

Review

Harnessing the chemokine system to home CAR-T cells into solid tumors

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SUMMARY

CAR-T cell therapy has been heralded as a breakthrough in the field of immunotherapy, but to date, this success has been limited to hematological malignancies. By harnessing the chemokine system and taking into consideration the chemokine expression profile in the tumor microenvironment, CAR-T cells may be homed into tumors to facilitate direct tumor cell cytotoxicity and overcome a major hurdle in generating effective CAR-T cell responses to solid cancers.

INTRODUCTION

The solid tumor microenvironment is highly complex, housing a plethora of tumor-infiltrating immune cells, reprogrammed stromal cells, and highly heterogeneous malignant cells in a densely barricaded and hostile tissue architecture. These features play critical roles in facilitating or restricting anti-tumor immune responses and in the successful application of chimeric antigen receptor (CAR)-T cell therapies. The trafficking of CAR-T cells into tumors is considered one of the first challenges in developing effective CAR-T cell therapy against solid cancers, as there is often a mismatch or lack of the appropriate migratory signals.

The chemokine system naturally plays a crucial role in the migration of hematopoietic cells during cellular development and effector function,^{1,2} which can be harnessed to home CAR-T cells directly to solid tumors. Many tumors derived from different tissue origins share similar patterns of chemokine ligand expression, raising the possibility of enhancing CAR-T cell trafficking and infiltration into a diverse array of cancers using select chemokine receptors.^{3,4} Directed trafficking of CAR-T cells into tumors also provides significant safety advantages as it lowers the required cell dose and reduces dissemination of CAR-T cells into the periphery where on-target off-tumor toxicity may occur. In this review, we discuss the current landscape of CAR-T cell therapies against solid cancers with emphasis on the mechanisms by which the architecture of solid tumors prevent T cell entry, the key chemokine axes relevant in tumor microenvironments, and how we might enhance CAR-T cell therapies by harnessing these chemokine axes.

CHEMOKINE-MEDIATED CONTROL OF IMMUNE RESPONSES

Chemokine system overview

The immune system is fundamental in protecting living organisms against invading pathogens, clearing dysfunctional or

defective cells, and participating in wound repair. These biological processes rely on the intricate and precise spatiotemporal migration of functionally diverse immune cell subsets to sub-anatomical niches.

The choreographed migration of immune cells is largely orchestrated by the chemokine superfamily; a group of small, secreted proteins that function in both homeostatic cellular trafficking and the controlled recruitment of specific cells in response to inflammatory signals. Chemokines also have important roles in the positioning of immune cells within tissues and in lymphoid tissue development,^{1–3} ensuring cells receive the correct signals to develop and function. Chemokines act as ligands for a family of cognate receptors that are mainly expressed by cells of hematopoietic origin to migrate toward increasing gradients of their respective chemokines. Structurally, conventional chemokine receptors are seven-transmembrane G protein-coupled receptors containing a canonical and highly conserved DRYLAIV motif in the third intracellular loop, which couples to heterotrimeric G proteins to initiate downstream signaling cascades. These signals lead to cell polarization, remodeling of the actin cytoskeleton, and conformational changes in integrins, which together stimulate directed cell migration and haptotaxis.^{5,6} Aside from cell migration, chemokine-mediated signaling and activation can directly transduce intracellular signaling pathways involved in cell proliferation and survival.⁴ Chemokine receptor activity is tightly regulated, with chemokine binding often leading to desensitization and internalization of receptors.⁴ The chemokine system is further regulated by atypical chemokine receptors (ACKRs), which are structurally distinguished from conventional chemokine receptors by an absent or otherwise modified DRYLAIV motif. This uncouples ACKRs from classical G protein-mediated signaling and results in ACKRs fulfilling a distinct role in regulating chemokine bioavailability by scavenging and internalizing mutual chemokine ligands to which conventional chemokine receptors bind.^{7–9} The tightly regulated spatiotemporal expression of both conventional chemokine receptors and ACKRs defines migratory



patterns at certain stages of cellular development and effector function, including immune responses in solid tumors. Within the tumor microenvironment (TME), it has been well-established that distinct chemokine receptors recruit a variety of leukocyte subsets into tumors, with naive T cells recruited via CCR7, effector T cells recruited via CXCR3/CCR5/CCR2/CCR6, monocytes and macrophages recruited via CCR2/CCR5, myeloid-derived suppressor cells (MDSCs) recruited via CXCR1/CXCR2/CXCR4/CCR2, and neutrophils recruited via CCR2/CXCR2.^{3,10} However, solid tumors utilize many mechanisms to evade the infiltration of anti-tumorigenic leukocytes, which will be discussed below.

Inefficient trafficking and penetration of cytotoxic T cells into solid tumors

In many cancers, limited T cell infiltration of tumors, particularly by CD8⁺ T cells, is correlated with poor clinical outcomes, whereas enhanced recruitment of T cells into the TME is strongly correlated with improved survival.^{11–16} Tumors exercise many mechanisms to restrict T cell entry and penetration. Physical barriers such as abnormal tumor vasculature and a densely packed extracellular matrix are frequently observed in the stroma of the TME, sequestering T cells outside the tumor parenchyma and preventing their direct contact with tumor cells.^{17,18} Type I collagen and fibronectin are the main structural components of the extracellular matrix of tumor stroma, forming fibers that dictate the trajectory and speed of T cell migration. *Ex vivo* imaging of viable sections of human non-small cell lung cancer (NSCLC) revealed that T cells migrate along fibers running parallel to blood vessels and epithelial regions, and that the presence of dense fibers immediately adjacent to tumor islets spatially blocks T cell entry.¹⁸ Furthermore, tumor vasculature frequently exhibits reduced expression of chemokines and adhesion molecules compared with normal vasculature, which limits T cell arrest in tumors.¹⁹ Other components within the tumor stroma and parenchyma may also sequester and distract T cells from forming contact with tumor cells.¹⁸ For instance, two-photon imaging of tumors in a methylcholanthrene (MCA)-induced ovalbumin (OVA)-positive fibrosarcoma mouse model revealed that dendritic cells (DCs) in the tumor parenchyma locally trap T cells by forming long-lasting antigen-specific interactions.²⁰ Similarly, in a spontaneous MMTV-PyMT mammary carcinoma mouse model, CD68⁺ CD11c⁺ macrophages in the TME formed long-lasting interactions with CD8⁺ T cells resulting in reduced motility and hostage of CD8⁺ T cells in the tumor stroma.¹⁶

Mismatch of migratory signaling elements, particularly between the chemokine profile expressed by tumors and chemokine receptor profile expressed by antigen-specific T cells, is a significant obstacle in T cell trafficking to tumors. By understanding the role of key chemokine receptor axes, these axes may be perceptively co-opted to recruit immune cell subsets to specific sub-anatomical niches, such as solid cancers. Some studies have also demonstrated that CD8⁺ effector T cells may bypass the requirement for chemokines, by expressing high levels of LFA-1 to form stable adhesions within endothelial vessels in non-tumorigenic inflammatory settings.^{21,22} However, significant evidence highlights chemokines as the main mediators of T cell trafficking into tumors. For instance, gene expression profiling of

44 human metastatic melanoma biopsies showed a significant correlation between T cell-associated genes and chemokine genes. Further RT-PCR and protein arrays revealed that the chemokines CCL2, CCL3, CCL4, CCL5, and CXCL9 were more highly expressed in tumors with increased T cell presence, implying that these chemokines likely promote T cell infiltration.²³ Conversely, poor infiltration of low-grade gliomas and melanomas by CD8⁺ T cells was significantly correlated with decreased expression of the CXCR3 ligands, CXCL9, CXCL10, and CXCL11 and the adhesion molecule ICAM-1 but had no correlation to immunogenic antigens in humans.^{23,24} Indeed, CXCR3 signaling is frequently downregulated in the TME in many cancers. In a mouse model of human primary ovarian cancer, it was found that epigenetic silencing of T_H1-type chemokine expression, mediated by histone modifications and DNA methylation, in tumor cells resulted in diminished effector T cell trafficking into the TME. Administration of epigenetic modifiers to prevent T_H1-type chemokine suppression enhanced intratumoral T cell infiltration, and this effect was abrogated when combined with anti-CXCR3.¹⁵ Furthermore, immunoediting by IFN γ was demonstrated to select for tumor variants that had lost CXCL9 expression in MCA-induced fibrosarcoma and melanoma models.²⁵ CXCR3 expression by CD8⁺ T cells was also shown to be suppressed by TGF β , restricting tumor infiltration in preclinical colorectal cancer mouse models.²⁶ These studies are consistent with findings in mouse melanoma models in which adoptively transferred CXCR3-deficient OT-I cells displayed reduced infiltration into tumors and decreased control of tumor growth compared with WT OT-I cells.^{27,28} In one of these studies, it was found CXCR3 was involved in stabilizing intravascular adhesion and extravasation of adoptively transferred effector cells through the tumor-vascular interface.²⁸ In contrast, the expression of certain chemokines has been associated with T cell exclusion from tumor islets. For example, CXCL12 derived from cancer-associated fibroblasts was found to limit intratumoral T cell infiltration.²⁹ This may be due to the chemo-repulsive effect of particularly high concentrations of CXCL12, as observed in a mouse melanoma model,³⁰ or by CXCL12-mediated sequestration of T cells in the stroma of the TME.^{16,31}

Chemokines in the tumor microenvironment

The TME consists of a complex interplay of cells, the recruitment of which is orchestrated by the expression of specific chemokines. These chemokines can be expressed by tumor cells, stromal cells, and infiltrating leukocytes. The specific chemokine profile expressed dictates to a large extent which immune cell subsets migrate into the TME and thus contributes to the type of immune response initiated. Aside from mediating trafficking into the TME, chemokines have also been demonstrated to directly regulate tumor cell proliferation, invasiveness, and metastasis.³

CD8⁺ T cells, T_H1 cells, and NK cells represent major players in anti-tumor immunity, with these cells commonly expressing the chemokine receptor CXCR3. CXCR3 on circulating T cells and expression of CXCL9 and CXCL10 in tumor sites has been extensively correlated with increased accumulation of intratumoral T cells and improved survival in many cancers.^{12,23,32,33} In an extensive meta-analysis of 5,953 carcinoma specimens from a range of solid tumors including breast, colorectal, ovarian, lung,

and melanoma; CXCL9, CXCL10, and CXCL11 were positively correlated with the density of tumor-infiltrating CD8⁺ T cells, T_H1 cells, and NK cells but were inversely correlated with neutrophils at the mRNA level.³⁴ CXCR3 ligands are expressed by monocytes, endothelial cells, fibroblasts, DC subsets, and certain cancer cells in response to IFN γ provided by T_H1 cells, cytotoxic T cells, and NK cells.^{35,36} However, a study that sorted YFP-labeled tumor cells, non-hematopoietic CD45⁻ stromal cells, and distinct populations of APCs in an autochthonous murine melanoma tumor model identified Batf3⁺ CD103⁺ cDC1s as the predominant source of CXCL9 and CXCL10. Chemokine production by CD103⁺ cDC1s recruited anti-tumor CD8⁺ T cells to tumors,³⁷ but this was dampened by tumor-intrinsic Wnt/ β -catenin pathway activation, which deterred CD103⁺ DC recruitment into the TME. These DCs were also found to be CCR5⁺ and recruited to tumors via CCL4 expression by malignant cells.³⁸ Given the crucial role of CXCL9 and CXCL10 in T cell intratumoral trafficking, several approaches have been explored to increase CXCR3 ligand expression in the TME. This has been achieved via direct delivery methods such as intratumoral injection of recombinant protein or vaccinia virus, which resulted in subsequent tumor regression.^{39,40} Furthermore, co-administration of a specific inhibitor against macrophages and anti-PD-1 was shown to increase CXCL9 and CXCL10 levels in tumors,¹⁶ and delivery of an oncolytic viral vaccine expressing GM-CSF resulted in a significant increase in CXCL10 and CXCL2.⁴¹

In addition to CXCR3, CCR2 and CCR5 have also been implicated in T cell trafficking to tumors. Many cancers constitutively express the ligand for CCR2, CCL2, as a result of chronic activation of the NF- κ B pathway. The expression of inflammatory mediators IL-1, IL-6, TNF α and the immunosuppressive cytokine TGF β may further promote CCL2 expression.⁴² CCL2 can be expressed by a diverse range of cells in the TME including endothelial cells, epithelial cells, fibroblasts, myeloid cells, T cells, and tumor cells.⁴³ CCL2 has been correlated with tumor progression⁴⁴ and attracts immunosuppressive and pro-metastatic TAMs and MDSCs in many cancers including glioblastoma, ovarian, lung, and breast cancers.^{45,46} CCR2 is also used by T_{reg} cells to traffic from tumor-draining lymph nodes into tumors, as demonstrated in murine breast cancer models, and CCR2⁺ T_{reg} cells were found to be enriched in tumors in oral squamous cell carcinoma patients.⁴⁶ Meanwhile, CCL5 has been detected in many hematological malignancies and solid tumors, playing several pro-tumorigenic roles such as stimulating angiogenesis, promoting metastasis, and driving recruitment of stromal and inflammatory cells. Similar to CCL2, CCL5 is also driven by activation of the NF- κ B pathway. CCL5 is expressed by T cells, macrophages, platelets, and certain malignant cells.⁴⁷ Despite high concentrations of CCL2 and CCL5 in the tumor intravascular space and functional chemokine receptor expression by T cells, CCR2 and CCR5 were found to be redundant in intratumoral T cell trafficking, as ablation of these receptors on adoptively transferred OT-I cells did not compromise their entry into tumors in a mouse melanoma model.²⁸ However, CCL5 may elicit anti-tumorigenic activity in other ways. Integral to optimal anti-tumor responses are cDC1s that cross-present tumor antigens to antigen-specific CD8⁺ T cells in tumor-draining lymph nodes,^{48,49} recruit tumor-infiltrating T cells,³⁷ and provide support to T cell function by

secreting IL-12.⁵⁰ It was recently shown cDC1 recruitment is largely dependent on the secretion of CCL5 and XCL1 by tumor-infiltrating NK cells. However, tumor-derived prostaglandin E2 may block cDC1 recruitment by impairing NK cell viability and chemokine production and downregulating CCR5 and XCR1 chemokine receptor expression by cDC1 cells.⁵¹

Other chemokines produced by tumors drive recruitment of leukocyte subsets that promote tumor growth. For instance, neutrophils and MDSCs are recruited into the TME by CXCL1, CXCL5, CXCL6, CXCL8, and CXCL12.^{52,53} Similar to CCL2 and CCL5, constitutive activation of NF- κ B in many tumors drives production of CXCL8, a ligand for CXCR1 and CXCR2.⁵⁴ CXCL8 can significantly impact tumor cell biology, enhancing proliferative, invasive, and migratory capacities of tumor cells through activation of phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways.⁵⁵ CXCL8 produced by melanoma cells was shown to promote transendothelial migration and lung metastasis by interacting with β 2-integrin and ICAM-1, anchoring tumor cells to the vascular endothelium.⁵⁶ Tumor-derived CXCL8 also sequesters CXCR1⁺ and CXCR2⁺ DCs in tumors, preventing egress and tumor antigen presentation in lymph nodes.⁵⁷ Other CXCR2 ligands, CXCL1 and CXCL2, were found to promote breast cancer metastasis by enhancing tumor cell survival via recruitment of CD11b⁺ Gr1⁺ myeloid cells. These myeloid cells secreted alarmins and myeloid-related proteins 8 and 14 to attract monocytes that further support cancer cell survival.^{58,59} Furthermore, tumor-derived CXCL8 was shown to induce NET formation by granulocytic MDSCs and neutrophils, which has a demonstrated role in capturing circulating cells and facilitating metastasis.⁶⁰⁻⁶² Expectedly, given the many pro-tumorigenic roles of CXCL8, the expression of CXCL8 has been detected in many cancers.⁶³⁻⁷⁰

The bioavailability of chemokines in the TME may be further regulated by atypical chemokine receptors such as ACKR4. By scavenging CCL21 in tumors, ACKR4 expressed by gp38⁺ CD31⁻ fibroblasts in the tumor stroma was found to regulate CCR7⁺ CD103⁺ cDC1 migration into the TME in a murine breast cancer model. Loss of ACKR4 resulted in DC retention in tumors due to accumulation of CCL21, thereby limiting DC egress to tumor-draining lymph nodes and potentially promoting local anti-tumor CD8⁺ T cell responses.⁹ Aside from ACKR4, ACKR2 and ACKR3 also have demonstrated roles in shaping tumor biology via chemokine scavenging.^{71,72}

In summary, chemokines act to orchestrate migration of diverse leukocyte subsets into the TME, the cellular functions of which dictate the type of immune response generated. The influence of this specific immune response on tumors is now appreciated to have important consequences for driving or inhibiting tumorigenesis. The next section will explore how anti-tumor immune responses may be enhanced therapeutically in the form of cancer immunotherapy.

ENHANCING CAR-T CELL INFILTRATION INTO SOLID TUMORS

Immunotherapy overview

Until relatively recently, intense focus of cancer therapies was directed toward tumor-intrinsic mechanisms, such as targeting

the hyperproliferative nature of tumors using chemotherapy and radiotherapy. However, as understanding of the role of the TME and immune contexture has improved, more specific and novel targets have been identified. For instance, tumors frequently exploit checkpoint pathways to dampen anti-tumor T cell activation and function. Checkpoint inhibitor therapy is a class of immunotherapy that acts to prevent this exploitation, which has received significant attention in the past two decades.

T cell-targeted modulators such as α -CTLA-4 and α -PD-1 are currently used as a first or second line of treatment in around 50 cancer types, particularly metastatic melanoma. However, despite long-lasting remission and disappearance of detectable metastases in 20% of metastatic melanoma patients, a large proportion of patients remain partially responsive or non-responsive.^{73,74} An analysis of 209 advanced melanoma patients identified multiple parameters that correlate with prolonged survival following ipilimumab (α -CTLA-4) treatment, including low baseline serum lactate dehydrogenase levels and monocyte, eosinophil, and lymphocyte abundance.⁷⁵ Furthermore, CyTOF analysis of peripheral blood from melanoma patients identified predictive biomarkers for non-responders to α -PD-1 and α -CTLA-4 therapies including an absence of tumor-infiltrating T cells and CD69⁺ CCL4-secreting NK cells.⁷⁶ This is consistent with the correlation between high levels of IFN γ and IFN γ -inducible genes, such as CXCL9, with α -PD-L1 responsiveness in melanoma, NSCLC, and renal cell carcinoma patients.⁷⁷ It is also consistent with the finding that the anti-tumor immune response induced by α -PD-1 is dependent on CXCL9 produced by intratumoral CD103⁺ DCs, which potentially facilitates CXCR3-dependent DC-T cell interactions in the TME.⁷⁸ Furthermore, single cell analysis of 16,291 individual immune cells from 48 melanoma patients treated with checkpoint inhibitor immunotherapy identified a subset of Tcf7⁺ TIM-3⁻ CD39⁻ CD8⁺ tumor-infiltrating lymphocytes that respond to α -PD-1 immunotherapy.⁷⁹ While the stratification of patients likely to respond to α -PD-1, α -CTLA-4, and related therapies using these parameters may simplify the treatment process, it emphasizes a great need for alternative methods to treat patients who are unresponsive or acquire resistance to such therapies.

Adoptive T cell therapies represent a promising avenue, utilizing *ex vivo* activated patient-derived cells, which have been expanded to sufficient numbers for therapeutic use. Pioneering work by Rosenberg and colleagues demonstrated that IL-2-expanded T cells significantly inhibited tumor progression in a mouse model of colorectal cancer and in patients with metastatic melanoma.^{80,81} However, antigen-specific T cell responses remained a key obstacle, due to the lack of tumor antigen recognition and MHC class I downregulation by tumors. More recent achievements in adoptive T cell therapy have circumvented the need for MHC-dependent activation, achieving antigen-specific recognition of tumor cells in the form of CAR-T cell therapy. Advancements within this field will be discussed in detail in the next section.

CAR-T cell therapy directed toward hematological and solid cancers

CD19 was the first of a series of B cell markers to be targeted with CAR-T cell therapy to treat a range of hematological malig-

nancies. CD19 is widely expressed on B cells in both mice and humans and is a co-receptor involved in fine-tuning pre-BCR/BCR signaling and B cell fate decisions.⁸² Clinical trials of CD19-targeting CAR-T cells in B cell acute lymphoblastic leukemia (B-ALL) demonstrated unprecedented clinical responses, reaching 80%–93% complete remission rates in patients with relapsed or refractory B-ALL^{83,84} and a long-term remission of 20 months in patients with a low disease burden of relapsed B-ALL.⁸⁵ The unprecedented success observed in these clinical trials culminated in the FDA approval of tisagenlecleucel (Kymriah; Novartis), a CD19-targeting CAR-T cell therapy for the treatment of relapsed or refractory B-ALL in 2017.⁸⁶ This was followed with the FDA approval of axicabtagene ciloleucel (Yescarta; Gilead) to treat large B cell lymphoma also in 2017 and brexucabtagene autoleucel (Tecartus; Gilead) to treat mantle cell lymphoma in 2020. Most recently in February 2021, lisocabtagene maraleucel (Breyanzi; Bristol Myers Squibb) was approved for patients with relapsed or refractory large B cell lymphoma.⁸⁷ All of these therapies are directed toward CD19.

Despite remarkable clinical outcomes of CAR-T cell therapy against hematological malignancies, this success has not yet translated to solid tumors. Solid tumors present numerous challenges that are absent in disseminated hematological cancers such as: heterogeneous antigen expression, physical and molecular barriers that block T cell entry, acidic and hypoxic conditions, and a highly immunosuppressive TME.⁸⁸ These features are illustrated in Figure 1. While there are a growing number of clinical trials currently investigating CAR-T cell therapies against solid tumors, this is disproportionate to the large number of those investigating these therapies in hematological cancers.⁸⁹ Meta-analysis of 22 clinical studies revealed a 9% overall response rate following CAR-T cell treatment in patients with solid tumors.⁹⁰ The first challenge in developing an effective CAR-T cell therapy against solid cancers is the identification of suitable tumor antigens that are either not expressed or expressed at lower levels on healthy cells to avoid on-target off-tumor complications. Consideration of both the identity and antigen density of a select tumor antigen is crucial in developing an effective CAR-T cell therapy as target antigen density on tumor cells has been positively correlated with increased activation and cytokine production of CAR-T cells.⁸⁹ However, antigen density on malignant cells relative to healthy cells also dictates the optimal affinity of the CAR such that healthy cells can be spared from CAR-T cell-mediated cytotoxicity. It is also important to emphasize that although there has been limited success reported in clinical trials of CAR-T cell therapies against solid cancers, most are in phase I trials, with the primary objective to test safety, dosing, and engraftment rather than efficacy. The most commonly targeted solid tumor antigens in current preclinical and clinical trials include GD2, EGFR, ROR1, PSMA, EpCAM, HER2, and mesothelin. The results from preclinical and clinical testing of CAR-T cells targeting some of these antigens are described in detail below. However, as this list is non-exhaustive, further information on CAR-T cell clinical trials against solid cancers can be found at <http://www.clinicaltrials.gov>.

GD2 is a ganglioside abundantly expressed on neuroblastoma cells, which has been the subject of many clinical trials of CAR-T

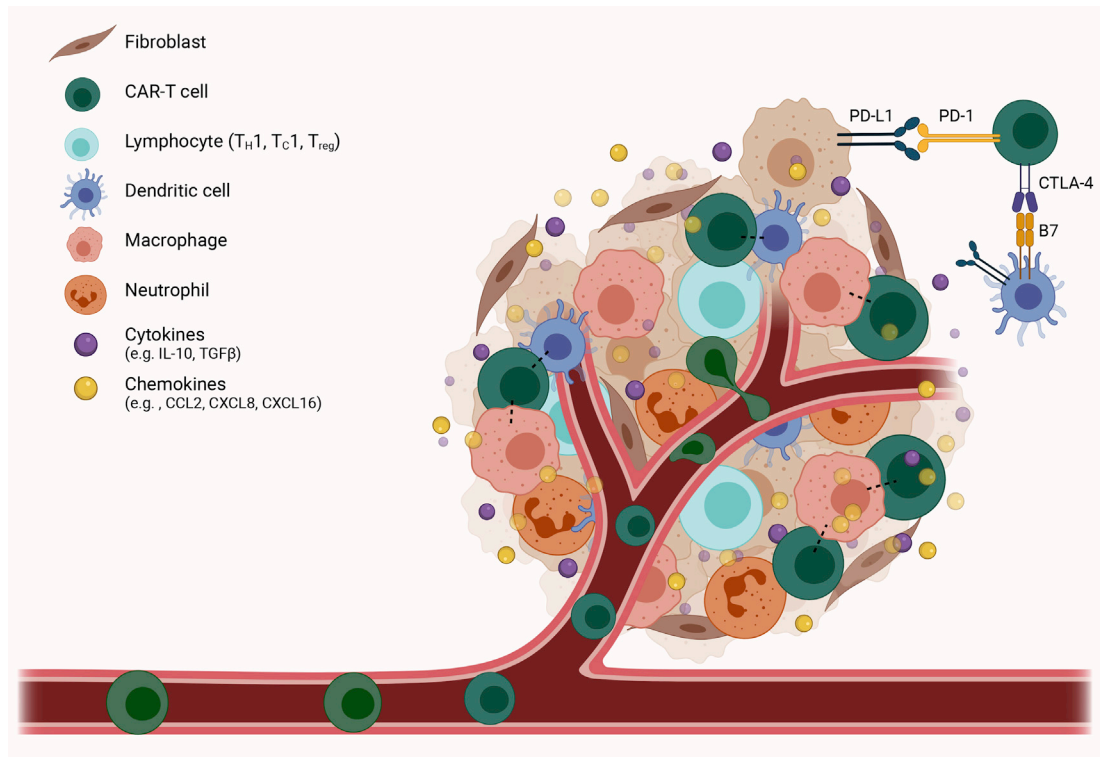


Figure 1. CAR-T cells versus the solid tumor microenvironment

Successful CAR-T cell therapy against solid tumors requires numerous challenges to be met. The initial challenge lies in the identification of a suitable target antigen, which is crucial as solid tumors exist as highly heterogeneous populations, with significant variability in the presence and expression level of specific antigens. Physical barriers, aberrant vasculature, and mismatched migratory signals prevent spatial recognition of tumors by CAR-T cells and block their infiltration into the tumor parenchyma. Macrophages and DCs may also sequester CAR-T cells in the tumor-associated stroma, preventing intratumoral infiltration by forming long-lasting interactions with T cells. After overcoming these many challenges, CAR-T cells that are able to infiltrate the TME are faced with strong immunosuppression perpetuated by recruited T_{reg} cells, MDSCs, neutrophils, and an inhibitory cytokine milieu. Acidification, hypoxia, and glucose deprivation further detrimentally impact T cell survival and effector function in the TME. Underpinning the whole process are requisite CAR-T cell-intrinsic properties such as the ability to specifically recognize and effectively kill tumor cells, self-renew, combat, or ignore immunosuppressive pressures and persist long-term *in vivo*. T_H1 , $CD4^+$ T helper type 1 cell; T_C1 , $CD8^+$ T cytotoxic (type 1) cell; T_{reg} , regulatory T cell; dashed lines represent direct interaction leading to T cell sequestration

cell therapy. Neuroblastoma is an aggressive and debilitating form of childhood brain cancer with a 5-year survival rate of only around 50% with current treatments. GD2 is also expressed by melanoma, lung cancer, and other brain cancer subtypes.⁹¹ However, low level expression by the basal regions of the brain, cerebellum, and peripheral nerves have resulted in fatal toxicity in preclinical, and possibly, clinical trials. For instance, in a preclinical model of human neuroblastoma xenografts in immunocompromised NOD-*scid* IL2R γ^{null} (NSG) mice, extensive infiltration and proliferation of high affinity GD2-targeting CAR-T cells resulted in lethal neuronal destruction, which was also observed in non-tumor-bearing mice injected with CAR-T cells.⁹² However, this has been challenged by a separate group that used a CAR with the same high affinity GD2 binder and reported no neurotoxicity in NSG mice.⁹³ Safety of GD2-targeting CAR-T cells has also been demonstrated in several clinical trials.^{94,95} To improve safety, a recent clinical trial designed and tested GD2-targeting CAR-T cells with an intermediate affinity GD2 binding domain and “suicide” gene composed of two epitopes that bind to the α -CD20 antibody, rituximab, in neuroblastoma patients. Despite evidence of CAR-T cell activation and anti-tumor responses in a

proportion of these patients, no neurological toxicity was observed. However, in this study, none of the patients showed objective clinical responses 28 days after infusion,⁹¹ highlighting the need to promote CAR-T cell persistence, which has been a recurring challenge in developing effective GD2-directed CAR-T cell therapies.^{91,95,96}

EGFR is a transmembrane receptor tyrosine kinase expressed in tissues including the skin, kidneys, and gastrointestinal tract, which is abnormally upregulated in numerous epithelial tumors, such as lung, pancreatic, colorectal, breast, and head and neck squamous cell carcinomas.⁹⁷ Overexpression of EGFR by tumors is correlated with tumor progression and has been the focus of numerous cancer therapies, including CAR-T cell therapy. The most common oncogenic mutant of EGFR is EGFR variant III (EGFRvIII), which is expressed in 24%–67% of glioblastomas, a highly aggressive subtype of brain tumors. CAR-T cells targeting EGFRvIII were found to produce IFN γ and lyse glioma stem cell lines in response to tumor antigen stimulation.⁹⁸ Furthermore, in preclinical mouse models of glioblastoma, delivery of EGFRvIII-targeting CAR-T cells reduced tumor growth and prolonged survival compared with irrelevant, control CD19-targeting CAR-T

cells when tumors were injected subcutaneously. MRI following intracranial implantation of tumor cells revealed significant reduction in the tumor mass, which was associated with enhanced CAR-T cell presence in the bone marrow, spleen, and brain.⁹⁹ However, despite promising preclinical results, limited success has been reported in clinical trials, with one trial reporting a 6% complete response rate in patients with EGFR-positive, advanced biliary tract cancers¹⁰⁰ and another reporting a 5.6% 6-month progression-free survival rate in patients with glioblastoma.¹⁰¹

The receptor tyrosine kinase-like orphan receptor 1 (ROR1) has also received attention as a target of CAR-T cell therapy as it is expressed by a range of cancers including B-lymphoid cancers and epithelial cancers such as lung, breast, colorectal, pancreatic, and ovarian. It is also expressed by some immature B cell precursors and adipocytes, but it is not expressed on normal mature B cells. ROR1-directed CAR-T cells effectively lysed ROR1⁺ breast cancer and kidney cancer cell lines *in vitro* and inhibited tumor growth to the same extent as CD19-targeting CAR-T cells in a human mantle cell lymphoma mouse model.¹⁰² ROR1-directed CAR-T cells have also demonstrated potent anti-tumor activity in three-dimensional microphysiologic A549 lung and MDA-MB-231 breast cancer models, which recapitulate certain architectural and phenotypical features of tumors. This was determined by quantification of IFN γ and IL-2 secretion into the culture medium, tumor cell apoptosis, and T cell proliferation.¹⁰³ A clinical trial of ROR1-targeting CAR-T cells in patients with hematological and epithelial cancers is currently ongoing (NCT02706392).

Prostate-specific membrane antigen (PSMA) is a membrane-localized carboxypeptidase, which is expressed ubiquitously in prostate tissue and is upregulated in prostate cancer.¹⁰⁴ However, it is also expressed in various organs including the kidney and brain, which has resulted in lethal on-target off-tumor toxicities in clinical trials. In one clinical study, two out of five patients with advanced hormone-refractory prostate cancer displayed only partial clinical responses to PSMA-targeting CAR-T cells.¹⁰⁵ To overcome the highly immunosuppressive TME, PSMA-directed CAR-T cells have been developed with a TGF β -dominant negative receptor, referred to as DNRII, which lacks the intracellular domains required for downstream TGF β signaling.¹⁰⁶ DNRII-modified PSMA-directed CAR-T cells exhibited enhanced proliferation upon stimulation with PSMA-expressing cancer cell lines, which was associated with an increased frequency of central memory CD8⁺ T cells and decreased FoxP3 expression. Furthermore, these cells were also able to significantly inhibit the growth of PC3-PSMA⁺ tumors in NSG mice compared with conventional PSMA-targeting CAR-T cells.¹⁰⁶ However, DNRII-modified PSMA-targeting CAR-T cells have also been associated with fatal immune effector cell-associated neurotoxicity syndrome in a recent clinical trial (NCT03089203).

While some CAR-T cell therapies are directed to tumor antigens expressed by multiple tumor types, such as EGFR, GD2, and ROR1, a large proportion target only one or a few cancer subtypes. The relatively small pool of known solid tumor antigens results in current CAR-T cell therapies acting on a limited number of solid cancers. Solid cancer treatments that act universally on different cancers have major advantages in reducing cost and

production time, which is crucial for patients with poor prognosis. One potential method to develop a universal CAR-T cell therapy is through sophisticated synthetic design of CAR-T cells, such that their antigen binding capacity is separated from the signaling domains to allow for target antigen flexibility. The two component system introduces an “ON” switch as a soluble antigen binding adaptor must be administered for the CAR-T cells to become activated. Moreover, multiple tumor antigens may be targeted via simultaneous administration of multiple antigen binding adaptors, without further modification of T cells. This may be highly beneficial in combating the heterogeneous expression of antigens often seen in solid tumors. The first example of this utilized biotin-binding immune receptors (BBIR), which comprised an extracellular-modified avidin protein linked to an intracellular T cell signaling domain, engineered onto T cells. Here, the BBIR binds to cancer cells pre-targeted with specific biotinylated molecules.¹⁰⁷ Recently, a more tailored approach was developed that utilized leucine zippers to bind the scFv portion to intracellular signaling domains. Upon administration of multiple scFv adaptors, these split, universal, and programmable (SUPRA) CAR-T cells were able to target multiple antigens,¹⁰⁸ which was demonstrated to prevent the emergence of antigen loss or escape variants, as similarly observed with bispecific or tri-specific CD19/CD20/CD22-targeting CAR-T cells.^{109,110}

As discussed, target antigen selection is often a limiting step in targeting solid cancers with CAR-T cell therapy, as antigens are often heterogeneously expressed, expressed by only one or a few cancer subtypes, or are also present on healthy tissues. Careful consideration of tumor target antigens and innovative CAR-T cell design have provided promising avenues in developing effective and potentially universal therapies against solid cancers. Upon selection of a suitable target antigen, the next major challenge is in homing CAR-T cell infiltration into often densely barricaded and hostile solid tumors.

Chemokine-directed CAR-T cell therapy

T cell trafficking into tumors remains a major hurdle in generating an effective CAR-T cell therapy against solid cancers. In some mouse tumor models, less than 1%–2% of adoptively transferred T cells were found to infiltrate solid tumors.^{27,111} Indeed, spatiotemporal imaging of PSMA-targeting CAR-T cell migration in prostate tumor-bearing mice revealed a large proportion were present in the thyroid, salivary glands, stomach, and bladder, with only 0.2% of total transferred cells located in the tumor.¹¹² Directed trafficking of CAR-T cells also provides significant safety advantages as it lowers the required cell dose and reduces dissemination in the periphery where on-target off-tumor toxicity may occur. Harnessing the chemokine system, which naturally plays a crucial role in the migration of hematopoietic cells, has therefore been identified as an attractive method to improve the activity of CAR-T cells by enhancing their trafficking into tumors. Chemokine receptor axes that have been studied as an approach to enhance intratumoral T cell trafficking are illustrated in Figure 2.

Understanding the expression profile of chemokines in the TME may inform which chemokine axes can be utilized to enhance CAR-T cell trafficking into solid tumors. Given the

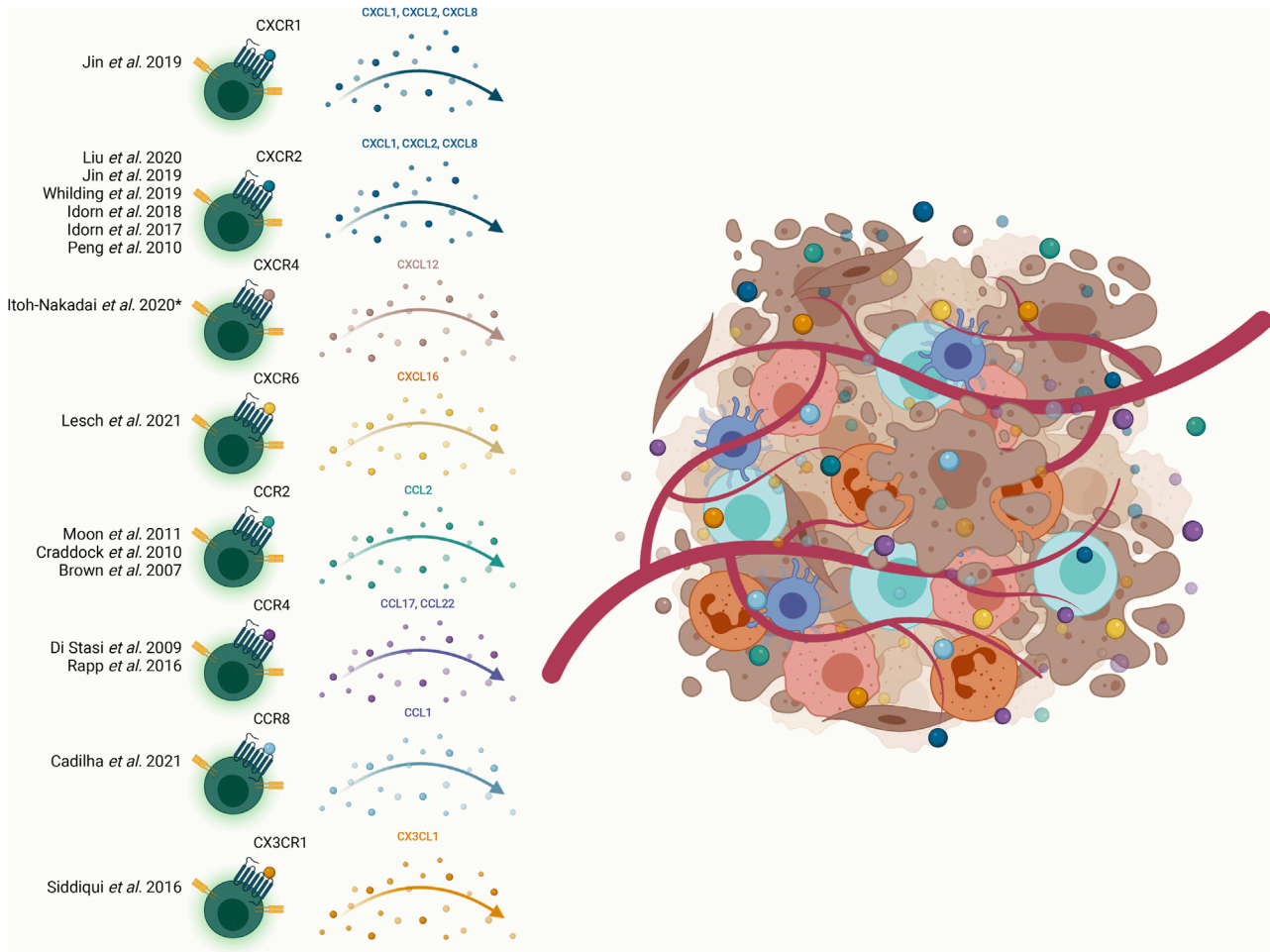


Figure 2. Co-opting chemokine receptor axes to enhance trafficking of CAR-T cells into solid tumors

Many tumors derived from different tissue origins share similar patterns of chemokine expression, raising the possibility of enhancing CAR-T cell infiltration into a diverse array of cancers using key chemokine/chemokine receptor axes. Chemokine receptors that have been utilized to guide T cell trafficking into solid tumors and specific anatomical niches include CXCR1,⁶⁶ CXCR2,^{64–66,68–70} CXCR4,¹¹³ (NCT04727008), CXCR6,¹¹⁴ CCR2,^{115,116,117} CCR4,^{118,119} CCR8,¹²⁰ and CX3CR1.¹²¹ Furthermore, CAR-T cells may be modified to express CCL19 and CCL21 to promote recruitment of endogenous DCs and T cells into tumors.^{122,123} *In this study, CAR-T cells were modified to express CXCR4 to enhance their recruitment into the CXCL12-rich bone marrow in a patient-derived acute myeloid leukemia mouse model.

numerous pathological effects of CXCL8 in angiogenesis, cancer stem cell survival, and recruitment of immunosuppressive myeloid cells, it is unsurprising that CXCL8 is strongly correlated with disease burden in many solid cancers including melanoma, renal cell carcinoma, NSCLC, pancreatic, breast, and ovarian.^{63–65,67,124–127} CXCL1 and CXCL2 are also highly expressed in many solid cancers.^{127–129} Indeed, the chemokine receptors that bind to these ligands, CXCR1 and CXCR2 have been co-opted as tumor-homing signals in several preclinical CAR-T cell therapies. For example, CXCR1- and CXCR2-modified CD70-targeting CAR-T cells showed enhanced intratumoral accumulation and persistence in a glioblastoma xenograft model, resulting in complete tumor regression and long-lasting immunological memory in rechallenge experiments compared with non-modified CAR-T cells. In this model, fractionated local radiation increased expression of CCL2, CCL20, and CXCL1, 2,

and 8 in tumors, and early intratumoral infiltration occurring in 3 days or less post-T cell transfer was a reliable indicator of survival. Furthermore, CXCR1/2-modified CAR-T cells siphoned off CXCL8 *in vitro*, potentially neutralizing pro-tumorigenic CXCL8 from tumors and improving the efficacy of other anti-tumor therapies following co-administration.⁶⁶ Similarly, transduction of pmel-1 transgenic T cells to express CXCR2 resulted in a 2-fold increase in T cell presence in gp100-transduced colorectal tumors by 6 days post-transfer. This was associated with a 50% reduction in tumor growth compared with administration of non-CXCR2-modified T cells when both were combined with DC vaccination and IL-2 administration.⁶⁹ In a separate study, transduction of lymphocytes isolated from the tumor ascites of ovarian cancer patients to express CXCR2 resulted in T cell migration into autologous and allogeneic ascites *in vitro*, which were found to highly express CXCL1 and CXCL8.⁶⁴ These

results are consistent with another study in which CXCR2 transduction of antigen-specific T cells enhanced their presence in human melanoma xenografts, which were found to express CXCL1 and CXCL8 in an immunocompromised mouse model.⁶⁵ Moreover, CXCR2-expressing CAR-T cells directed to integrin $\alpha\text{v}\beta 6$ controlled tumor growth in a human pancreatic tumor xenograft mouse model more effectively compared with non-CXCR2-expressing CAR-T cells.⁷⁰ However it is important to note that in many of these studies, in which T cell infiltration is quantified several days post-transfer (at the earliest), the underlying mechanism, whether that be increased trafficking or proliferation, increased retention within tumors, or decreased apoptosis of CAR-T cells was not differentiated.

Another chemokine axis of interest in enhancing intratumoral trafficking of adoptive cell immunotherapy is CCL2-CCR2. For instance, GD2-targeting CAR-T cells were transduced to express CCR2b after CCL2 was identified to be highly expressed by neuroblastoma cell lines and primary tumor biopsies. This resulted in increased CAR-T cell intratumoral accumulation in subcutaneous human neuroblastoma xenografts in SCID mice at d2 and d3 post-T cell transfer, which was accompanied by increased CAR-T cell proliferation and persistence and reduced tumor burden compared with mice receiving GD2-targeting CAR-T cells without CCR2b. However, manufacture involved sequential transduction of two separate vectors containing the GD2 CAR and CCR2b, resulting in low transduction efficiency.¹¹⁵ Patient pleural fluids and cell lines of malignant pleural mesothelioma (MPM) were also found to express high levels of CCL2. Therefore, CAR-T cells targeting mesothelin, a glycoprotein expressed at high levels on MPM, ovarian cancer, and pancreatic cancer, were transduced to express CCR2b. This resulted in improved anti-tumor efficacy against advanced human MPM tumor xenografts in immunocompromised mice compared with CCR2-negative CAR-T cells. Improved anti-tumor efficacy was associated with a 12.5-fold increase in the presence of CAR-T cells within tumors 5 days following T cell transfer. However, similar to the GD2-targeting CAR-T cells described in the previous study, lentiviral transduction with two separate vectors resulted in low efficiency with 30% CAR-positive in the control mesothelin-targeting CAR-T cell population and 19% CAR- and CCR2-double positive in the dual-transduced population.¹¹⁶ Aside from neuroblastoma and MPM, CCL2 is also constitutively highly expressed by other tumors including glioma, melanoma, lung cancer, and Hodgkin's lymphoma,¹¹⁷ positioning CCR2 as a potential candidate for enhancing intratumoral infiltration of adoptive cell therapies to these cancers.

The use of CXCL16-CXCR6, CXCL12-CXCR4, and CCL1-CCR8 chemokine axes to improve adoptive T cell intratumoral trafficking has also received some attention in a limited number of studies. CXCL16 exists in two distinct forms, whereby transmembrane CXCL16 is converted into a soluble form via ADAM10- and ADAM17-mediated cleavage and subsequent shedding.^{130,131} Transmembrane CXCL16 mediates cell adhesion, whereas soluble CXCL16 acts as a chemoattractant.¹¹⁴ As CXCL16 was found to be highly expressed by OVA-expressing murine pancreatic cancer models and patient pancreatic ductal adenocarcinoma (PDAC) tumor biopsies, OT-I cells, murine EpCAM-targeting CAR-T cells, and human mesothelin-targeting

CAR-T cells were transduced to express CXCR6. CXCR6-transduced EpCAM-targeting CAR-T cells induced significant tumor regression with complete tumor rejection in 40% of mice, which was superior to the regression observed with unmodified control, CXCR3-transduced, or CCR4-transduced EpCAM-targeting CAR-T cells. Here, CXCR3 and CCR4 were tested in parallel due to high expression of their ligands in murine pancreatic mouse models, and tumor cells were transduced to express EpCAM. Intravital and two-photon imaging confirmed accumulation of CXCR6-transduced OT-I cells within murine pancreatic tumors. Meanwhile CXCR6-transduced mesothelin-targeting CAR-T cells significantly inhibited tumor growth of patient-derived pancreatic cancer xenografts in immunocompromised mice and produced significantly higher levels of IFN γ upon *in vitro* stimulation with patient-derived organoids (compared with control CAR-T cells and control T cells, respectively).¹¹⁴ Aside from pancreatic cancer, CXCL16 was also found to be highly expressed in multiple cancer types such as ovarian, breast, prostate, cervical, lung, and colorectal cancers according to analysis of The Cancer Genome Atlas.¹¹⁴ However, stratification of transmembrane versus soluble CXCL16 is crucial as the soluble form acts as a chemoattractant and frequently correlates with invasion and metastasis, whereas the transmembrane form may inhibit tumor proliferation, which is associated with CXCR6⁺ lymphocyte accumulation in the TME.^{114,131,132} CXCR6 may also have an additional role, as reported in a separate study that demonstrated CXCR6 expression was critical for sustained anti-tumor efficacy of cytotoxic T cells in an antigen-specific model of murine melanoma. In that model, it was revealed cytotoxic CXCR6⁺ CD8⁺ T cells were positioned in a perivascular niche of the tumor stroma, localized with CCR7⁺ cDCs that express CXCL16. Through cell contact-dependent *trans*-presentation of IL-15 by cDCs, interaction with cDCs provided key survival and proliferation signals to cytotoxic T cells.¹³³

Similarly to CXCL16, the ligand for CXCR4, CXCL12 was found to be highly expressed by cancer-associated fibroblasts in PDAC, NSCLC, and breast cancer,^{29,134,135} and both CXCR4 and CXCL12 have been reported to be upregulated in many cancers.^{136–138} Indeed, CXCL12 along with its receptors CXCR4 and CXCR7 have been strongly correlated with enhanced proliferation, angiogenesis, and metastasis of multiple cancers.¹³⁹ To enhance homing of CAR-T cells into the CXCL12-rich bone marrow of mice transplanted with patient-derived acute myeloid leukemia, CD25-targeting CAR-T cells were modified to co-express CXCR4. Administration of CXCR4-modified CD25-targeting CAR-T cells resulted in decreased tumor burden, although differences in trafficking were not explicitly investigated.¹¹³ Currently, CXCR4-modified B cell maturation antigen (BCMA)-targeting CAR-T cells are being investigated in a clinical trial of multiple myeloma (NCT04727008).

Known mechanisms involved in recruitment to the TME, such as the CCL1-CCR8 axis used by T_{reg} cells, may also be co-opted to enhance CAR-T cell trafficking. The use of CCL1-CCR8 by T_{reg} cells has been demonstrated in analyses of human breast cancer¹⁴⁰ and PDAC biopsies,¹²⁰ which found a strong correlation between CCL1 and FoxP3 expression and highlighted an upregulation of CCL1 in cancer tissues. However, exploitation of the CCL1-CCR8 axis may expose CAR-T cells to immunosuppression

by localizing these cells with TGF β -expressing T_{reg} cells, which utilize the same chemokine axis. Thus, to simultaneously combat immunosuppression and improve trafficking into solid tumors, CCR8 and the TGF β -insensitive receptor, DNR11, were engineered onto murine EpCAM-targeting CAR-T cells. This resulted in significant tumor inhibition and improved survival in a murine pancreatic tumor model, which was transduced to express the antigen EpCAM, compared with unmodified CAR-T cells. In contrast, CAR-T cells that were engineered to express CCR8 alone had a minimal effect on tumor growth, likely due to TGF β -mediated immunosuppression. Similarly, dual CCR8-and DNR11-expressing mesothelin-targeting CAR-T cells elicited improved tumor regression and survival against human pancreatic cancer xenografts, which were transduced to express mesothelin. Consistent with the murine setting, significant tumor regression was only observed for CAR-T cells that expressed both CCR8 and DNR11 and not CCR8-expressing CAR-T cells, which showed no additional improvement over unmodified CAR-T cells. Enhanced tumor regression was associated with increased presence of dual DNR11- and CCR8-expressing CAR-T cells within tumors compared with CAR-T cells expressing CCR8 or DNR11 alone.¹²⁰ However, as with the previous studies involving chemokine receptor modified CAR-T cells, it is unclear whether the findings in this study are a result of increased proliferation, trafficking, survival, or intratumoral retention, as analysis was performed 19 days post-T cell administration in endpoint tumors.¹²⁰

Furthermore, the elevated expression of other chemokines such as CCL5, CXCL5, CX3CL1, CCL17, CCL20, and CCL22 across various tumor models and patient biopsies may also provide potential avenues to enhance CAR-T cell trafficking into tumors.^{28,44,63,118,119,121,141–144} For instance, following the finding that multi-nucleated Reed-Sternberg cells of Hodgkin's lymphoma express the ligands for CCR4, CCL17, and CCL22, a study found CCR4 co-expression on CD30-targeting CAR-T cells enhanced anti-tumor efficacy against a subcutaneous Hodgkin's lymphoma human xenograft model.¹¹⁸ In a separate study, the CCR4-CCL22 axis was also utilized to enhance intratumoral recruitment of cytotoxic T cells in an antigen-specific murine pancreatic tumor model, resulting in enhanced anti-tumor efficacy of CCR4-transduced T cells.¹¹⁹ Similarly, following the identification of CX3CL1 expression in human colorectal cancer biopsies and cancer cell lines, transduction of human primary T cells to express CX3CR1 resulted in enhanced intratumoral accumulation of these cells in endpoint human colorectal tumor xenografts in immunocompromised mice.¹²¹

To supplement CAR-T cell function, the chemokine system has also been used to recruit endogenous immune subsets to tumors. For instance, CD20-targeting CAR-T cells engineered to simultaneously express IL-7 and CCL19 were found to recruit T cells and DCs to tumors, mimicking the role of fibroblastic reticular cells in the T cell zone of secondary lymphoid organs. *Ex vivo* analysis of modified CAR-T cells showed increased diversity of TCR repertoires compared with that at pre-injection, indicating epitope spreading to recognize different tumor antigens. This observation was consistent with results in rechallenge experiments, which showed delayed tumor growth of parental CD20-negative mast cell tumors in mice previously injected with CD20-positive tumor cells and IL-7- and CCL19-expressing CD20-targeting CAR-T

cells.¹²² Similarly, CAR-T cells targeting claudin-18.2 (CLDN18.2) were engineered to co-express IL-7 and CCL21 in a separate study. In parallel analysis, IL-7- and CCL21-expressing CAR-T cells were more effective than IL-7- and CCL19-expressing CAR-T cells in suppressing tumor growth in murine pancreatic, breast, and liver tumor models, which was associated with enhanced CAR-T cell and DC infiltration into tumors. This may be related to differing chemotactic abilities of CCL19 and CCL21. Furthermore, reduced tumor angiogenesis was observed following administration of IL-7- and CCL21-expressing CAR-T cells, possibly due to the angiostatic activity of CCL21.¹²³ However, increased concentrations of CCL21 in the TME may play a detrimental role in some contexts. In a separate study, tumor-derived CCL21 was found to shift the immune response from immunogenic to tolerogenic in mouse melanoma models, associated with the recruitment of T_{reg} cells, MDSCs, and naive T cells that are subsequently polarized toward T_{reg} differentiation within the TGF β -enriched immunosuppressive cytokine milieu.¹⁴⁵

While the local delivery of chemokines into the TME enhanced the recruitment of immune subsets in these studies, this process may be highly complex and context-dependent. This was exemplified in a separate study, which showed that delivery of CXCL11 by a first dose of mesothelin-targeting CAR-T cells did not improve anti-tumor efficacy of a second dose of CAR-T cells in a human malignant mesothelioma xenograft mouse model. This was accompanied by a significant reduction in human CD3⁺ cell accumulation in endpoint tumors compared with mice receiving two doses of control mesothelin-targeting CAR-T cells. In contrast, delivery of CXCL11 via an oncolytic vaccinia virus in a murine subcutaneous lung cancer model resulted in an increased frequency of CAR-T cells in endpoint tumors and improved anti-tumor efficacy. The differences between the two delivery methods was proposed to be due to chronic secretion of CXCL11 by CAR-T cells driving continuous autocrine activation of CXCR3 and sustained calcium signaling.⁴⁰ Indeed, this may be an important consideration for all CAR-T cells transduced to express chemokine receptors as sustained calcium signaling has been reported to induce T cell anergy in a calcineurin-dependent pathway.¹⁴⁶ Furthermore, CXCL11 has been shown to inhibit angiogenesis, which may have resulted in reduced T cell trafficking into tumors in the previous study.⁴⁰ However, this contrasts the results presented by Luo and colleagues, in which CCL21 delivery by CAR-T cells led to reduced angiogenesis but increased CAR-T cell and DC recruitment into tumors.¹²³ The conflicting results presented in these studies highlights the complicated mechanisms by which chemokine-mediated signaling may influence CAR-T cell recruitment and activity.

Aside from chemokine receptors, adhesion molecules such as integrins and their ligands have also been utilized to enhance lymphocyte homing. For instance, purified glycosylphosphatidylinositol-anchored proteins were used to express α v β 3 integrin ligands on the cell surface of lymphocytes, resulting in enhanced arrest on the tumor vascular endothelium, which expresses high levels of the α v β 3 integrin.¹⁴⁷ Other methods have utilized CAR-T cell therapies to combat the dense structural network surrounding solid tumors in efforts to improve T cell infiltration. These include fibroblast activation protein (FAP)-directed CAR-T cells, which deplete FAP-expressing cancer-associated fibroblasts¹⁴⁸

or CAR-T cells engineered to deliver ECM-modifying enzymes such as heparanase¹⁴⁹ and hyaluronidase¹⁵⁰ into the TME.

Taken together, these preclinical studies highlight the potential in harnessing the chemokine system to home CAR-T cells into tumors and facilitate direct tumor cell cytotoxicity, resulting in significant improvements in CAR-T cell efficacy against solid cancers. Delivery of chemokines such as CCL19 and CCL21 into the TME by CAR-T cells may also act as a strategy to promote anti-tumorigenic DC and T cell recruitment into solid tumors while targeting architectural components of the TME via ECM-modifying enzymes and depletion of cancer-associated fibroblasts may further enhance intratumoral infiltration.

CONCLUSIONS

Recent clinical advances have demonstrated the efficacy of immunotherapy in the eradication of tumors. While CAR-T cell therapy has achieved unprecedented success in treating hematological malignancies, solid tumors and their associated tumor microenvironment present many challenges that prevent the delivery of effective therapy. One of the major challenges lies in targeting CAR-T cell migration into often densely barricaded and hostile solid tumors. The chemokine system naturally plays a critical role in the migration of hematopoietic cells, which can be harnessed to home CAR-T cell therapy directly into tumors. Identification of key chemokine axes such as CXCL8/CXCR2 and CCL2/CCR2 in the TME has led to promising preclinical results demonstrating enhanced infiltration of chemokine receptor-modified CAR-T cells into solid tumor models, which is associated with significant improvement in anti-tumor efficacy. Furthermore, many chemokines such as CCL2, CXCL8, and CXCL16 are widely expressed in different solid tumors, potentially providing an avenue to target CAR-T cells into diverse cancer types using select chemokine receptors. By addressing this initial challenge, CAR-T cell therapies may be developed that are efficiently recruited to, and specifically recognize, a large repertoire of malignant cells within solid tumor contexts and begin the translation of CAR-T cell therapy from hematological malignancies to solid cancers.

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AUTHOR CONTRIBUTIONS

Writing—original draft, J.F.; writing—review & editing, J.F., I.C., S.R.M.; funding acquisition, S.R.M. and I.C.

DECLARATION OF INTERESTS

J.F. and S.R.M. are employed by Carina Biotech. S.R.M. and I.C. are inventors on patent US-2020-0,054,677-A1, which is licensed to Carina Biotech. S.R.M. is on the scientific advisory board at Tizona Therapeutics.

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