CAR-T cell trafficking to tumors, limited expansion and persistence, tumor relapse due to antigen loss or heterogeneity, and an immunosuppressive tumor microenvironment (TME) that dampens CAR-T cell functions. In this review, we discuss insights from recent successful clinical trials in advanced solid tumors and highlight groundbreaking strategies integrating synthetic biology and gene engineering to enhance CAR-T cell fitness, potency, and persistence, activate host immunity, reprogram the TME, and enable multi-antigen targeting. We examine strengths and weaknesses of current preclinical models for assessing the efficacy and safety of CAR-T cell therapies, including human xenografts in immunodeficient mice and humanized or syngeneic models. The array of cutting-edge approaches employed in next-generation CAR-T cell therapies is expected to transform the treatment landscape of solid tumors.

CAR-T CELL THERAPY FOR SOLID TUMORS

CAR-T cell therapy has revolutionized the treatment and prognosis of hematologic tumors, achieving remarkable, and sometimes curable, results in certain blood cancers. As a testament to this clinical success, seven CAR-T cell products have been approved by the US Food and Drug Administration (FDA). In contrast, there are no FDA-approved CAR-T cells for solid tumors despite two T cell-based therapies being recently approved: lifileucel, derived from tumor-infiltrating lymphocytes,[1] for advanced melanoma and afamitresgene autoleucel, a T cell receptor (TCR)-engineered therapy targeting melanoma-associated antigen 4 (MAGE-A4), for synovial cell sarcoma.[2,3]

Attempts to translate CAR-T cell therapy to solid tumors have yielded disappointing clinical results and revealed key challenges that constrain therapeutic efficacy, including the limited ability of CAR-T cells to traffic to and infiltrate the tumors. This limitation is primarily due to the lack of optimal chemokines and inflammatory signals released by the tumor, along with physical barriers, such as a dense extracellular matrix surrounding the tumor. Once inside the tumors, CAR-T cells encounter a hostile tumor microenvironment (TME), which diminishes the ability of engineered T cells to persist, expand, and exert cytotoxic functions. Lastly, tumors can quickly adapt to escape CAR-T cell recognition by antigen loss, mutation, or down-regulation or due to heterogeneous antigen expression.

This review discusses current strategies to overcome the key obstacles for CAR-T cell therapy in solid tumors. We examine recent successful clinical trials in advanced solid tumors and highlight strategies to enable multi-antigen targeting and enhance CAR-T cell trafficking. We also explore cutting-edge gene engineering and armoring approaches to increase CAR-T cell fitness and efficacy and counteract the immunosuppressive TME. Furthermore, we evaluate the strengths and limitations of current preclinical models to test these modifications. Leveraging this knowledge, we provide a roadmap for future CAR-T cell therapies.

PROMISING OUTCOMES FROM RECENT CLINICAL TRIALS OF CAR-T CELLS IN SOLID TUMORS: WHAT WE CAN LEARN FROM THEM

After a decade of discouraging clinical outcomes using CAR-T cells against a variety of solid tumors, recent clinical trials have yielded encouraging results in brain, gastric, liver, sarcoma, neuroblastoma, pleural, and claudin6-positive (CLDN6) tumors (Table S1).

In the context of brain tumors, which are typically refractory to most treatments, significant progress has been made, with three CAR-T cell products published in 2024. One study demonstrated the safety of repetitive local infusions of interleukin (IL)-13Rα2-CAR-T cells and reported promising efficacy data.[4] They also observed an increase in median survival from 7.7 to 10.2 months for the clinical arm treated with CAR-T cells manufactured from $CD62L^+$ naive, stem cell memory, and central memory T (Tn/mem) cells as the starting populations.[4] A second study, published by our group, used epidermal growth factor receptor

(EGFR)-vIII-CAR-T cells engineered to secrete a T cell-engaging antibody molecule (TEAM) targeting CD3 on T cells and the wild-type EGFR antigen on tumor cells (i.e., CARv3-TEAM-E T cells)[5] to improve tumor targeting. We reported dramatic and rapid (within 24–48 h) anti-tumor responses in three patients with glioblastoma receiving a single intraventricular infusion of CARv3-TEAM-E T cells.[6] Notably, one patient remained in prolonged remission at the 104-day follow-up, while the remaining two responses were transient. Lastly, a clinical trial of intrathecal administration of CAR-T cells targeting IL-13Rα2 and EGFR reported tumor volume reductions in 8/13 patients, with one qualifying as a partial response.[7] Although most reductions did not meet the criteria for an objective radiographic response, CAR-T cells and cytokines were detected in the cerebrospinal fluid, suggesting bioactivity. One patient had stable disease ongoing for 16 months. Importantly, all three studies demonstrated the safety and feasibility of delivering CAR-T cells into the central nervous system (CNS), with only one dose-limiting toxicity (DLT) occurring across all patients.

Significant progress has also been reported for pediatric patients with glioma. A phase 1 clinical study of disialoganglioside (GD2)-CAR-T cells reported clinical improvements and tumor volume reductions in 9/11 patients given two consecutive doses (intravenous and intracerebroventricular), with one patient achieving complete remission lasting 30 months.[8] No DLTs were observed at the lower doses, but high-grade cytokine release syndrome (CRS) occurred in three patients treated with the highest dose. Another trial with repetitive intracerebroventricular administrations of B7-H3-CAR-T cells reported grade 1–2 adverse events, with only one grade 4 event, suggesting that repetitive infusions of CAR-T cell are well tolerated. MRI imaging indicated 1 partial response (6%) and 15 stable diseases (83%), with 3 patients still alive at more than 40 months from diagnosis.[9]

Compared to previous trials, key features of these clinical studies include locoregional administration of CAR-T cells,[4,6–9] repetitive CAR-T cell infusions,[4,6–9] and multi-antigen targeting.[6,7] Locoregional CAR-T cell delivery associated with rapid anti-tumor responses and the detection of high concentrations of engineered T cells in the cerebrospinal fluid, which was accompanied with early-onset tumor inflammation-associated neurotoxicity. Administering multiple doses of CAR-T cells and multi-antigen targeting further ensured more effective and sustained anti-tumor responses and was shown to be safe in these initial trials. While all studies demonstrated the feasibility of manufacturing enough CAR-T cells for their target doses, the B7-H3 trial uniquely used a methotrexate-resistant dihydrofolate reductase (DHFR) mutein to enrich CAR-T cells *ex vivo* and successfully generated cells from a single apheresis in all patients, including one who received an impressive 81 doses (3×10^9 CAR-T cells).[9] The study with IL-13Rα2-CAR-T cells also reported increased CAR-T cell numbers and improved survival when starting from $CD62L^+$ Tn/mem cells. Therefore, optimizing CAR-T cell manufacturing will contribute to the success of multi-dose trials.

For solid tumors not involving the brain, two studies of claudin18.2-CAR-T cells in gastrointestinal cancer[10] and GD2-CAR-T cells in patients with neuroblastoma[11] reached promising outcomes (Table S1). Both trials employed higher doses of CAR-T cells and reported higher levels of circulating CAR-T cells that persisted longer as compared to previous trials in solid tumors. Notably, the GD2-CAR-T cells used a third-generation CAR incorporating both CD28 and a 4-1BB co-stimulatory domain and reported a median CAR-T cell persistence of 3 months, with a maximal persistence of more than 2 years in two patients. In both trials, the extended CAR-T cell persistence correlated, albeit not significantly, with better responses.

Another approach to improve CAR-T cell engraftment is the use of lymphodepleting chemotherapy. In pediatric and adolescent patients with sarcoma, multiple infusions of human epidermal growth factor receptor 2 (HER2)-CAR-T cells in combination with lymphodepletion resulted in 50% disease control, including complete responses in three patients.[12] Lymphodepletion associated with better CAR-T cell expansion compared to historical controls receiving the same dose of CAR-T cells in the absence of chemotherapy.[12,13] This approach was also safe, despite the known risk of on-target off-tumor toxicity previously observed with HER2-CAR-T cells.[14] Manageable grade 1–2 CRS was observed in 79% of patients, with grade 3–4 CRS observed only in the two patients receiving the highest CAR-T cell dose. The lack of toxicities could reflect the short persistence of circulating CAR-T cells and the use of the FRP5-derived single-chain fragment variable (scFv) as the antigen-binding domain of the CAR instead of the trastuzumab-derived scFv.

Others have used vaccination to improve CAR-T cell persistence and expansion. This can be achieved through direct engagement of the CAR molecule[15] or the use of mRNA vaccines, which are taken up and translated by dendritic cells (DCs), resulting in the surface expression of the encoded protein. A major advantage of vaccination is that CAR-T cells will encounter their cognate antigen in secondary lymphoid organs, where co-stimulation is maximal and immunosuppression is minimal, as compared to the TME of solid tumors. BioNTech has adopted its proprietary RNA-lipoplex (LPX) delivery system to deliver mRNAs encoding for mutated neo-antigens or endogenous self-antigens to the lymphoid compartment.[16] Interim results of the ongoing phase 1/2 clinical trial combining claudin-6 (CLDN6)-CAR-T cells with a CLDN6-LPX vaccine (CARVac) reported manageable side effects and encouraging efficacy (Table S1).[17] Together, results from preclinical and clinical studies suggest that the combination of CAR-T cells with booster vaccines can significantly expand and prolong the survival of CAR-T cells. A limitation to the broad applicability of the CARVac approach is the difficulty in identifying suitable antigens that can be safely targeted without increasing the risk of on-target off-tumor toxicity or the induction of auto-reactive antibodies and T cells. Indeed, many antigens expressed on solid tumors and targeted by CAR-T cells are also present, albeit at lower levels, on normal vital tissues.

ADDRESSING TUMOR ANTIGEN HETEROGENEITY

Despite these promising clinical results, there is still a need for improved tumor targeting by CAR-T cells. One way to achieve this is by targeting multiple tumor antigens. Most solid tumors do not uniformly express tumor antigens, leading to the selection

of antigen-negative clones that escape CAR-T cell recognition. Tumors can also evolve to down-regulate or lose the targeted antigen, resulting in the relapse of antigen-low or negative tumors.[18] Targeting multiple antigens could lead to a more effective anti-tumor response, while minimizing the risk of antigen escape. Bi-specific CAR-T cells have been successfully tested in clinical trials for hematologic malignancies.[19–21] They can be achieved by infusing a mixed population of single-targeting CAR-T cells (pooled CAR), by expressing two different CARs on the surface of the same T cell (dual-targeting CAR), or by linking two antigen-binding domains in a single CAR molecule (TanCAR). The use of pooled CAR-T cells is demanding in terms of costs and regulatory requirements, because it entails the manufacturing of two separate products. Additionally, this strategy has proven less effective compared to dual-targeting CARs, possibly due to competition and the preferential expansion of one CAR-T cell population over the other in the TME.[22]

Dual-targeting CAR-T cells can be generated by expressing two CARs from a bicistronic vector or by co-transducing T cells with two different CAR-encoding vectors. However, similar to the pooled CAR-T cell strategy, co-transduction can result in a mixed population of cells and preferential selection of one CAR-T cell population over the other *in vivo*. Dual-targeting EGFR/IL-13Rα2 CAR-T cells showed promising results in patients with glioblastoma.[7] In preclinical models, unique dual-targeting designs have been used to improve CAR-T cell efficacy. For example, dual-targeting GD2/B7-H3 CAR-T cells with a shared CD3z domain and split CD28/4-1BB co-stimulatory domains provided improved CAR-T cell metabolic fitness and superior anti-tumor activity and prevented tumor escape against a neuroblastoma xenograft model.[23]

TanCARs targeting EGFRvIII/IL-13Rα2[24] or HER2/IL-13Rα2[25] have successfully addressed tumor antigen heterogeneity in preclinical models of glioblastoma. A major drawback of TanCARs is the need to optimize the CAR structure for the selected tumor antigen pair. This optimization aims to position the scFvs for optimal binding of the antigen, minimize steric hindrance, and allow proper formation of a functional immunological synapse. Although current TanCARs have relied on the use of two scFvs linked together, camelid nanobodies (variable domain of a heavy-chain, VHHs) could offer a promising alternative. VHHs are smaller and more stable than scFvs, making them easier to incorporate into a dual-targeting design. Additionally, while the light and heavy chains of two scFvs could potentially mispair during protein assembly, no such risk exists with VHHs, which consist of a single heavy chain. The adoption of VHHs could be especially relevant for the design of tri-specific CAR-T cells, which incorporate three antigen-binding domains.

An alternative strategy to address tumor antigen heterogeneity is through engineering CAR-T cells to secrete bi-specific antibodies against additional tumor antigens.[5,26] An advantage of this strategy is that the secreted T cell engager (TCE) can redirect the cytotoxicity of both tumor-infiltrating CAR^+ and bystander (CAR negative) T cells, thereby enhancing therapeutic efficacy. The TCE is also capable of eliciting degranulation of T regulatory cells, which can be abundant in the TME, enabling additional help in killing the tumor.[5] Additionally, the preferential accumulation of CAR-T cells in the tumor and the short half-life of TCE molecules in the bloodstream can help minimizing on-target off-tumor toxicity when the targeted antigen is also expressed in healthy vital tissues.[5] As described earlier, this strategy has been recently tested in a phase 1 clinical trial in patients with glioblastoma resulting in significant anti-tumor responses.[6] In the future, engineering CAR-T cells to secrete tri-specific TCE could further expand tumor antigen targeting and help addressing tumor escape.

Lastly, an alternative approach to address tumor heterogeneity is modular CAR-T cells, where tumor cell killing is dependent on an adapter molecule. In principle, these approaches are highly versatile, allowing for control of CAR-T cell activity by simply switching or modulating the availability of the adapter molecule(s) without the need to re-engineer the T cells. A wide array of adapter-mediated CARs has been developed, including biotin-, fluorescein isothiocyanate (FITC)-, and general control nondepressible-4 (GCN4)-binding CARs, where tumor cell killing relies on the administration of biotinylated, FITC-labeled, and GCN4-tagged tumor-targeting antibodies, respectively.[27] Two major limitations of these first adapter-regulated CARs are the potential immunogenicity of the adapter recognition domain and the need to empirically test the size and geometry of each tumor-targeting adapter molecule to achieve optimal synapse formation and CAR-T cell activation.[27] Improved versions of adapter-regulated CARs include universal CARs (UniCAR) and split, universal, and programmable (SUPRA) CARs. UniCARs are composed of a 5B9-targeting scFv that constitutes the extracellular domain of the CAR and is recognized by an E5B9 peptide-tagged tumor-targeting adapter molecule,[28] which is non-immunogenic. This peptide has been successfully linked to various tumor-targeting adapter molecules, including antibodies, affibodies, soluble TCRs, and small molecules.[29] In preclinical models, UniCARs have been shown to address tumor heterogeneity, minimize antigen escape, and prevent CAR-T cell toxicity.[29] However, it remains to be determined whether UniCARs will be effective in patients. Two clinical trials are currently ongoing (NCT04230265 and NCT04633148).

SUPRA CARs incorporate an inert leucine zipper receptor (zipCAR), which can bind to a tumor-targeting leucine zipper adapter scFv (zipFv). Administering multiple zipFv combinations can be used as a versatile OR gate strategy to prevent cancer evasion.[30] Additionally, using zipFvs at different doses or affinities can regulate CAR-T cell signaling and cytotoxicity, with lower doses or reduced affinity zipFvs leading to decreased interferon-γ (IFN-γ) release by the engineered T cells.[30] CAR-T cell activation can also be dampened by combining leucine zipper pairs with different affinities and administering a zipFv inhibitor that sequesters activating zipFvs.[30] Lastly, the SUPRA CAR system can be integrated with AND gate Boolean logic by combining two mutually exclusive zipCAR/zipFv pairs into a split CAR design where binding to both antigens on the same tumor cell is required to mediate functional T cell signaling and tumor cell killing.[30] While multi-antigen targeting zipFvs are meant to address tumor antigen heterogeneity, the split SUPRA CARs are meant to improve tumor antigen specificity, which reduces on-target off-tumor toxicity at the risk of tumor antigen escape upon loss of either antigen.

On-target off-tumor toxicity is a major concern with multi-antigen targeting. Identifying antigens uniquely expressed in solid

tumors but absent in normal tissues remains a key challenge for safe and effective CAR-T cell therapy and is an area of ongoing investigation. Recent efforts have also explored expanding the antigen repertoire to peptide-major histocompatibility complexes (MHCs) derived from intracellular proteins, viral antigens, and neo-antigens.[31–35] To improve the safety of CAR-T cells, kill switches have been incorporated in the CAR design to selectively eliminate the engineered cells upon treatment with a switch activator. Examples include inducible caspase-9 (iCasp9)[36] or herpes simplex virus (HSV) tyrosine kinase,[37] both of which are activated with rimiducid or ganciclovir, or inactive receptors, such as truncated EGFR,[38] truncated HER2,[39] and the CD20 epitope,[40] which are recognized by commercially available depleting antibodies (cetuximab, trastuzumab, and rituximab). These strategies have been administered in the clinic with mixed results, identifying limitations to their use. For instance, in two clinical trials, the iCasp9 approach successfully diminished CAR-T cells in patients treated with rimiducid[11,41]; however, in one case, the effect was transient since the CAR-T cells re-expanded after 6 weeks and remained detectable for 30 months after infusion.[11] This could be due to the preferential elimination of T cells with high, but not low, levels of iCasp9 expression. Another limitation to iCasp9 is that the dimerizing drug rimiducid is not commercially available. The viral-derived HSV tyrosine kinase kill switch is limited by its immunogenicity, reliance on cell proliferation for activity, delayed kinetics, potential off-target toxicity of ganciclovir, and interference with antiviral treatments.[42] A major limitation in the use of EGFR-, HER2-, and CD20-based safety switches is that cell clearance relies on the efficiency of antibody-dependent cellular cytotoxicity or complement-mediated lysis, which may result in partial or inconsistent cell elimination, especially in immunocompromised patients. Our group incorporated a truncated EGFR (tEGFR) into CD37-targeting CAR-T cells, which were evaluated in a first-in-human phase 1 trial in patients with CD37-positive hematologic malignancies (NCT04136275). Two patients with prolonged, severe pancytopenia received cetuximab in an attempt to deplete CAR-37 T cells.[43] Although CAR-T cells were no longer detectable by flow cytometry, peripheral blood vector copy numbers remained unchanged, suggesting that cetuximab bound to tEGFR and masked its detection without inducing cell death, likely due to the absence of functional myeloid effector cells in these patients. In contrast, two subsequent trials using tEGFR-expressing CAR-T cells targeting Claudin18.2 in pancreatic cancer or CD5 in T cell lymphomas have demonstrated successful depletion of CAR-T cells upon cetuximab treatment in patients.[44,45]

An alternative strategy to improve tumor targeting and minimize on-target off-tumor toxicity is using the SynNotch technology,[46] or its improved and fully humanized version, SNIPR,[47] to regulate CAR expression. This strategy uses a synthetic notch receptor against a priming antigen, which has specific but heterogeneous expression in tumors. Recognition of the priming antigen induces the expression of a conventional CAR directed against an antigen with homogeneous but not entirely tumor-restricted expression. SynNotch-CARs improved safety and therapeutic efficacy in preclinical models where tumors and the susceptible vital tissues were not co-localized.[46,48,49] Notably, the SNIPR technology also responds to soluble cues, opening the possibility to regulate CAR expression upon recognition of exogenously administered or tumor-restricted soluble molecules.[50] SynNotch-CARs are currently in clinical testing (NCT06186401). Additional emerging tunable safety technologies include transcriptionally regulatable CAR expression systems, split CAR designs, protease-activatable CARs, drug-inducible protein degradation platforms, logic-gated CARs, and transient CAR expression via mRNA electroporation.[42]

ENHANCING TRAFFICKING AND INFILTRATION OF CAR-T CELLS INTO SOLID TUMORS

Another key factor impacting therapeutic efficacy is the ability of CAR-T cells to traffic to and infiltrate solid tumors. Their entrance is limited by the lack of optimal inflammatory and chemokine signals secreted by cancer cells, the abnormal tumor vasculature, and the presence of physical barriers such as a dense stroma surrounding the tumor cells. Additionally, once infused intravenously in patients, CAR-T cells may home to lymphoid organs, where they will not encounter the cognate antigen and cannot be primed by DCs, potentially leading to reduced CAR-T cell proliferation, differentiation into effector T cells, and decreased trafficking to the tumor site. One strategy to address limited CAR-T cell trafficking is locoregional administration of CAR-T cells. In preclinical models of brain tumors, locoregional delivery achieved effective anti-tumor responses with a 10-fold lower dose of CAR-T cells.[51–53] In clinical trials, local administration[4,6,7] of CAR-T cells significantly improved therapeutic efficacy in patients with glioblastoma compared to intravenous administration.[54] Similarly, administration of CAR-T cells in the pleural cavity achieved promising results in combination with PD1-blockade therapy in patients with pleural tumors.[55] CAR-T cells have also been locally delivered in a fibrin gel within the tumor cavity to target minimal residual disease post incomplete surgery in preclinical models of breast cancer, showing prolonged intra-tumoral CAR-T cell persistence and reduced incidence of on-target off-tumor lung toxicity compared to CAR-T cells injected directly into the tumor cavity.[56] This strategy has also been shown to improve therapeutic efficacy in a preclinical model of partially resected glioblastoma[57] and is currently being tested in patients with metastatic triple-negative breast cancer (NCT05623488).

Alternative strategies designed to improve CAR-T cell trafficking and infiltration rely on the overexpression of chemokines, chemokine receptors, or adhesion molecules on CAR-T cells and have shown promising results in preclinical studies.[58] These include overexpression of C-X-C chemokine receptor 2 (CXCR2), C-C chemokine receptor 2b (CCR2b), CXCR6, CXCR5, C-X3-C chemokine receptor 1 (CX3CR1), CCR4, C-C chemokine ligand 2 (CCL2), and CCR9 on CAR-T cells. A major challenge to this approach is finding a chemokine axis that is restricted to tumors, which is critical to prevent unwanted T cell migration toward healthy tissues. Additionally, chronic expression of chemokines or chemokine receptors can result in chronic calcium signaling and subsequent T cell anergy.[58] Some of these strategies are under clinical investigation in multiple myeloma and non-small cell lung cancer (NCT04727008, NCT05060796).

CAR-T cell infiltration is also limited by the presence of a dense stroma surrounding solid tumors. To address this, two studies have targeted fibroblast-activating protein (FAP)-expressing cancer-associated fibroblasts (CAFs), which are primarily responsible for the deposition of this extracellular matrix. One strategy employed FAP-specific CAR-T cells, which rendered pancreatic tumors more susceptible to being killed by mesothelin-targeting CAR-T cells administered as a subsequent treatment.[59] The second strategy, performed in our lab, engineered mesothelin-targeting CAR-T cells to secrete a CD3/FAP TCE, efficiently redirecting CAR$^+$ and bystander T cells against FAP-expressing CAFs.[60] Other groups have engineered CAR-T cells to express enzymes that break down the extracellular matrix, such as heparinase.[61] Tumor-infiltrating stromal cells, macrophages, and myeloid-derived suppressor cells can also directly suppress CAR-T cell functions and drive therapeutic resistance, as recently shown in patients with glioblastoma and aggressive B cell lymphoma.[62,63] This is an emerging area of ongoing investigation.

GENE EDITING OF CAR-T CELLS TO IMPROVE FITNESS, PERSISTENCE, AND FUNCTIONALITY

In tumors, adoptively transferred CAR-T cells can progressively acquire an exhausted or dysfunctional phenotype due to chronic antigen exposure, nutrient deprivation, low pH, hypoxia, and immunosuppressive cues in the TME. Exhausted T cells are characterized by distinct transcriptional and epigenetic profiles, elevated and sustained expression of checkpoint inhibitory molecules, and loss of effector functions.[64] CAR-T cell dysfunction can either be acquired at the tumor site or be pre-existent when CAR-T cells are manufactured from T cells of heavily pre-treated patients or patients with a high tumor burden. Accordingly, variability in the chromatin landscape in the CAR-T cell product at the time of infusion is significantly associated with the therapeutic outcome.[65] The importance of epigenetics in regulating CAR-T cell function became evident when integration of a CAR-encoding lentiviral vector caused a loss-of-function mutation in the ten-eleven translocation 2 (*TET2*) gene in combination with a naturally occurring mutation in the patient's other *TET2* allele. The mutation resulted in massive CAR-T cell expansion and induction of a complete response for the chronic lymphocytic leukemia (CLL) patient.[66] Subsequent studies purposefully inducing this mutation in CAR-T cells revealed that the bi-allelic loss of *TET2* improved tumor control in mice, but it also led to antigen-independent uncontrolled clonal expansion, which was dependent on sustained basic leucine zipper ATF-like transcription factor 3 (*BATF3*) expression, raising potential safety concerns.[67]

Several strategies have aimed to increase CAR-T cell fitness by modulating the epigenome, transcriptome, and phenotype of engineered T cells. To achieve these modifications, clustered regularly interspaced palindromic repeats (CRISPR)-Cas9 and base editing technologies have been used to introduce single or multiplex gene editing. CAR-T cells with knockout of epigenetic regulators, such as DNA methyltransferase 3 alpha (*DNMT3A*),[68] suppressor of variegation 3-9 homolog 1 (*SUV39H1*),[69] thymocyte selection-associated high mobility group box protein (*TOX*), and *TOX2*[70]; transcriptional regulators, such as nuclear receptor subfamily 4 group A2 (*NR4A2*),[71] inhibitor of DNA binding 3 (*ID3*),[72] and SRY-box transcription factor 4 (*SOX4*)[72]; and inhibitory receptors, such as cytotoxic T lymphocyte-associated protein 4 (*CTLA4*),[73] programmed cell death 1 (*PDCD1*),[74,75] and lymphocyte activation gene 3 (*LAG3*),[76] have improved CAR-T cell antitumor activity in preclinical mouse models. In human and mouse TCR-engineered T cells, additional sex combs like 1 (*ASXL1*) knockout enhanced T cell longevity and anti-tumor efficacy and synergized with PDL1 blockade therapy, highlighting *ASXL1* as a potential target to improve CAR-T cell fitness.[77] It remains to be determined whether these gene modifications will also be beneficial in patients. Indeed, knocking out *PDCD1* (encoding programmed cell death protein 1, PD1) improved efficacy in preclinical studies but failed to confer clinical benefit in patients treated with New York esophageal squamous cell carcinoma 1 (NY-ESO-1)-targeting TCR-engineered T cells.[78] This result was not completely unexpected since studies in mice have revealed the importance of fine-tuning PD1 expression to counteract excessive TCR signaling, prevent immunopathology, and allow optimal memory formation.[79] CAR-T cell fitness can also be improved by overexpressing transcriptional regulators such as JUN,[80] forkhead box O1 (FOXO1),[81,82] and basic leucine zipper ATF-like transcription factor (BATF).[83] Interestingly, the choice of which starting T cell population is genetically manipulated significantly influences the outcome, due to functional, transcriptional, and epigenetic differences between each T cell subset. For example, overexpression of the transcription factor runt-related transcription factor 2 (*RUNX2*) enhanced CAR-T cell potency and reduced exhaustion in naive but not memory T cells.[84] Thus, prior T cell antigen experience should be considered when designing these gene-editing approaches.

Besides targeting *ad hoc* genes, large-scale *in vitro* CRISPR screens systematically identify genes that, when expressed or deleted in T cells, enhance their proliferation, persistence, cytokine production, cytotoxicity, or metabolism or render them resistant to exhaustion (Table S2).[85–96] These *in vitro* CRISPR screens are typically performed by culturing gene-edited T cells under immunosuppressive conditions or through single or repetitive rounds of antigen stimulation to induce exhaustion (Figure 1). Under immunosuppressive conditions, *RASA2* was identified as a negative regulator of T cell proliferation and cytotoxicity, and its deletion improved CAR-T cell efficacy in a B cell leukemia model.[89] Similarly, mediator complex subunit 12 (*MED12*) deletion improved CAR-T cell fitness and anti-tumor efficacy *in vivo*.[92] Screening of naturally occurring mutations commonly found in T cell neoplasms also led to the expression of caspase recruitment domain family member 11(*CARD11*)-phosphoinositide-3-kinase regulatory subunit 3 (*PIK3R3*) gene fusion in CAR-T cells, which improved therapeutic efficacy.[97] More recently, base editing screens have been used *in vitro* in primary human T cells to identify protein domains and amino acid residues that regulate T cell activation and cytokine production.[98] These gain- or loss-of-function mutations were able to tune T cell functions, identifying another method that may be employed to improve immunotherapies.

In addition to *in vitro* screens, *in vivo* CRISPR screens will be important to identify key regulators of CAR-T cell fitness in a

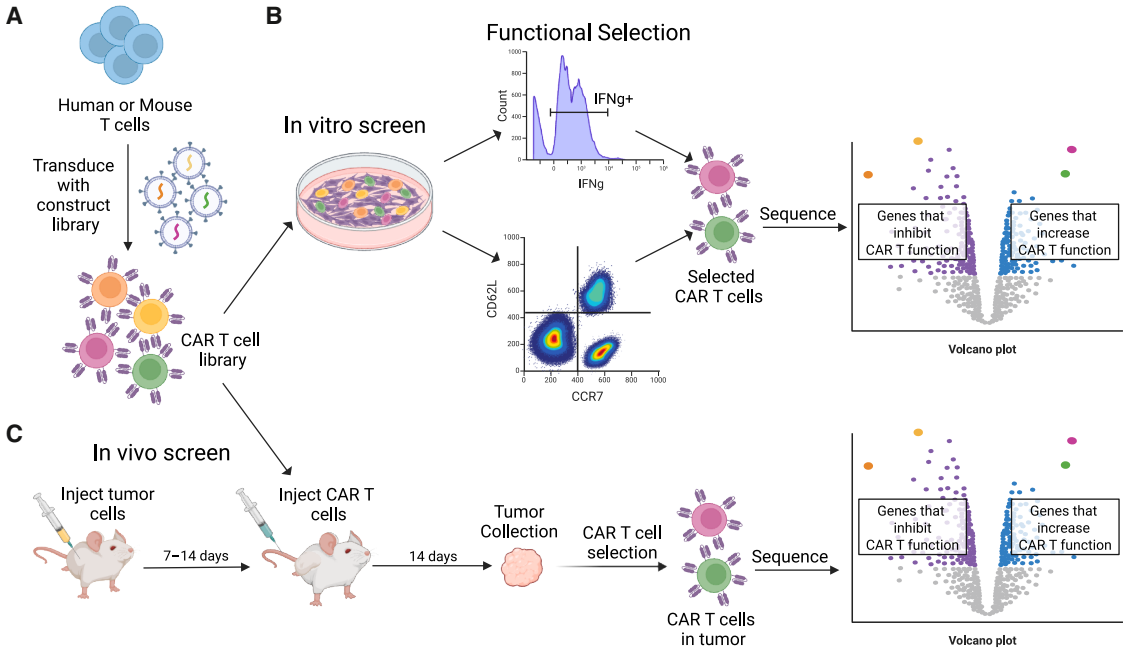


Figure 1. CRISPR screen methodology for CAR-T cells *in vitro* and *in vivo*

(A) A library of CRISPR-modified CAR-T cells is prepared by transfection with one or more vector containing the CAR and guide RNAs targeting the entire genome or select genes. Each cell typically receives a single guide, creating a unique perturbation. Multiple guides per gene are included to increase confidence in gene-level hits during analysis.

(B) *In vitro* screens involve co-culturing CAR-T cells with tumor cells and applying a functional selection pressure (i.e., cytokine secretion after chronic stimulation or phenotype changes after defined culture conditions, etc.). Enriched or depleted gene perturbations are identified by comparing post-selection samples to the input library or to control conditions via sequencing.

(C) *In vivo* screens involve injecting the CRISPR-modified CAR-T cell library into tumor-bearing mice. After a defined period, CAR-T cells are harvested from the tumors and sequenced to identify genetic modifications that are enriched or depleted relative to the injected library, revealing gene perturbations that influence CAR-T cell function *in vivo*.

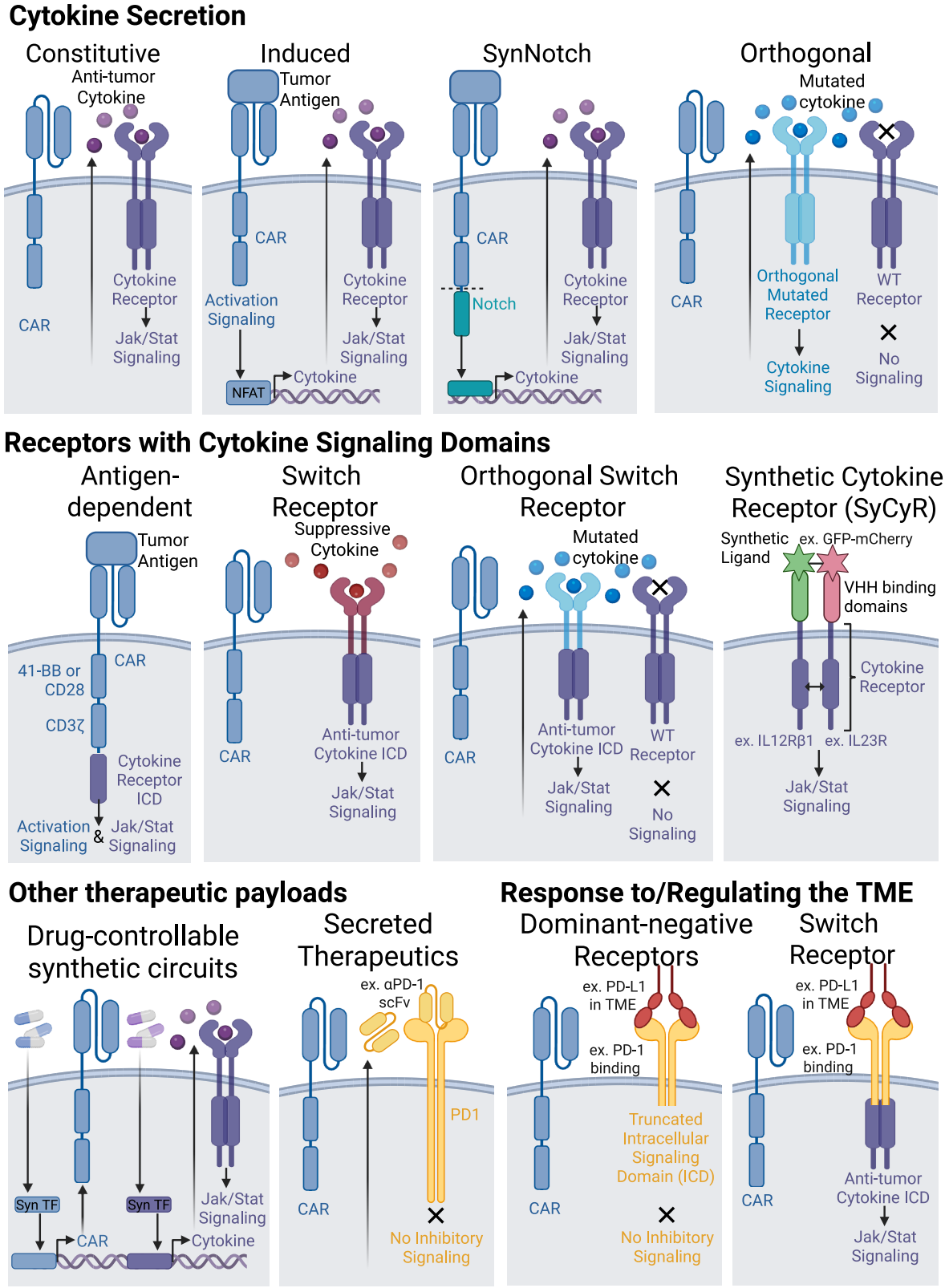
setting that better mimics the physiological conditions found in patients. So far, *in vivo* CRISPR screens have mainly been performed using TCR-engineered mouse T cells in syngeneic mice (Table S2; Figure 1),[99–104] and some of the candidate genes identified, such as Regnase-1,[101] have also been validated in CAR-T cells. Interestingly, double deletion of Roquin-1 and Regnase-1 was more effective at enhancing CAR-T cell activity as compared to the single knockouts but was accompanied by the development of a lymphoproliferative syndrome and toxicity in some mice.[105] Our group performed an *in vivo* loss-of-function CRISPR screen in human B cell maturation antigen (BCMA)-targeting CAR-T cells using a library of selected genes known to be involved in normal T cell function. We identified a novel gene target (cyclin-dependent kinase inhibitor 1B, *CDKN1B*) that, when knocked out, increased CAR-T cell persistence and anti-tumor function *in vivo.*[106] Importantly, the genes identified as top hits *in vitro* varied from the hits enriched *in vivo*, demonstrating the need for *in vivo* screening to better identify targets with therapeutic potential.

Reciprocal CRISPR-screens have also identified gene dependencies in both tumor and CAR-T cells. For instance, deletion of ikaros family zinc finger 2 (*IKZF2*) and transducin-like enhancer protein 4 (*TLE4*) in CAR-T cells or of v-rel avian reticuloendotheliosis viral oncogene homolog A (*RELA*) and nuclear protein localization protein 4 homolog (*NPLOC4*) in glioblastoma stem cells improved tumor sensitivity to CAR-T cell killing and therapeutic efficacy.[86] Similarly, our group discovered that an intact interferon (IFN)-γ receptor signaling pathway in tumor cells is requisite for effective CAR-T cell killing in solid but not hematologic tumors.[107] Collectively, these studies emphasize that effective CAR-T cell killing and the gene dependencies for optimal CAR-T cell potency are influenced by the tumor type.

To efficiently target multiple genes at a time, the multiplexed effector guide arrays (MEGA) platform allows for multiplex gene knockout. This platform employs CRISPR-Cas13d, which has RNA-guided RNA endonuclease activity, allowing regulation of the T cell transcriptome without cutting the genome and potentially improving the safety of gene editing.[108] Further regulation of Cas13d using a drug-regulated version allowed T cell perturbations upon trimethoprim administration, an approach that could be relevant to fine-tune the expression of genes whose constitutive deletion is detrimental. Further studies are required to evaluate the feasibility of MEGA for clinical translation.

ARMORING CAR-T CELLS TO ENHANCE EFFICACY AND TARGET THE IMMUNOSUPPRESSIVE TME

Armoring CAR-T cells to secrete therapeutic payloads or express synthetic receptors that integrate new signals is another strategy



(legend on next page)

to target the immunosuppressive TME, enhance CAR-T cell potency and persistence, and activate endogenous immunity.

Cytokines have been incorporated into CAR-T cells as a separate transgene, which is either expressed constitutively or upon CAR-T cell activation, or by incorporating the cytokine signaling domain(s) in the CAR molecule (Figure 2). CAR-T cells engineered with a third signal derived from cytokine engagement are commonly referred to as T cells redirected for universal cytokine-mediated killing or as fourth-generation CAR-T cells. Many of these cytokines belong to the common gamma chain family, including IL-15, IL-7, IL-2, and IL-21. In preclinical models of lung cancer, neuroblastoma, and glioblastoma, IL-15-secreting GD2-CAR-T cells showed increased accumulation in the TME and enhanced cytotoxicity.[109,110] Importantly, recent clinical trials comparing conventional (NCT02932956 and NCT02905188) versus IL-15-secreting glypican-3 (GPC3)-CAR-T cells (NCT04377932 and NCT05103631) in patients with solid tumors reported increased expansion of the armored CAR-T cells, which resulted in increased disease control and anti-tumor responses (Table S1).[41] This improved efficacy was accompanied by higher incidence of CRS that required treatment with IL-1/IL-6 inhibitors or depletion of the engineered CAR-T cells via activation of the iCasp9 safety switch.

IL-7 promotes homeostatic T cell expansion and survival and supports memory T cell formation. Since exogenous IL-7 can lead to systemic toxicity, a constitutively active synthetic receptor composed of the extracellular domain of CD34 and the intracellular domain of the IL-7 receptor has been developed to safely mediate IL-7 signaling in the absence of IL-7.[111] This strategy is being tested in two clinical trials with GD2 CAR-T cells for solid tumors (NCT04099797 and NCT03635632). Similarly, our group has developed a membrane-bound drug-regulated degradable IL-7, which resulted in improved CAR-T cell expansion and persistence.[112]

IL-2 has also garnered significant interest due to its role in T cell activation and expansion, but its use in patients has been hindered by DLTs and the preferential expansion of T regulatory cells upon its systemic administration. To overcome these challenges, synthetic gene circuits based on the SynNotch technology have been developed and have demonstrated increased efficacy compared to systemic IL-2 delivery or CAR-T cell-secreted IL-2 (constitutive or activation dependent).[113] Alternative approaches include drug-regulated synthetic circuits that control the release of multiple payloads independently[114] or a synthetic split cytokine that becomes active only when the split components colocalize upon recognition of two tumor antigens.[115] To induce common gamma chain cytokine signaling, the truncated intracellular domain of the IL-2β receptor has been linked to the signal transducer and activator of transcription 3 (STAT3) binding motif and expressed in a second-generation CAR, resulting in superior *in vivo* persistence and anti-tumor effects.[116]

Recently, the development of orthogonal cytokine systems (engineered cytokines that bind exclusively to modified receptors) has allowed for restricted cytokine signaling to the engineered cells, thereby limiting systemic toxicity.[117] For example, an orthogonal human IL-2 that selectively activates an orthogonal IL-2β receptor expressed on CAR-T cells[118,119] is currently being tested in a phase 1 clinical trial of CD19-CAR-T cells (NCT05665062). Building on this system, the extracellular domain of an orthogonal IL-2 receptor has been fused to the intracellular signaling domain of the IL-9 receptor, creating a new orthogonally acting synthetic receptor.[120] Similarly, synthetic cytokine receptors (SyCyRs) incorporating the intracellular domain of IL-23 and IL-11/IL-6 receptors delivered canonical cytokine signals upon ligation of GFP-Cherry fusion proteins, providing the proof of principle that SyCyRs can deliver physiological cytokine signals in response to non-physiological ligands and opening the possibility to initiate the third signal in response to any artificial ligand.[121]

IL-21 is another common gamma chain cytokine that has gained interest due to its pleiotropic effects on multiple immune cells. In GPC3-targeting CAR-T cells, co-expression of IL-15 and IL-21 upon CAR activation enhanced expansion, persistence, and anti-tumor responses against human hepatocellular carcinoma and was superior to conventional GPC3-CAR-T or CAR-T cells engineered to secrete only one of the two cytokines.[122] A clinical trial is currently evaluating the safety and efficacy of this strategy in patients with liver cancer (NCT04715191 and NCT02932956). Interestingly, IL-10, a cytokine commonly known for its immunosuppressive functions, was also found to improve CAR-T cell fitness by reprogramming the T cell metabolism and preventing exhaustion when exogenously administered as an IL-10-Fc fusion protein[123] or when secreted by the CAR-T cells.[124]

Besides common gamma chain cytokines, pro-inflammatory cytokines including IL-12, IL-18, and IL-23 have been tested to enhance CAR-T cell effector functions and reprogram the TME. Early attempts to integrate constitutive IL-12 signaling into TCR- or CAR-engineered T cells demonstrated severe DLT, which could only be partly mitigated by restricting its release upon T cell activation. Indeed, the first trial employing

Figure 2. Armored CAR-T cell designs

Armored CAR-T cells incorporate an additional engineering strategy to enhance their anti-tumor function. Cytokines, such as common gamma chain (IL-15, IL-7, IL-2, or IL-21), pro-inflammatory (IL-12, IL-18, and IL-23), or cytokines/secreted factors that affect the TME (IL-36, IL-7, CCL9, and Flt3L), can be secreted constitutively, induced by CAR activation or induced by a SynNotch system to act on endogenous cytokine receptors. Orthogonal cytokines enhance the safety of potent cytokines with broad effects by expressing a mutated cytokine that only binds to an orthogonal mutated receptor (and not the wild-type [WT] receptor). Both are expressed along with the CAR-T to enhance function only in the CAR-T cells and not endogenous cells. Cytokine signaling can also be induced in CAR-T cells through expression of their intracellular signaling domains (ICDs) either in the CAR itself, leading to antigen-dependent signaling, or in chimeric receptors. The ICDs can be used to switch a suppressive signal to an activating signal (switch receptors and orthogonal switch receptors) or activate cytokine signaling in response to a fully synthetic molecule (SyCyR). CAR-T cells can also be engineered with synthetic transcription factors that, upon activation with a drug, bind to promoters that trigger the production of a CAR or a cytokine, thus controlling when CAR-T cells are activated. They can also be used to deliver therapeutic payloads, such as scFvs to block signaling of suppressive receptors. Finally, CAR-T cells can be armored against suppressive signals in the TME through dominant-negative or switch receptors that act as a sink for the suppressive signal or turn the suppressive signal into an activating signal.

TCR-engineered T cells secreting IL-12 upon activation (NFAT-regulated IL-12) resulted in objective responses in 63% of patients in the absence of exogenous IL-2 administration and with cell doses that were 10- to 100-fold lower than the doses commonly used in trials with conventional TILs. However, severe liver toxicity was observed in all patients, precluding further development.[125] New strategies to mitigate systemic IL-12 toxicity include the use of membrane-bound IL-12,[126] the incorporation of IL-12 in the CAR ectodomain,[127] the direct delivery of IL-12 in the TME,[128] or the site-specific insertion of the cytokine gene into endogenous loci that have tumor-restricted expression.[129]

IL-18 secretion also improved CD19-CAR-T cell persistence and enhanced the anti-tumor response through both autocrine effects and paracrine signaling on endogenous immune cells.[130,131] IL-18 signaling also enhanced CAR-T cell anti-tumor responses in syngeneic models of multiple myeloma expressing low levels of targeted tumor antigen[132] and small-cell lung cancer.[133] Three phase 1 clinical trials in patients with hematologic malignancies are underway (NCT04684563, NCT05989204, and NCT06287528).

IL-23 is a heterodimer composed of the IL-23α (p19) and IL-12β (p40) subunits, which promote T cell proliferation and survival. Since activated CAR-T cells up-regulate the IL-23 receptor and the IL-23α (p19) subunit, engineering CAR-T cells to express the p40 subunit enables selective IL 23-signaling upon CAR-T cell activation. This strategy enhanced CAR-T cell expansion and efficacy and showed reduced side effects as compared to CAR-T cells engineered to express either IL-15 or IL-18 cytokines.[134]

Another approach to target the immunosuppressive TME and enhance CAR-T cell efficacy was to armor CAR-T cells to locally deliver fms-like tyrosine kinase 3 ligand (FLT3L), which promoted the expansion of intra-tumoral DCs and the induction of endogenous immunity.[135] Similarly, incorporating IL-36 into CAR-T cells not only improved CAR-T cell expansion and persistence but also remodeled the TME and elicited secondary anti-tumor responses, which addressed antigen-negative tumor relapse.[136] CAR-T cells secreting IL-7 and CCL19 have displayed improved survival, enhanced endogenous T cell and DC recruitment, and stronger therapeutic responses against lung and pancreatic tumors.[137] This strategy is currently being tested in hematologic tumors (NCT03778346, NCT03929107, NCT04833504, and NCT05659628) and has shown promising preliminary safety and efficacy data in patients with hepatocellular, pancreatic, and ovarian carcinoma.[138] Together, strategies aimed at enhancing host immunity could address tumor antigen heterogeneity, ultimately improving CAR-T cell efficacy against solid tumors.

CAR-T cells have also been engineered to secrete therapeutic payloads or to express synthetic receptors that provide additional co-stimulation (chimeric co-stimulatory receptors) or convert inhibitory or neutral signals in the TME into positive signals (switch receptors) or act as a sink for immunosuppressive cytokines or pathways (dominant-negative receptors) (Figure 2), for instance, CAR-T cells secreting PD1-blocking scFvs to target the PD1/programmed cell death ligand 1 (PDL1) inhibitory pathway in the TME.[139] CAR-T cells equipped with dominant-negative receptors protect them from the immunosuppressive effect of transforming growth factor β (TGF-β)[140] or the PD1/PDL1 interaction[141] or the pro-apoptotic effect of the Fas/Fas-ligand interaction.[142] Switch receptors have been engineered by fusing the ectodomain of the immunosuppressive IL-4 cytokine with the endodomain of pro-survival cytokines IL-2 or IL-7,[143] the extracellular domain of the PD1 inhibitory receptor with the intracellular domain of the CD28 co-stimulatory receptor,[144] or the TGF-β receptor II ectodomain with the intracellular domain of the 4-11B co-stimulatory receptor.[145] A CAR with a TGF-β switch receptor converted this immunosuppressive signal into a potent T cell activation signal.[146] Chimeric co-stimulatory receptors have also been introduced into CAR-T cells to provide additional co-stimulation upon recognition of a second tumor antigen.[147]

PRECLINICAL MOUSE MODELS

To implement these novel CAR-T cell designs in the clinic, they must first be examined in preclinical models. However, it is difficult to develop models that accurately recapitulate human tumors and, therefore, accurately predict clinical outcomes in patients. Current preclinical models primarily rely on immunodeficient mice, most commonly the Nod-SCID Il2Rγ-knockout (NSG) strain.[148] These mice sustain engraftment with a broad range of human tumor cell lines and patient-derived xenografts along with human CAR-T cells, without the need for pre-conditioning. Although all approved CAR-T cell products have undergone proof-of-concept efficacy studies using these xenograft models, the absence of an intact immune system and human stromal cells and the incomplete cross-reactivity between mouse and human cytokines, chemokines, survival factors, and adhesion molecules prevent the assessment of a complete TME and host immunity on CAR-T cell efficacy. Additionally, immunodeficient mice do not recapitulate immuno-mediated toxicities associated with CAR-T cell therapy. Indeed, the clinical manifestations of CRS, immune effector cell-associated neurotoxicity syndrome (ICANS), hemophagocytic lympho-histiocytosis (HLH), and macrophage activation syndrome are driven by immune-mediated systemic inflammatory responses. Long-term studies in NSG mice are also limited by the development of xenogenic graft-versus-host disease, which can be partly mitigated using immunodeficient mice in which the murine beta-2-microglobulin (*B2M*) gene or the MHC class I (heavy chains *H2-K* and *H2-D*) and class II (*H2-IA* and *H2-IE*) genes have been knocked out.[148]

To study CAR-T cell therapies in the presence of a human immune system, humanized mice have been generated by engrafting human hematopoietic stem cells into immunodeficient mice.[148] However, these models are challenging due to their high costs, incomplete human immune reconstitution, and increased risk of spontaneous tumor rejection due to alloreactive responses from reconstituted human immune cells. Moreover, ensuring full human leukocyte antigen matching between CAR-T cells, bone marrow cells, and tumor cells is rarely feasible, especially in the context of solid tumors. SGM3 mice that constitutively express human stem cell factor, granulocyte-macrophage colony-stimulating factor (GM-CSF), and

IL-3 cytokines have been developed to ensure better reconstitution and functionality of human T, B, and myeloid cells. This model has been used to study CAR-T cell toxicities, including CRS and ICANS. In this model, xeno-tolerant human CAR-T cells displayed anti-tumor efficacy and induced CRS and ICANS symptoms, which were managed by depleting human monocytes or blocking IL-6 with tocilizumab or the IL-1 receptor with anakinra.[149] SCID-beige mice, which retain functional murine myeloid cells, have also been used to demonstrate the role of macrophages in mediating CRS through the release of IL-6, IL-1, and nitric oxide.[150] Humanized SGM3 mice have also shown that toxicity of CD19-CAR-T cells can be mitigated with emapalumab an IFN-γ-neutralizing antibody, without compromising the efficacy.[151]

An alternative to humanized mice is the use of syngeneic mouse models, where mouse CAR-T cells are transferred into immunocompetent mice bearing murine tumors. This approach allows for CAR-T cell studies in the presence of an intact TME, host immunity, and fully functional primary and secondary lymphoid organs. These models have been instrumental in evaluating the effects of conditioning on CAR-T cell efficacy,[152] the activity of cytokine-armored CAR-T cells on the TME or host immunity,[130,132,133,135–137,153] the efficacy of CAR-T cells targeting the tumor stroma,[59] and the influence of combining CAR-T cells with vaccines[15,16] or immunomodulators.[154,155] One drawback to syngeneic models is that mouse T cells have functional and phenotypic differences with respect to human T cells. For instance, murine CAR-T cells exhibit more limited persistence *in vivo*, possibly due to the *in vitro* engineering and culture processes, lower expansion capacity in culture, and difficulties in manufacturing. Mouse T cells are not susceptible to the transduction with lentiviral vectors, requiring ecotropic retroviral vectors, and are highly sensitive to cryopreservation, losing viability. Recently, Ark313, a synthetic variant of the adeno-associated virus-6, has enabled high transduction and gene editing of murine T cells.[156]

Additionally, the CAR constructs used to engineer mouse T cells incorporate murine signaling domains, which do not reflect the final product that will be translated into patients. Most CAR constructs used in murine T cells utilize a binder optimized to recognize the human tumor antigen, while also retaining varying degrees of cross-reactivity with the mouse homolog, which may result in reduced anti-tumor efficacy. To address this issue, the human antigen is often overexpressed on mouse tumors, leading to supraphysiological levels of antigen expression and potential immunogenicity when the homology between the mouse and human proteins is low. Other groups have instead developed CARs incorporating binders that are highly specific for the mouse antigen[157,158] or transgenic mice with the human gene encoding for the tumor antigen knocked into the mouse gene homolog. Such mice have been used to investigate dose-dependent CRS and neurotoxicity[159] and on-target off-tumor toxicity[56] using mouse CAR-T cells. Syngeneic mice have also successfully recapitulated late-onset HLH toxicity using perforin-deficient CAR-T cells.[160]

CONCLUDING REMARKS AND OUTLOOK

CAR-T cell therapy has emerged as a groundbreaking treatment modality capable of inducing impressive responses in previously untreatable cancers. The long-awaited success recently achieved against aggressive solid tumors has renewed enthusiasm and generated new hope within the field. Lessons from recent clinical trials emphasize the importance of local administration, lymphodepletion, and the need for repeated doses of CAR-T cells to enhance both efficacy and safety. Furthermore, clinical trial data suggest that earlier interventions could maximize the therapeutic benefit of CAR-T cell therapy. In this review, we have highlighted various engineering strategies aimed at improving CAR-T cell fitness, persistence, expansion, and trafficking to solid tumors. Approaches to safely target multiple tumor antigens will be essential to address tumor antigen heterogeneity and prevent tumor escape. Armoring strategies to improve CAR-T cell fitness and target the immunosuppressive TME are also expected to drive future clinical advances against solid tumors, potentially reducing the need for lymphodepleting chemotherapy. The rapid pace of developing new CAR-T products underscores the need for a framework to quickly identify which gene modification will ultimately benefit patients with cancer. Current preclinical models, including immunodeficient xenograft models, humanized mice, and fully immunocompetent syngeneic mice, should be used appropriately and in combination, considering the complementary strengths and weaknesses of each model. We anticipate that the continued integration of synthetic biology, gene editing, novel delivery methods, improved manufacturing processes, and rational combinations with other treatment modalities will further enhance the efficacy and safety of CAR-T cell therapies for solid tumors, while simultaneously reducing costs and increasing accessibility.

ACKNOWLEDGMENTS

Work in the author's laboratory (M.V.M.) is supported by grants from the NIH (R01CA238268, R01CA252940, R01CA249062, R01CA249071, and UG3UCA283619) and DOD W81XWH-20-1-0315. G.E. is recipient of a Gilead Research Scholar Award.

AUTHOR CONTRIBUTIONS

G.E., T.R.B., and M.V.M. wrote and edited the manuscript. All authors have read and approved the final manuscript.

DECLARATION OF INTERESTS

M.V.M. is an inventor on patents related to adoptive cell therapies, held by Massachusetts General Hospital (some licensed to ProMab and Luminary) and University of Pennsylvania (some licensed to Novartis). M.V.M. holds equity in 2SeventyBio, A2Bio, AffyImmune, BendBio, Cargo, GBM newco, Model T bio, NexImmune, and Oncternal. M.V.M. receives Grant/Research support from Kite Pharma, Moderna, and Sobi. M.V.M. has served as a consultant for multiple companies involved in cell therapies. M.V.M.'s competing interests are managed by Mass General Brigham.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2025.102353.

REFERENCES

1. Chesney, J., Lewis, K.D., Kluger, H., Hamid, O., Whitman, E., Thomas, S., Wermke, M., Cusnir, M., Domingo-Musibay, E., Phan, G.Q., et al. (2022).

Efficacy and safety of lifileucel, a one-time autologous tumor-infiltrating lymphocyte (TIL) cell therapy, in patients with advanced melanoma after progression on immune checkpoint inhibitors and targeted therapies: pooled analysis of consecutive cohorts of the C-144-01 study. J. Immunother. Cancer *10*, e005755. https://doi.org/10.1136/jitc-2022-005755.

2. D'Angelo, S.P., Araujo, D.M., Abdul Razak, A.R., Agulnik, M., Attia, S., Blay, J.-Y., Carrasco Garcia, I., Charlson, J.A., Choy, E., Demetri, G.D., et al. (2024). Afamitresgene autoleucel for advanced synovial sarcoma and myxoid round cell liposarcoma (SPEARHEAD-1): an international, open-label, phase 2 trial. Lancet *403*, 1460–1471. https://doi.org/10.1016/S0140-6736(24)00319-2.
3. Hong, D.S., Van Tine, B.A., Biswas, S., McAlpine, C., Johnson, M.L., Olszanski, A.J., Clarke, J.M., Araujo, D., Blumenschein, G.R., Kebriaei, P., et al. (2023). Autologous T cell therapy for MAGE-A4+ solid cancers in HLA-A*02+ patients: a phase 1 trial. Nat. Med. *29*, 104–114. https://doi.org/10.1038/s41591-022-02128-z.
4. Brown, C.E., Hibbard, J.C., Alizadeh, D., Blanchard, M.S., Natri, H.M., Wang, D., Ostberg, J.R., Aguilar, B., Wagner, J.R., Paul, J.A., et al. (2024). Locoregional delivery of IL-13Rα2-targeting CAR-T cells in recurrent high-grade glioma: a phase 1 trial. Nat. Med. *30*, 1001–1012. https://doi.org/10.1038/s41591-024-02875-1.
5. Choi, B.D., Yu, X., Castano, A.P., Bouffard, A.A., Schmidts, A., Larson, R.C., Bailey, S.R., Boroughs, A.C., Frigault, M.J., Leick, M.B., et al. (2019). CAR-T cells secreting BiTEs circumvent antigen escape without detectable toxicity. Nat. Biotechnol. *37*, 1049–1058. https://doi.org/10.1038/s41587-019-0192-1.
6. Choi, B.D., Gerstner, E.R., Frigault, M.J., Leick, M.B., Mount, C.W., Balaj, L., Nikiforow, S., Carter, B.S., Curry, W.T., Gallagher, K., and Maus, M.V. (2024). Intraventricular CARv3-TEAM-E T Cells in Recurrent Glioblastoma. N. Engl. J. Med. *390*, 1290–1298. https://doi.org/10.1056/nejmoa2314390.
7. Bagley, S.J., Desai, A.S., Fraietta, J.A., Silverbush, D., Chafamo, D., Freeburg, N.F., Gopikrishna, G.K., Rech, A.J., Nabavizadeh, A., Bagley, L.J., et al. (2025). Intracerebroventricular bivalent CAR T cells targeting EGFR and IL-13Rα2 in recurrent glioblastoma: a phase 1 trial. Nat. Med. *31*, 2778–2787. https://doi.org/10.1038/s41591-025-03745-0.
8. Monje, M., Mahdi, J., Majzner, R., Yeom, K.W., Schultz, L.M., Richards, R.M., Barsan, V., Song, K.W., Kamens, J., Baggott, C., et al. (2025). Intravenous and intracranial GD2-CAR T cells for H3K27M+ diffuse midline gliomas. Nature *637*, 708–715. https://doi.org/10.1038/s41586-024-08171-9.
9. Vitanza, N.A., Ronsley, R., Choe, M., Seidel, K., Huang, W., Rawlings-Rhea, S.D., Beam, M., Steinmetzer, L., Wilson, A.L., Brown, C., et al. (2025). Intracerebroventricular B7-H3-targeting CAR T cells for diffuse intrinsic pontine glioma: a phase 1 trial. Nat. Med. *31*, 861–868. https://doi.org/10.1038/s41591-024-03451-3.
10. Qi, C., Liu, C., Gong, J., Liu, D., Wang, X., Zhang, P., Qin, Y., Ge, S., Zhang, M., Peng, Z., et al. (2024). Claudin18.2-specific CAR T cells in gastrointestinal cancers: phase 1 trial final results. Nat. Med. *30*, 2224–2234. https://doi.org/10.1038/s41591-024-03037-z.
11. Del Bufalo, F., De Angelis, B., Caruana, I., Del Baldo, G., De Ioris, M.A., Serra, A., Mastronuzzi, A., Cefalo, M.G., Pagliara, D., Amicucci, M., et al. (2023). GD2-CART01 for Relapsed or Refractory High-Risk Neuroblastoma. N. Engl. J. Med. *388*, 1284–1295. https://doi.org/10.1056/nejmoa2210859.
12. Hegde, M., Navai, S., DeRenzo, C., Joseph, S.K., Sanber, K., Wu, M., Gad, A.Z., Janeway, K.A., Campbell, M., Mullikin, D., et al. (2024). Autologous HER2-specific CAR T cells after lymphodepletion for advanced sarcoma: a phase 1 trial. Nat. Cancer *5*, 880–894. https://doi.org/10.1038/s43018-024-00749-6.
13. Ahmed, N., Brawley, V.S., Hegde, M., Robertson, C., Ghazi, A., Gerken, C., Liu, E., Dakhova, O., Ashoori, A., Corder, A., et al. (2015). Human Epidermal Growth Factor Receptor 2 (HER2) -Specific Chimeric Antigen Receptor-Modified T Cells for the Immunotherapy of HER2-Positive Sarcoma. J. Clin. Oncol. *33*, 1688–1696. https://doi.org/10.1200/JCO.2014.58.0225.
14. Morgan, R.A., Yang, J.C., Kitano, M., Dudley, M.E., Laurencot, C.M., and Rosenberg, S.A. (2010). Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol. Ther. *18*, 843–851. https://doi.org/10.1038/mt.2010.24.
15. Ma, L., Hostetler, A., Morgan, D.M., Maiorino, L., Sulkaj, I., Whittaker, C.A., Neeser, A., Pires, I.S., Yousefpour, P., Gregory, J., et al. (2023). Vaccine-boosted CAR T crosstalk with host immunity to reject tumors with antigen heterogeneity. Cell *186*, 3148–3165.e20. https://doi.org/10.1016/j.cell.2023.06.002.
16. Reinhard, K., Rengstl, B., Oehm, P., Michel, K., Billmeier, A., Hayduk, N., Klein, O., Kuna, K., Ouchan, Y., Wöll, S., et al. (2020). An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors. Science *367*, 446–453. https://doi.org/10.1126/science.aay5967.
17. Mackensen, A., Haanen, J.B.A.G., Koenecke, C., Alsdorf, W., Wagner-Drouet, E., Borchmann, P., Heudobler, D., Ferstl, B., Klobuch, S., Bokemeyer, C., et al. (2023). CLDN6-specific CAR-T cells plus amplifying RNA vaccine in relapsed or refractory solid tumors: the phase 1 BNT211-01 trial. Nat. Med. *29*, 2844–2853. https://doi.org/10.1038/s41591-023-02612-0.
18. Majzner, R.G., and Mackall, C.L. (2018). Tumor Antigen Escape from CAR T-cell Therapy. Cancer Discov. *8*, 1219–1226. https://doi.org/10.1158/2159-8290.CD-18-0442.
19. Shi, M., Wang, J., Huang, H., Liu, D., Cheng, H., Wang, X., Chen, W., Yan, Z., Sang, W., Qi, K., et al. (2024). Bispecific CAR T cell therapy targeting BCMA and CD19 in relapsed/refractory multiple myeloma: a phase I/II trial. Nat. Commun. *15*, 3371. https://doi.org/10.1038/s41467-024-47801-8.
20. Spiegel, J.Y., Patel, S., Muffly, L., Hossain, N.M., Oak, J., Baird, J.H., Frank, M.J., Shiraz, P., Sahaf, B., Craig, J., et al. (2021). CAR T cells with dual targeting of CD19 and CD22 in adult patients with recurrent or refractory B cell malignancies: a phase 1 trial. Nat. Med. *27*, 1419–1431. https://doi.org/10.1038/s41591-021-01436-0.
21. Zhang, Y., Wang, Y., Liu, Y., Tong, C., Wang, C., Guo, Y., Ti, D., Yang, Q., Qiao, S., Wu, Z., and Han, W. (2022). Long-term activity of tandem CD19/CD20 CAR therapy in refractory/relapsed B-cell lymphoma: a single-arm, phase 1-2 trial. Leukemia *36*, 189–196. https://doi.org/10.1038/s41375-021-01345-8.
22. Ruella, M., Barrett, D.M., Kenderian, S.S., Shestova, O., Hofmann, T.J., Perazzelli, J., Klichinsky, M., Aikawa, V., Nazimuddin, F., Kozlowski, M., et al. (2016). Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. J. Clin. Investig. *126*, 3814–3826. https://doi.org/10.1172/JCI87366.
23. Hirabayashi, K., Du, H., Xu, Y., Shou, P., Zhou, X., Fucá, G., Landoni, E., Sun, C., Chen, Y., Savoldo, B., and Dotti, G. (2021). Dual Targeting CAR-T Cells with Optimal Costimulation and Metabolic Fitness enhance Antitumor Activity and Prevent Escape in Solid Tumors. Nat. Cancer *2*, 904–918. https://doi.org/10.1038/s43018-021-00244-2.
24. Schmidts, A., Srivastava, A.A., Ramapriyan, R., Bailey, S.R., Bouffard, A.A., Cahill, D.P., Carter, B.S., Curry, W.T., Dunn, G.P., Frigault, M.J., et al. (2023). Tandem chimeric antigen receptor (CAR) T cells targeting EGFRvIII and IL-13Rα2 are effective against heterogeneous glioblastoma. Neurooncol. Adv. *5*, vdac185. https://doi.org/10.1093/noajnl/vdac185.
25. Hegde, M., Mukherjee, M., Grada, Z., Pignata, A., Landi, D., Navai, S.A., Wakefield, A., Fousek, K., Bielamowicz, K., Chow, K.K.H., et al. (2016). Tandem CAR T cells targeting HER2 and IL13Rα2 mitigate tumor antigen escape. J. Clin. Investig. *126*, 3036–3052. https://doi.org/10.1172/JCI83416.
26. Yin, Y., Rodriguez, J.L., Li, N., Thokala, R., Nasrallah, M.P., Hu, L., Zhang, L., Zhang, J.V., Logun, M.T., Kainth, D., et al. (2022). Locally secreted BiTEs complement CAR T cells by enhancing killing of antigen

heterogeneous solid tumors. Mol. Ther. *30*, 2537–2553. https://doi.org/10.1016/j.ymthe.2022.05.011.

27. McCue, A.C., Yao, Z., and Kuhlman, B. (2022). Advances in modular control of CAR-T therapy with adapter-mediated CARs. Adv. Drug Deliv. Rev. *187*, 114358. https://doi.org/10.1016/j.addr.2022.114358.
28. Cartellieri, M., Feldmann, A., Koristka, S., Arndt, C., Loff, S., Ehninger, A., von Bonin, M., Bejestani, E.P., Ehninger, G., and Bachmann, M.P. (2016). Switching CAR T cells on and off: a novel modular platform for retargeting of T cells to AML blasts. Blood Cancer J. *6*, e458. https://doi.org/10.1038/bcj.2016.61.
29. Bachmann, M. (2019). The UniCAR system: A modular CAR T cell approach to improve the safety of CAR T cells. Immunol. Lett. *211*, 13–22. https://doi.org/10.1016/j.imlet.2019.05.003.
30. Cho, J.H., Collins, J.J., and Wong, W.W. (2018). Universal Chimeric Antigen Receptors for Multiplexed and Logical Control of T Cell Responses. Cell *173*, 1426–1438.e11. https://doi.org/10.1016/j.cell.2018.03.038.
31. Yarmarkovich, M., Marshall, Q.F., Warrington, J.M., Premaratne, R., Farrel, A., Groff, D., Li, W., di Marco, M., Runbeck, E., Truong, H., et al. (2023). Targeting of intracellular oncoproteins with peptide-centric CARs. Nature *623*, 820–827. https://doi.org/10.1038/s41586-023-06706-0.
32. Benton, A., Liu, J., Poussin, M.A., Lang Goldgewicht, A., Udawela, M., Bear, A.S., Wellhausen, N., Carreno, B.M., Smith, P.M., Beasley, M.D., et al. (2025). Mutant KRAS peptide targeted CAR-T cells engineered for cancer therapy. Cancer Cell *43*, 1365–1376.e5. https://doi.org/10.1016/j.ccell.2025.05.006.
33. Householder, K.D., Xiang, X., Jude, K.M., Deng, A., Obenaus, M., Zhao, Y., Wilson, S.C., Chen, X., Wang, N., and Garcia, K.C. (2025). De novo design and structure of a peptide-centric TCR mimic binding module. Science *389*, 375–379.
34. Haurum Johansen, K., Stephan Wolff, D., Scapolo, B., Fernández-Quintero, M.L., Risager Christensen, C., Loeffler, J.R., Rivera-de-Torre, E., Overath, M.D., Kjaergaard Munk, K., Morell, O., et al. (2025). De novo-designed pMHC binders facilitate T cell-mediated cytotoxicity toward cancer cells. Science *389*, 380–385.
35. Liu, B., Greenwood, N.F., Bonzanini, J.E., Motmaen, A., Meyerberg, J., Dao, T., Xiang, X., Ault, R., Sharp, J., Wang, C., et al. (2025). Design of high-specificity binders for peptide-MHC-I complexes. Science *389*, 386–391.
36. Straathof, K.C., Pulè, M.A., Yotnda, P., Dotti, G., Vanin, E.F., Brenner, M.K., Heslop, H.E., Spencer, D.M., and Rooney, C.M. (2005). An inducible caspase 9 safety switch for T-cell therapy. Blood *105*, 4247–4254. https://doi.org/10.1182/blood-2004-11-4564.
37. Bordignon, C., Bonini, C., Verzeletti, S., Nobili, N., Maggioni, D., Traversari, C., Giavazzi, R., Servida, P., Zappone, E., and Benazzi, E. (1995). Transfer of the HSV-tk gene into donor peripheral blood lymphocytes for in vivo modulation of donor anti-tumor immunity after allogeneic bone marrow transplantation. Hum. Gene Ther. *6*, 813–819. https://doi.org/10.1089/hum.1995.6.6-813.
38. Wang, X., Chang, W.-C., Wong, C.W., Colcher, D., Sherman, M., Ostberg, J.R., Forman, S.J., Riddell, S.R., and Jensen, M.C. (2011). A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells. Blood *118*, 1255–1263. https://doi.org/10.1182/blood-2011-02-337360.
39. Johnson, A.J., Wei, J., Rosser, J.M., Künkele, A., Chang, C.A., Reid, A.N., and Jensen, M.C. (2021). Rationally Designed Transgene-Encoded Cell-Surface Polypeptide Tag for Multiplexed Programming of CAR T-cell Synthetic Outputs. Cancer Immunol. Res. *9*, 1047–1060. https://doi.org/10.1158/2326-6066.CIR-20-0470.
40. Philip, B., Kokalaki, E., Mekkaoui, L., Thomas, S., Straathof, K., Flutter, B., Marin, V., Marafioti, T., Chakraverty, R., Linch, D., et al. (2014). A highly compact epitope-based marker/suicide gene for easier and safer T-cell therapy. Blood *124*, 1277–1287. https://doi.org/10.1182/blood-2014-01-545020.
41. Steffin, D., Ghatwai, N., Montalbano, A., Rathi, P., Courtney, A.N., Arnett, A.B., Fleurence, J., Sweidan, R., Wang, T., Zhang, H., et al. (2025). Interleukin-15-armoured GPC3 CAR T cells for patients with solid cancers. Nature *637*, 940–946. https://doi.org/10.1038/s41586-024-08261-8.
42. Lu, L., Xie, M., Yang, B., Zhao, W.-B., and Cao, J. (2024). Enhancing the safety of CAR-T cell therapy: Synthetic genetic switch for spatiotemporal control. Sci. Adv. *10*, eadj6251.
43. Frigault, M.J., Graham, C.E., Berger, T.R., Ritchey, J., Horick, N.K., El-Jawahri, A., Scarfò, I., Schmidts, A., Haradhvala, N.J., Wehrli, M., et al. (2024). Phase 1 study of CAR-37 T cells in patients with relapsed or refractory CD37+ lymphoid malignancies. Blood *144*, 1153–1167. https://doi.org/10.1182/blood.2024024104.
44. Lin, H., Cheng, J., Zhu, L., Zeng, Y., Dai, Z., Zhang, Y., Zhu, X., and Mu, W. (2024). Anti-CD5 CAR-T cells with a tEGFR safety switch exhibit potent toxicity control. Blood Cancer J. *14*, 98. https://doi.org/10.1038/s41408-024-01082-y.
45. Zhong, G., Zhang, X., Zhao, R., Guo, Z., Wang, C., Yu, C., Liu, D., Hu, K., Gao, Y., Zhao, B., et al. (2025). The high efficacy of claudin18.2-targeted CAR-T cell therapy in advanced pancreatic cancer with an antibody-dependent safety strategy. Mol. Ther. *33*, 2778–2788. https://doi.org/10.1016/j.ymthe.2025.01.012.
46. Roybal, K.T., Williams, J.Z., Morsut, L., Rupp, L.J., Kolinko, I., Choe, J.H., Walker, W.J., McNally, K.A., and Lim, W.A. (2016). Engineering T Cells with Customized Therapeutic Response Programs Using Synthetic Notch Receptors. Cell *167*, 419–432.e16. https://doi.org/10.1016/j.cell.2016.09.011.
47. Zhu, I., Liu, R., Garcia, J.M., Hyrenius-Wittsten, A., Piraner, D.I., Alavi, J., Israni, D.V., Liu, B., Khalil, A.S., and Roybal, K.T. (2022). Modular design of synthetic receptors for programmed gene regulation in cell therapies. Cell *185*, 1431–1443.e16. https://doi.org/10.1016/j.cell.2022.03.023.
48. Choe, J.H., Watchmaker, P.B., Simic, M.S., Gilbert, R.D., Li, A.W., Krasnow, N.A., Downey, K.M., Yu, W., Carrera, D.A., Celli, A., et al. (2021). SynNotch-CAR T cells overcome challenges of specificity, heterogeneity, and persistence in treating glioblastoma. Sci. Transl. Med. *13*, eabe7378.
49. Simic, M.S., Watchmaker, P.B., Gupta, S., Wang, Y., Sagan, S.A., Duecker, J., Shepherd, C., Diebold, D., Pineo-Cavanaugh, P., Haegelin, J., et al. (2024). Programming tissue-sensing T cells that deliver therapies to the brain. Science *386*, eadl4237. https://doi.org/10.1126/science.adl4237.
50. Piraner, D.I., Abedi, M.H., Duran Gonzalez, M.J., Chazin-Gray, A., Lin, A., Zhu, I., Ravindran, P.T., Schlichthaerle, T., Huang, B., Bearchild, T.H., et al. (2025). Engineered receptors for soluble cellular communication and disease sensing. Nature *638*, 805–813. https://doi.org/10.1038/s41586-024-08366-0.
51. Priceman, S.J., Tilakawardane, D., Jeang, B., Aguilar, B., Murad, J.P., Park, A.K., Chang, W.-C., Ostberg, J.R., Neman, J., Jandial, R., et al. (2018). Regional Delivery of Chimeric Antigen Receptor-Engineered T Cells Effectively Targets HER2+ Breast Cancer Metastasis to the Brain. Clin. Cancer Res. *24*, 95–105. https://doi.org/10.1158/1078-0432.CCR-17-2041.
52. Theruvath, J., Sotillo, E., Mount, C.W., Graef, C.M., Delaidelli, A., Heitzeneder, S., Labanieh, L., Dhingra, S., Leruste, A., Majzner, R.G., et al. (2020). Locoregionally administered B7-H3-targeted CAR T cells for treatment of atypical teratoid/rhabdoid tumors. Nat. Med. *26*, 712–719. https://doi.org/10.1038/s41591-020-0821-8.
53. Donovan, L.K., Delaidelli, A., Joseph, S.K., Bielamowicz, K., Fousek, K., Holgado, B.L., Manno, A., Srikanthan, D., Gad, A.Z., Van Ommeren, R., et al. (2020). Locoregional delivery of CAR T cells to the cerebrospinal fluid for treatment of metastatic medulloblastoma and ependymoma. Nat. Med. *26*, 720–731. https://doi.org/10.1038/s41591-020-0827-2.
54. O'Rourke, D.M., Nasrallah, M.P., Desai, A., Melenhorst, J.J., Mansfield, K., Morrissette, J.J.D., Martinez-Lage, M., Brem, S., Maloney, E., Shen,

A., et al. (2017). A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci. Transl. Med. *9*, eaaa0984. https://doi.org/10.1126/scitranslmed.aaa0984.

55. Adusumilli, P.S., Zauderer, M.G., Rivière, I., Solomon, S.B., Rusch, V.W., O'Cearbhaill, R.E., Zhu, A., Cheema, W., Chintala, N.K., Halton, E., et al. (2021). A Phase I Trial of Regional Mesothelin-Targeted CAR T-cell Therapy in Patients with Malignant Pleural Disease, in Combination with the Anti-PD-1 Agent Pembrolizumab. Cancer Discov. *11*, 2748–2763. https://doi.org/10.1158/2159-8290.CD-21-0407.
56. Uslu, U., Da, T., Assenmacher, C.-A., Scholler, J., Young, R.M., Tchou, J., and June, C.H. (2023). Chimeric antigen receptor T cells as adjuvant therapy for unresectable adenocarcinoma. Sci. Adv. *9*, eade2526.
57. Ogunnaike, E.A., Valdivia, A., Yazdimamaghani, M., Leon, E., Nandi, S., Hudson, H., Du, H., Khagi, S., Gu, Z., Savoldo, B., et al. (2021). Fibrin gel enhances the antitumor effects of chimeric antigen receptor T cells in glioblastoma. Sci. Adv. *7*, eabg5841. https://doi.org/10.1126/sciadv.abg5841.
58. Foeng, J., Comerford, I., and McColl, S.R. (2022). Harnessing the chemokine system to home CAR-T cells into solid tumors. Cell Rep. Med. *3*, 100543. https://doi.org/10.1016/j.xcrm.2022.100543.
59. Xiao, Z., Todd, L., Huang, L., Noguera-Ortega, E., Lu, Z., Huang, L., Kopp, M., Li, Y., Pattada, N., Zhong, W., et al. (2023). Desmoplastic stroma restricts T cell extravasation and mediates immune exclusion and immunosuppression in solid tumors. Nat. Commun. *14*, 5110. https://doi.org/10.1038/s41467-023-40850-5.
60. Wehrli, M., Guinn, S., Birocchi, F., Kuo, A., Sun, Y., Larson, R.C., Almazan, A.J., Scarfò, I., Bouffard, A.A., Bailey, S.R., et al. (2024). Mesothelin CAR T-cells secreting anti-FAP/anti-CD3 molecules efficiently target pancreatic adenocarcinoma and its stroma. Clin. Cancer Res. *30*, 1859–1877. https://doi.org/10.1158/1078-0432.CCR-23-3841.
61. Caruana, I., Savoldo, B., Hoyos, V., Weber, G., Liu, H., Kim, E.S., Ittmann, M.M., Marchetti, D., and Dotti, G. (2015). Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. Nat. Med. *21*, 524–529. https://doi.org/10.1038/nm.3833.
62. Gholamin, S., Natri, H.M., Zhao, Y., Xu, S., Aftabizadeh, M., Comin-Anduix, B., Saravanakumar, S., Masia, C., Wong, R.A., Peter, L., et al. (2025). Overcoming myeloid-driven resistance to CAR T therapy by targeting SPP1. bioRxiv. https://doi.org/10.1101/2025.04.01.646202.
63. Stahl, D., Gödel, P., Balke-Want, H., Gholamipoorfard, R., Segbers, P., Tetenborg, L., Koker, M., Dörr, J., Gregor, L., Bachurski, D., et al. (2025). CSF1R+ myeloid-monocytic cells drive CAR-T cell resistance in aggressive B cell lymphoma. Cancer Cell *43*, 1476–1494. https://doi.org/10.1016/j.ccell.2025.05.013.
64. Jenkins, E., Whitehead, T., Fellermeyer, M., Davis, S.J., and Sharma, S. (2023). The current state and future of T-cell exhaustion research. Oxf. Open Immunol. *4*, iqad006. https://doi.org/10.1093/oxfimm/iqad006.
65. Fraietta, J.A., Lacey, S.F., Orlando, E.J., Pruteanu-Malinici, I., Gohil, M., Lundh, S., Boesteanu, A.C., Wang, Y., O'connor, R.S., Hwang, W.T., et al. (2018). Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. Nat. Med. *24*, 563–571. https://doi.org/10.1038/s41591-018-0010-1.
66. Fraietta, J.A., Nobles, C.L., Sammons, M.A., Lundh, S., Carty, S.A., Reich, T.J., Cogdill, A.P., Morrissette, J.J.D., DeNizio, J.E., Reddy, S., et al. (2018). Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. Nature *558*, 307–312. https://doi.org/10.1038/s41586-018-0178-z.
67. Jain, N., Zhao, Z., Feucht, J., Koche, R., Iyer, A., Dobrin, A., Mansilla-Soto, J., Yang, J., Zhan, Y., Lopez, M., et al. (2023). TET2 guards against unchecked BATF3-induced CAR T cell expansion. Nature *615*, 315–322. https://doi.org/10.1038/s41586-022-05692-z.
68. Prinzing, B., Zebley, C.C., Petersen, C.T., Fan, Y., Anido, A.A., Yi, Z., Nguyen, P., Houke, H., Bell, M., Haydar, D., et al. (2021). Deleting DNMT3A in CAR T cells prevents exhaustion and enhances antitumor activity. Sci. Transl. Med. *13*, eabh0272. https://doi.org/10.1126/scitranslmed.abh0272.
69. Jain, N., Zhao, Z., Koche, R.P., Antelope, C., Gozlan, Y., Montalbano, A., Brocks, D., Lopez, M., Dobrin, A., Shi, Y., et al. (2024). Disruption of SUV39H1-Mediated H3K9 Methylation Sustains CAR T-cell Function. Cancer Discov. *14*, 142–157. https://doi.org/10.1158/2159-8290.CD-22-1319.
70. Seo, H., Chen, J., González-Avalos, E., Samaniego-Castruita, D., Das, A., Wang, Y.H., López-Moyado, I.F., Georges, R.O., Zhang, W., Onodera, A., et al. (2019). TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8+ T cell exhaustion. Proc. Natl. Acad. Sci. USA *116*, 12410–12415. https://doi.org/10.1073/pnas.1905675116.
71. Chen, J., López-Moyado, I.F., Seo, H., Lio, C.-W.J., Hempleman, L.J., Sekiya, T., Yoshimura, A., Scott-Browne, J.P., and Rao, A. (2019). NR4A transcription factors limit CAR T cell function in solid tumours. Nature *567*, 530–534. https://doi.org/10.1038/s41586-019-0985-x.
72. Good, C.R., Aznar, M.A., Kuramitsu, S., Samareh, P., Agarwal, S., Donahue, G., Ishiyama, K., Wellhausen, N., Rennels, A.K., Ma, Y., et al. (2021). An NK-like CAR T cell transition in CAR T cell dysfunction. Cell *184*, 6081–6100.e26. https://doi.org/10.1016/j.cell.2021.11.016.
73. Agarwal, S., Aznar, M.A., Rech, A.J., Good, C.R., Kuramitsu, S., Da, T., Gohil, M., Chen, L., Hong, S.-J.A., Ravikumar, P., et al. (2023). Deletion of the inhibitory co-receptor CTLA-4 enhances and invigorates chimeric antigen receptor T cells. Immunity *56*, 2388–2407.e9. https://doi.org/10.1016/j.immuni.2023.09.001.
74. Ren, J., Liu, X., Fang, C., Jiang, S., June, C.H., and Zhao, Y. (2017). Multiplex Genome Editing to Generate Universal CAR T Cells Resistant to PD1 Inhibition. Clin. Cancer Res. *23*, 2255–2266. https://doi.org/10.1158/1078-0432.CCR-16-1300.
75. Hu, W., Zi, Z., Jin, Y., Li, G., Shao, K., Cai, Q., Ma, X., and Wei, F. (2019). CRISPR/Cas9-mediated PD-1 disruption enhances human mesothelin-targeted CAR T cell effector functions. Cancer Immunol. Immunother. *68*, 365–377. https://doi.org/10.1007/s00262-018-2281-2.
76. Zhang, Y., Zhang, X., Cheng, C., Mu, W., Liu, X., Li, N., Wei, X., Liu, X., Xia, C., and Wang, H. (2017). CRISPR-Cas9 mediated LAG-3 disruption in CAR-T cells. Front. Med. *11*, 554–562. https://doi.org/10.1007/s11684-017-0543-6.
77. Kang, T.G., Lan, X., Mi, T., Chen, H., Alli, S., Lim, S.E., Bhatara, S., Vasandan, A.B., Ward, G., Bentivegna, S., et al. (2024). Epigenetic regulators of clonal hematopoiesis control CD8 T cell stemness during immunotherapy. Science *386*, eadl4492. https://doi.org/10.1126/science.adl4492.
78. Stadtmauer, E.A., Fraietta, J.A., Davis, M.M., Cohen, A.D., Weber, K.L., Lancaster, E., Mangan, P.A., Kulikovskaya, I., Gupta, M., Chen, F., et al. (2020). CRISPR-engineered T cells in patients with refractory cancer. Science *367*, eaba7365. https://doi.org/10.1126/science.aba7365.
79. Weiss, S.A., Huang, A.Y., Fung, M.E., Martinez, D., Chen, A.C.Y., LaSalle, T.J., Miller, B.C., Scharer, C.D., Hegde, M., Nguyen, T.H., et al. (2024). Epigenetic tuning of PD-1 expression improves exhausted T cell function and viral control. Nat. Immunol. *25*, 1871–1883. https://doi.org/10.1038/s41590-024-01961-3.
80. Lynn, R.C., Weber, E.W., Sotillo, E., Gennert, D., Xu, P., Good, Z., Anbunathan, H., Lattin, J., Jones, R., Tieu, V., et al. (2019). c-Jun overexpression in CAR T cells induces exhaustion resistance. Nature *576*, 293–300. https://doi.org/10.1038/s41586-019-1805-z.
81. Chan, J.D., Scheffler, C.M., Munoz, I., Sek, K., Lee, J.N., Huang, Y.-K., Yap, K.M., Saw, N.Y.L., Li, J., Chen, A.X.Y., et al. (2024). FOXO1 enhances CAR T cell stemness, metabolic fitness and efficacy. Nature *629*, 201–210. https://doi.org/10.1038/s41586-024-07242-1.
82. Doan, A.E., Mueller, K.P., Chen, A.Y., Rouin, G.T., Chen, Y., Daniel, B., Lattin, J., Markovska, M., Mozarsky, B., Arias-Umana, J., et al. (2024). FOXO1 is a master regulator of memory programming in CAR T cells. Nature *629*, 211–218. https://doi.org/10.1038/s41586-024-07300-8.

83. Seo, H., González-Avalos, E., Zhang, W., Ramchandani, P., Yang, C., Lio, C.-W.J., Rao, A., and Hogan, P.G. (2021). BATF and IRF4 cooperate to counter exhaustion in tumor-infiltrating CAR T cells. Nat. Immunol. *22*, 983–995. https://doi.org/10.1038/s41590-021-00964-8.

84. DeGolier, K.R., Danis, E., D'Antonio, M., Cimons, J., Yarnell, M., Kedl, R.M., Kohler, M.E., Scott-Browne, J.P., and Fry, T.J. (2025). Antigen experience history directs distinct functional states of CD8+ CAR T cells during the antileukemia response. Nat. Immunol. *26*, 68–81. https://doi.org/10.1038/s41590-024-02034-1.

85. Shifrut, E., Carnevale, J., Tobin, V., Roth, T.L., Woo, J.M., Bui, C.T., Li, P.J., Diolaiti, M.E., Ashworth, A., and Marson, A. (2018). Genome-wide CRISPR Screens in Primary Human T Cells Reveal Key Regulators of Immune Function. Cell *175*, 1958–1971.e15. https://doi.org/10.1016/j.cell.2018.10.024.

86. Wang, D., Prager, B.C., Gimple, R.C., Aguilar, B., Alizadeh, D., Tang, H., Lv, D., Starr, R., Brito, A., Wu, Q., et al. (2021). CRISPR Screening of CAR T Cells and Cancer Stem Cells Reveals Critical Dependencies for Cell-Based Therapies. Cancer Discov. *11*, 1192–1211. https://doi.org/10.1158/2159-8290.CD-20-1243.

87. Shang, W., Jiang, Y., Boettcher, M., Ding, K., Mollenauer, M., Liu, Z., Wen, X., Liu, C., Hao, P., Zhao, S., et al. (2018). Genome-wide CRISPR screen identifies FAM49B as a key regulator of actin dynamics and T cell activation. Proc. Natl. Acad. Sci. USA *115*, E4051–E4060. https://doi.org/10.1073/pnas.1801340115.

88. Wu, J.E., Manne, S., Ngiow, S.F., Baxter, A.E., Huang, H., Freilich, E., Clark, M.L., Lee, J.H., Chen, Z., Khan, O., et al. (2023). In vitro modeling of CD8+ T cell exhaustion enables CRISPR screening to reveal a role for BHLHE40. Sci. Immunol. *8*, eade3369. https://doi.org/10.1126/sciimmunol.ade3369.

89. Carnevale, J., Shifrut, E., Kale, N., Nyberg, W.A., Blaeschke, F., Chen, Y.Y., Li, Z., Bapat, S.P., Diolaiti, M.E., O'Leary, P., et al. (2022). RASA2 ablation in T cells boosts antigen sensitivity and long-term function. Nature *609*, 174–182. https://doi.org/10.1038/s41586-022-05126-w.

90. McCutcheon, S.R., Swartz, A.M., Brown, M.C., Barrera, A., McRoberts Amador, C., Siklenka, K., Humayun, L., Ter Weele, M.A., Isaacs, J.M., Reddy, T.E., et al. (2023). Transcriptional and epigenetic regulators of human CD8+ T cell function identified through orthogonal CRISPR screens. Nat. Genet. *55*, 2211–2223. https://doi.org/10.1038/s41588-023-01554-0.

91. Renauer, P., Park, J.J., Bai, M., Acosta, A., Lee, W.-H., Lin, G.H., Zhang, Y., Dai, X., Wang, G., Errami, Y., et al. (2023). Immunogenetic Metabolomics Reveals Key Enzymes That Modulate CAR T-cell Metabolism and Function. Cancer Immunol. Res. *11*, 1068–1084. https://doi.org/10.1158/2326-6066.CIR-22-0565.

92. Freitas, K.A., Belk, J.A., Sotillo, E., Quinn, P.J., Ramello, M.C., Malipatlolla, M., Daniel, B., Sandor, K., Klysz, D., Bjelajac, J., et al. (2022). Enhanced T cell effector activity by targeting the Mediator kinase module. Science *378*, eabn5647. https://doi.org/10.1126/science.abn5647.

93. Belk, J.A., Yao, W., Ly, N., Freitas, K.A., Chen, Y.-T., Shi, Q., Valencia, A.M., Shifrut, E., Kale, N., Yost, K.E., et al. (2022). Genome-wide CRISPR screens of T cell exhaustion identify chromatin remodeling factors that limit T cell persistence. Cancer Cell *40*, 768–786.e7. https://doi.org/10.1016/j.ccell.2022.06.001.

94. Long, L., Wei, J., Lim, S.A., Raynor, J.L., Shi, H., Connelly, J.P., Wang, H., Guy, C., Xie, B., Chapman, N.M., et al. (2021). CRISPR screens unveil signal hubs for nutrient licensing of T cell immunity. Nature *600*, 308–313. https://doi.org/10.1038/s41586-021-04109-7.

95. Ye, L., Park, J.J., Peng, L., Yang, Q., Chow, R.D., Dong, M.B., Lam, S.Z., Guo, J., Tang, E., Zhang, Y., et al. (2022). A genome-scale gain-of-function CRISPR screen in CD8 T cells identifies proline metabolism as a means to enhance CAR-T therapy. Cell Metab. *34*, 595–614.e14. https://doi.org/10.1016/j.cmet.2022.02.009.

96. Schmidt, R., Steinhart, Z., Layeghi, M., Freimer, J.W., Bueno, R., Nguyen, V.Q., Blaeschke, F., Ye, C.J., and Marson, A. (2022). CRISPR activation and interference screens decode stimulation responses in primary human T cells. Science *375*, eabj4008. https://doi.org/10.1126/science.abj4008.

97. Garcia, J., Daniels, J., Lee, Y., Zhu, I., Cheng, K., Liu, Q., Goodman, D., Burnett, C., Law, C., Thienpont, C., et al. (2024). Naturally occurring T cell mutations enhance engineered T cell therapies. Nature *626*, 626–634. https://doi.org/10.1038/s41586-024-07018-7.

98. Schmidt, R., Ward, C.C., Dajani, R., Armour-Garb, Z., Ota, M., Allain, V., Hernandez, R., Layeghi, M., Xing, G., Goudy, L., et al. (2024). Base-editing mutagenesis maps alleles to tune human T cell functions. Nature *625*, 805–812. https://doi.org/10.1038/s41586-023-06835-6.

99. Dong, M.B., Wang, G., Chow, R.D., Ye, L., Zhu, L., Dai, X., Park, J.J., Kim, H.R., Errami, Y., Guzman, C.D., et al. (2019). Systematic Immunotherapy Target Discovery Using Genome-Scale In Vivo CRISPR Screens in CD8 T Cells. Cell *178*, 1189–1204.e23. https://doi.org/10.1016/j.cell.2019.07.044.

100. LaFleur, M.W., Nguyen, T.H., Coxe, M.A., Miller, B.C., Yates, K.B., Gillis, J.E., Sen, D.R., Gaudiano, E.F., Al Abosy, R., Freeman, G.J., et al. (2019). PTPN2 regulates the generation of exhausted CD8+ T cell subpopulations and restrains tumor immunity. Nat. Immunol. *20*, 1335–1347. https://doi.org/10.1038/s41590-019-0480-4.

101. Wei, J., Long, L., Zheng, W., Dhungana, Y., Lim, S.A., Guy, C., Wang, Y., Wang, Y.-D., Qian, C., Xu, B., et al. (2019). Targeting REGNASE-1 programs long-lived effector T cells for cancer therapy. Nature *576*, 471–476. https://doi.org/10.1038/s41586-019-1821-z.

102. Chen, Z., Arai, E., Khan, O., Zhang, Z., Ngiow, S.F., He, Y., Huang, H., Manne, S., Cao, Z., Baxter, A.E., et al. (2021). In vivo CD8+ T cell CRISPR screening reveals control by Fli1 in infection and cancer. Cell *184*, 1262–1280.e22. https://doi.org/10.1016/j.cell.2021.02.019.

103. Ye, L., Park, J.J., Dong, M.B., Yang, Q., Chow, R.D., Peng, L., Du, Y., Guo, J., Dai, X., Wang, G., et al. (2019). In vivo CRISPR screening in CD8 T cells with AAV-Sleeping Beauty hybrid vectors identifies membrane targets for improving immunotherapy for glioblastoma. Nat. Biotechnol. *37*, 1302–1313. https://doi.org/10.1038/s41587-019-0246-4.

104. Manguso, R.T., Pope, H.W., Zimmer, M.D., Brown, F.D., Yates, K.B., Miller, B.C., Collins, N.B., Bi, K., LaFleur, M.W., Juneja, V.R., et al. (2017). In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target. Nature *547*, 413–418. https://doi.org/10.1038/nature23270.

105. Mai, D., Johnson, O., Reff, J., Fan, T.-J., Scholler, J., Sheppard, N.C., and June, C.H. (2023). Combined disruption of T cell inflammatory regulators Regnase-1 and Roquin-1 enhances antitumor activity of engineered human T cells. Proc. Natl. Acad. Sci. USA *120*, e2218632120. https://doi.org/10.1073/pnas.2218632120.

106. Knudsen, N.H., Escobar, G., Korell, F., Kienka, T., Nobrega, C.N., Anderson, S., Cheng, A.Y., Zschummel, M., Armstrong, A., Bouffard, A., et al. (2025). In vivo CCRISPR screens identify key modifiers of CAR-T cell function in myeloma. Preprint at bioRxiv. https://doi.org/10.1101/2024.11.19.624352.

107. Larson, R.C., Kann, M.C., Bailey, S.R., Haradhvala, N.J., Llopis, P.M., Bouffard, A.A., Scarfó, I., Leick, M.B., Grauwet, K., Berger, T.R., et al. (2022). CAR T cell killing requires the IFNγR pathway in solid but not liquid tumours. Nature *604*, 563–570. https://doi.org/10.1038/s41586-022-04585-5.

108. Tieu, V., Sotillo, E., Bjelajac, J.R., Chen, C., Malipatlolla, M., Guerrero, J.A., Xu, P., Quinn, P.J., Fisher, C., Klysz, D., et al. (2024). A versatile CRISPR-Cas13d platform for multiplexed transcriptomic regulation and metabolic engineering in primary human T cells. Cell *187*, 1278–1295.e20. https://doi.org/10.1016/j.cell.2024.01.035.

109. Gargett, T., Ebert, L.M., Truong, N.T.H., Kollis, P.M., Sedivakova, K., Yu, W., Yeo, E.C.F., Wittwer, N.L., Gliddon, B.L., Tea, M.N., et al. (2022). GD2-targeting CAR-T cells enhanced by transgenic IL-15 expression are an effective and clinically feasible therapy for glioblastoma. J. Immunother. Cancer *10*, e005187. https://doi.org/10.1136/jitc-2022-005187.

110. Reppel, L., Tsahouridis, O., Akulian, J., Davis, I.J., Lee, H., Fucà, G., Weiss, J., Dotti, G., Pecot, C.V., and Savoldo, B. (2022). Targeting disialoganglioside GD2 with chimeric antigen receptor-redirected T cells in lung cancer. J. Immunother. Cancer *10*, e003897. https://doi.org/10.1136/jitc-2021-003897.

111. Shum, T., Omer, B., Tashiro, H., Kruse, R.L., Wagner, D.L., Parikh, K., Yi, Z., Sauer, T., Liu, D., Parihar, R., et al. (2017). Constitutive Signaling from an Engineered IL7 Receptor Promotes Durable Tumor Elimination by Tumor-Redirected T Cells. Cancer Discov. *7*, 1238–1247. https://doi.org/10.1158/2159-8290.CD-17-0538.

112. Kann, M.C., Schneider, E.M., Almazan, A.J., Lane, I.C., Bouffard, A.A., Supper, V.M., Takei, H.N., Tepper, A., Leick, M.B., Larson, R.C., et al. (2024). Chemical genetic control of cytokine signaling in CAR-T cells using lenalidomide-controlled membrane-bound degradable IL-7. Leukemia *38*, 590–600. https://doi.org/10.1038/s41375-023-02113-6.

113. Allen, G.M., Frankel, N.W., Reddy, N.R., Bhargava, H.K., Yoshida, M.A., Stark, S.R., Purl, M., Lee, J., Yee, J.L., Yu, W., et al. (2022). Synthetic cytokine circuits that drive T cells into immune-excluded tumors. Science *378*, eaba1624. https://doi.org/10.1126/science.aba1624.

114. Li, H.-S., Israni, D.V., Gagnon, K.A., Gan, K.A., Raymond, M.H., Sander, J.D., Roybal, K.T., Joung, J.K., Wong, W.W., and Khalil, A.S. (2022). Multidimensional control of therapeutic human cell function with synthetic gene circuits. Science *378*, 1227–1234. https://doi.org/10.1126/science.ade0156.

115. Quijano-Rubio, A., Bhuiyan, A.M., Yang, H., Leung, I., Bello, E., Ali, L.R., Zhangxu, K., Perkins, J., Chun, J.-H., Wang, W., et al. (2023). A split, conditionally active mimetic of IL-2 reduces the toxicity of systemic cytokine therapy. Nat. Biotechnol. *41*, 532–540. https://doi.org/10.1038/s41587-022-01510-z.

116. Kagoya, Y., Tanaka, S., Guo, T., Anczurowski, M., Wang, C.-H., Saso, K., Butler, M.O., Minden, M.D., and Hirano, N. (2018). A novel chimeric antigen receptor containing a JAK-STAT signaling domain mediates superior antitumor effects. Nat. Med. *24*, 352–359. https://doi.org/10.1038/nm.4478.

117. Sockolosky, J.T., Trotta, E., Parisi, G., Picton, L., Su, L.L., Le, A.C., Chhabra, A., Silveria, S.L., George, B.M., King, I.C., et al. (2018). Selective targeting of engineered T cells using orthogonal IL-2 cytokine-receptor complexes. Science *359*, 1037–1042. https://doi.org/10.1126/science.aar3246.

118. Aspuria, P.-J., Vivona, S., Bauer, M., Semana, M., Ratti, N., McCauley, S., Riener, R., de Waal Malefyt, R., Rokkam, D., Emmerich, J., et al. (2021). An orthogonal IL-2 and IL-2Rβ system drives persistence and activation of CAR T cells and clearance of bulky lymphoma. Sci. Transl. Med. *13*, eabg7565. https://doi.org/10.1126/scitranslmed.abg7565.

119. Zhang, Q., Hresko, M.E., Picton, L.K., Su, L., Hollander, M.J., Nunez-Cruz, S., Zhang, Z., Assenmacher, C.-A., Sockolosky, J.T., Garcia, K. C., and Milone, M.C. (2021). 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. *13*, eabg6986. https://doi.org/10.1126/scitranslmed.abg6986.

120. Kalbasi, A., Siurala, M., Su, L.L., Tariveranmoshabad, M., Picton, L.K., Ravikumar, P., Li, P., Lin, J.-X., Escuin-Ordinas, H., Da, T., et al. (2022). Potentiating adoptive cell therapy using synthetic IL-9 receptors. Nature *607*, 360–365. https://doi.org/10.1038/s41586-022-04801-2.

121. Engelowski, E., Schneider, A., Franke, M., Xu, H., Clemen, R., Lang, A., Baran, P., Binsch, C., Knebel, B., Al-Hasani, H., et al. (2018). Synthetic cytokine receptors transmit biological signals using artificial ligands. Nat. Commun. *9*, 2034. https://doi.org/10.1038/s41467-018-04454-8.

122. Batra, S.A., Rathi, P., Guo, L., Courtney, A.N., Fleurence, J., Balzeau, J., Shaik, R.S., Nguyen, T.P., Wu, M.-F., Bulsara, S., et al. (2020). Glypican-3-Specific CAR T Cells Coexpressing IL15 and IL21 Have Superior Expansion and Antitumor Activity against Hepatocellular Carcinoma. Cancer Immunol. Res. *8*, 309–320. https://doi.org/10.1158/2326-6066.CIR-19-0293.

123. Guo, Y., Xie, Y.-Q., Gao, M., Zhao, Y., Franco, F., Wenes, M., Siddiqui, I., Bevilacqua, A., Wang, H., Yang, H., et al. (2021). Metabolic reprogramming of terminally exhausted CD8+ T cells by IL-10 enhances anti-tumor immunity. Nat. Immunol. *22*, 746–756. https://doi.org/10.1038/s41590-021-00940-2.

124. Zhao, Y., Chen, J., Andreatta, M., Feng, B., Xie, Y.-Q., Wenes, M., Wang, Y., Gao, M., Hu, X., Romero, P., et al. (2024). IL-10-expressing CAR T cells resist dysfunction and mediate durable clearance of solid tumors and metastases. Nat. Biotechnol. *42*, 1693–1704. https://doi.org/10.1038/s41587-023-02060-8.

125. Zhang, L., Morgan, R.A., Beane, J.D., Zheng, Z., Dudley, M.E., Kassim, S.H., Nahvi, A.V., Ngo, L.T., Sherry, R.M., Phan, G.Q., et al. (2015). Tumor-infiltrating lymphocytes genetically engineered with an inducible gene encoding interleukin-12 for the immunotherapy of metastatic melanoma. Clin. Cancer Res. *21*, 2278–2288. https://doi.org/10.1158/1078-0432.CCR-14-2085.

126. Lee, E.H.J., Murad, J.P., Christian, L., Gibson, J., Yamaguchi, Y., Cullen, C., Gumber, D., Park, A.K., Young, C., Monroy, I., et al. (2023). Antigen-dependent IL-12 signaling in CAR T cells promotes regional to systemic disease targeting. Nat. Commun. *14*, 4737. https://doi.org/10.1038/s41467-023-40115-1.

127. Hombach, A., Barden, M., Hannappel, L., Chmielewski, M., Rappl, G., Sachinidis, A., and Abken, H. (2022). IL12 integrated into the CAR exodomain converts CD8+ T cells to poly-functional NK-like cells with superior killing of antigen-loss tumors. Mol. Ther. *30*, 593–605. https://doi.org/10.1016/j.ymthe.2021.10.011.

128. Bhatia, V., Kamat, N.V., Pariva, T.E., Wu, L.-T., Tsao, A., Sasaki, K., Sun, H., Javier, G., Nutt, S., Coleman, I., et al. (2023). Targeting advanced prostate cancer with STEAP1 chimeric antigen receptor T cell and tumor-localized IL-12 immunotherapy. Nat. Commun. *14*, 2041. https://doi.org/10.1038/s41467-023-37874-2.

129. Chen, A.X.Y., Yap, K.M., Kim, J.S., Sek, K., Huang, Y.-K., Dunbar, P.A., Wiebking, V., Armitage, J.D., Munoz, I., Todd, K.L., et al. (2025). Rewiring endogenous genes in CAR T cells for tumour-restricted payload delivery. Nature *644*, 241–251. https://doi.org/10.1038/s41586-025-09212-7.

130. Hu, B., Ren, J., Luo, Y., Keith, B., Young, R.M., Scholler, J., Zhao, Y., and June, C.H. (2017). Augmentation of Antitumor Immunity by Human and Mouse CAR T Cells Secreting IL-18. Cell Rep. *20*, 3025–3033. https://doi.org/10.1016/j.celrep.2017.09.002.

131. Avanzi, M.P., Yeku, O., Li, X., Wijewarnasuriya, D.P., van Leeuwen, D.G., Cheung, K., Park, H., Purdon, T.J., Daniyan, A.F., Spitzer, M.H., and Brentjens, R.J. (2018). Engineered Tumor-Targeted T Cells Mediate Enhanced Anti-Tumor Efficacy Both Directly and through Activation of the Endogenous Immune System. Cell Rep. *23*, 2130–2141. https://doi.org/10.1016/j.celrep.2018.04.051.

132. Ng, B.D., Rajagopalan, A., Kousa, A.I., Fischman, J.S., Chen, S., Massa, A., Elias, H.K., Manuele, D., Galiano, M., Lemarquis, A.L., et al. (2024). IL-18-secreting multiantigen targeting CAR T cells eliminate antigen-low myeloma in an immunocompetent mouse model. Blood *144*, 171–186. https://doi.org/10.1182/blood.2023022293.

133. Jaspers, J.E., Khan, J.F., Godfrey, W.D., Lopez, A.V., Ciampricotti, M., Rudin, C.M., and Brentjens, R.J. (2023). IL-18-secreting CAR T cells targeting DLL3 are highly effective in small cell lung cancer models. J. Clin. Investig. *133*, e166028. https://doi.org/10.1172/JCI166028.

134. Ma, X., Shou, P., Smith, C., Chen, Y., Du, H., Sun, C., Porterfield Kren, N., Michaud, D., Ahn, S., Vincent, B., et al. (2020). Interleukin-23 engineering improves CAR T cell function in solid tumors. Nat. Biotechnol. *38*, 448–459. https://doi.org/10.1038/s41587-019-0398-2.

135. Lai, J., Mardiana, S., House, I.G., Sek, K., Henderson, M.A., Giuffrida, L., Chen, A.X.Y., Todd, K.L., Petley, E.V., Chan, J.D., et al. (2020). Adoptive cellular therapy with T cells expressing the dendritic cell growth factor Flt3L drives epitope spreading and antitumor immunity. Nat. Immunol. *21*, 914–926. https://doi.org/10.1038/s41590-020-0676-7.

136. Li, X., Daniyan, A.F., Lopez, A.V., Purdon, T.J., and Brentjens, R.J. (2021). Cytokine IL-36γ improves CAR T-cell functionality and induces endogenous antitumor response. Leukemia *35*, 506–521. https://doi.org/10.1038/s41375-020-0874-1.

137. Adachi, K., Kano, Y., Nagai, T., Okuyama, N., Sakoda, Y., and Tamada, K. (2018). IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. Nat. Biotechnol. *36*, 346–351. https://doi.org/10.1038/nbt.4086.

138. Pang, N., Shi, J., Qin, L., Chen, A., Tang, Y., Yang, H., Huang, Y., Wu, Q., Li, X., He, B., et al. (2021). IL-7 and CCL19-secreting CAR-T cell therapy for tumors with positive glypican-3 or mesothelin. J. Hematol. Oncol. *14*, 118. https://doi.org/10.1186/s13045-021-01128-9.

139. Rafiq, S., Yeku, O.O., Jackson, H.J., Purdon, T.J., van Leeuwen, D.G., Drakes, D.J., Song, M., Miele, M.M., Li, Z., Wang, P., et al. (2018). Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy in vivo. Nat. Biotechnol. *36*, 847–856. https://doi.org/10.1038/nbt.4195.

140. Kloss, C.C., Lee, J., Zhang, A., Chen, F., Melenhorst, J.J., Lacey, S.F., Maus, M.V., Fraietta, J.A., Zhao, Y., and June, C.H. (2018). Dominant-Negative TGF-β Receptor Enhances PSMA-Targeted Human CAR T Cell Proliferation And Augments Prostate Cancer Eradication. Mol. Ther. *26*, 1855–1866. https://doi.org/10.1016/j.ymthe.2018.05.003.

141. Cherkassky, L., Morello, A., Villena-Vargas, J., Feng, Y., Dimitrov, D.S., Jones, D.R., Sadelain, M., and Adusumilli, P.S. (2016). Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. J. Clin. Investig. *126*, 3130–3144. https://doi.org/10.1172/JCI83092.

142. Yi, F., Cohen, T., Zimmerman, N., Dündar, F., Zumbo, P., Eltilib, R., Brophy, E.J., Arkin, H., Feucht, J., Gormally, M.V., et al. (2025). CAR-engineered lymphocyte persistence is governed by a FAS ligand–FAS autoregulatory circuit. Nat. Cancer. https://doi.org/10.1038/s43018-025-01009-x.

143. Wilkie, S., Burbridge, S.E., Chiapero-Stanke, L., Pereira, A.C.P., Cleary, S., van der Stegen, S.J.C., Spicer, J.F., Davies, D.M., and Maher, J. (2010). Selective expansion of chimeric antigen receptor-targeted T-cells with potent effector function using interleukin-4. J. Biol. Chem. *285*, 25538–25544. https://doi.org/10.1074/jbc.M110.127951.

144. Qin, L., Cui, Y., Yuan, T., Chen, D., Zhao, R., Li, S., Jiang, Z., Wu, Q., Long, Y., Wang, S., et al. (2022). Co-expression of a PD-L1-specific chimeric switch receptor augments the efficacy and persistence of CAR T cells via the CD70-CD27 axis. Nat. Commun. *13*, 6051. https://doi.org/10.1038/s41467-022-33793-w.

145. Sukumaran, S., Watanabe, N., Bajgain, P., Raja, K., Mohammed, S., Fisher, W.E., Brenner, M.K., Leen, A.M., and Vera, J.F. (2018). Enhancing the Potency and Specificity of Engineered T Cells for Cancer Treatment. Cancer Discov. *8*, 972–987. https://doi.org/10.1158/2159-8290.CD-17-1298.

146. Chang, Z.L., Lorenzini, M.H., Chen, X., Tran, U., Bangayan, N.J., and Chen, Y.Y. (2018). Rewiring T-cell responses to soluble factors with chimeric antigen receptors. Nat. Chem. Biol. *14*, 317–324. https://doi.org/10.1038/nchembio.2565.

147. Katsarou, A., Sjöstrand, M., Naik, J., Mansilla-Soto, J., Kefala, D., Kladis, G., Nianias, A., Ruiter, R., Poels, R., Sarkar, I., et al. (2021). Combining a CAR and a chimeric costimulatory receptor enhances T cell sensitivity to low antigen density and promotes persistence. Sci. Transl. Med. *13*, eabh1962. https://doi.org/10.1126/scitranslmed.abh1962.

148. Chuprin, J., Buettner, H., Seedhom, M.O., Greiner, D.L., Keck, J.G., Ishikawa, F., Shultz, L.D., and Brehm, M.A. (2023). Humanized mouse models for immuno-oncology research. Nat. Rev. Clin. Oncol. *20*, 192–206. https://doi.org/10.1038/s41571-022-00721-2.

149. Norelli, M., Camisa, B., Barbiera, G., Falcone, L., Purevdorj, A., Genua, M., Sanvito, F., Ponzoni, M., Doglioni, C., Cristofori, P., et al. (2018). Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. Nat. Med. *24*, 739–748. https://doi.org/10.1038/s41591-018-0036-4.

150. Giavridis, T., van der Stegen, S.J.C., Eyquem, J., Hamieh, M., Piersigilli, A., and Sadelain, M. (2018). CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. Nat. Med. *24*, 731–738. https://doi.org/10.1038/s41591-018-0041-7.

151. Manni, S., Del Bufalo, F., Merli, P., Silvestris, D.A., Guercio, M., Caruso, S., Reddel, S., Iaffaldano, L., Pezzella, M., Di Cecca, S., et al. (2023). Neutralizing IFNγ improves safety without compromising efficacy of CAR-T cell therapy in B-cell malignancies. Nat. Commun. *14*, 3423. https://doi.org/10.1038/s41467-023-38723-y.

152. Srivastava, S., Furlan, S.N., Jaeger-Ruckstuhl, C.A., Sarvothama, M., Berger, C., Smythe, K.S., Garrison, S.M., Specht, J.M., Lee, S.M., Amezquita, R.A., et al. (2021). Immunogenic Chemotherapy Enhances Recruitment of CAR-T Cells to Lung Tumors and Improves Antitumor Efficacy when Combined with Checkpoint Blockade. Cancer Cell *39*, 193–208.e10. https://doi.org/10.1016/j.ccell.2020.11.005.

153. Agliardi, G., Liuzzi, A.R., Hotblack, A., De Feo, D., Núñez, N., Stowe, C.L., Friebel, E., Nannini, F., Rindlisbacher, L., Roberts, T.A., et al. (2021). Intratumoral IL-12 delivery empowers CAR-T cell immunotherapy in a pre-clinical model of glioblastoma. Nat. Commun. *12*, 444. https://doi.org/10.1038/s41467-020-20599-x.

154. Uslu, U., Sun, L., Castelli, S., Finck, A.V., Assenmacher, C.A., Young, R.M., Chen, Z.J., and June, C.H. (2024). The STING agonist IMSA101 enhances chimeric antigen receptor T cell function by inducing IL-18 secretion. Nat. Commun. *15*, 3933. https://doi.org/10.1038/s41467-024-47692-9.

155. Johnson, L.R., Lee, D.Y., Eacret, J.S., Ye, D., June, C.H., and Minn, A.J. (2021). The immunostimulatory RNA RN7SL1 enables CAR-T cells to enhance autonomous and endogenous immune function. Cell *184*, 4981–4995.e14. https://doi.org/10.1016/j.cell.2021.08.004.

156. Nyberg, W.A., Wang, C.H., Ark, J., Liu, C., Clouden, S., Qualls, A., Caryotakis, S., Wells, E., Simon, K., Garza, C., et al. (2025). In vivo engineering of murine T cells using the evolved adeno-associated virus variant Ark313. Immunity *58*, 499–512.e7. https://doi.org/10.1016/j.immuni.2025.01.009.

157. Davila, M.L., Kloss, C.C., Gunset, G., and Sadelain, M. (2013). CD19 CAR-targeted T cells induce long-term remission and B Cell Aplasia in an immunocompetent mouse model of B cell acute lymphoblastic leukemia. PLoS One *8*, e61338. https://doi.org/10.1371/journal.pone.0061338.

158. Kochenderfer, J.N., Yu, Z., Frasheri, D., Restifo, N.P., and Rosenberg, S.A. (2010). Adoptive transfer of syngeneic T cells transduced with a chimeric antigen receptor that recognizes murine CD19 can eradicate lymphoma and normal B cells. Blood *116*, 3875–3886. https://doi.org/10.1182/blood-2010-01-265041.

159. Pennell, C.A., Barnum, J.L., McDonald-Hyman, C.S., Panoskaltsis-Mortari, A., Riddle, M.J., Xiong, Z., Loschi, M., Thangavelu, G., Campbell, H.M., Storlie, M.D., et al. (2018). Human CD19-Targeted Mouse T Cells Induce B Cell Aplasia and Toxicity in Human CD19 Transgenic Mice. Mol. Ther. *26*, 1423–1434. https://doi.org/10.1016/j.ymthe.2018.04.006.

160. Ishii, K., Pouzolles, M., Chien, C.D., Erwin-Cohen, R.A., Kohler, M.E., Qin, H., Lei, H., Kuhn, S., Ombrello, A.K., Dulau-Florea, A., et al. (2020). Perforin-deficient CAR T cells recapitulate late-onset inflammatory toxicities observed in patients. J. Clin. Investig. *130*, 5425–5443. https://doi.org/10.1172/JCI130059.