Paving the road to make chimeric antigen receptor-T-cell therapy effective against solid tumors

Keishi Adachi | Koji Tamada

Abstract
The three major standard therapies, that is, surgery, chemotherapy, and radiation therapy have conventionally been applied to the treatments for cancers and have saved many patients. In addition, for intractable, refractory, or advanced malignancies that cannot be cured by the three standard therapies, immunotherapy is an important subject of basic and clinical researches. Immune checkpoint inhibitor therapy (ICI) has shown significant therapeutic efficacies on some types of tumors in large-scale randomized clinical trials, making a major impact on clinical oncology by scientifically proving and establishing the effectiveness of an immunotherapy. In 2018, ICI was awarded the Nobel Prize in Physiology or Medicine, and immunotherapy is now becoming the “fourth” standard therapy for cancers. Recently, adoptive cell therapies, in which genetically modified T cells with enhanced reactivity against tumors are infused into the patients, have been attracting considerable attention as a hopeful immunotherapy following ICI. Particularly, chimeric antigen receptor (CAR)-T-cell therapies demonstrate marked therapeutic efficacies against some hematologic malignancies, and have been approved in many countries. However, current CAR-T-cell therapy is considered to be little effective against solid tumors, which is one of the challenging issues to be overcome in CAR-T-cell therapy. In this review, we at first introduce CAR and CAR-T cell, and then focus on the recent progress of CAR-T-cell therapy against solid tumors as well as the novel concept on a role of CAR-T cells, aiming to further understandings of the novel cancer immunotherapies.

KEYWORDS
antigen heterogeneity, chimeric antigen receptor, migration, solid tumors, tumor microenvironment

Abbreviations: Ags, antigens; AMPK, adenosine monophosphate-activated protein kinase; BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; CD, cluster of differentiation; CRISPR, clustered regularly interspaced short palindromic repeat; CXCR/CCR, CXC/CC chemokine receptor; DCs, dendritic cells; ECs, endothelial cells; EphA2, erythropoietin-producing hepatocellular receptor A2; Fli3L, Fms-Like Tyrosine Kinase 3 Ligand; GLUT1, glucose transporter type 1; HER2, human epidermal growth factor receptor; HIF1α, hypoxia inducible factor-1α; ICAM-1, intercellular adhesion molecule 1; ICI, immune checkpoint inhibitor; IL, interleukin; MDSCs, myeloid-derived suppressor cells; OVA, ovalbumin; PD-L1/PD-L2, programmed cell death 1 ligand 1/2; PDX, patient-derived xenograft; PI3K, phosphatidylinositol-3 kinase; scFvs, single-chain variable fragment; SUPRA CAR, split universal and programmable CAR; TCR, T-cell receptor; TGF-β, transforming growth factor-β; TME, tumor microenvironment; TRCs, T-zone fibroblastic reticular cells; Tregs, regulatory T cells; VCAM-1, vascular cell adhesion molecule 1.
INTRODUCTION

The prognosis for patients with intractable, refractory, or advanced cancer remains extremely poor, and the development of the innovative therapies against those cancers is an urgent issue. Immunotherapies, which take advantage of a patient’s own anti-tumor immunity to eliminate tumor, are predicted to have different mechanisms of action and spectra of toxicities from the three conventional standard therapies, and has long been the subject of research and development. The superior efficacy of ICI therapy and gene-modified T-cell (e.g., CAR-T cell and TCR-T cell) therapy, which began to be reported in the early 2010s, has attracted significant attention worldwide and has had a major impact on clinical oncology. Immune checkpoint inhibitors exert their anti-tumor effects by nonspecifically disarming the immunosuppressive conditions in cancer patients, whereas gene-modified T-cell therapies do so specifically by recognizing tumor Ags.1-5

CAR-T cells were developed based on the concept of conferring the capacities to eliminate cancer cells by exogenously expressing artificial receptors derived from tumor Ag-specific monoclonal antibodies to the patient’s own T cells that were not specific for the tumor Ags or that were no longer responsive to the cancer cells. CAR-T cells (formerly also referred to as T-bodies) whose CARs were composed of an extracellular domain derived from a tumor Ag-specific monoclonal antibody and a signaling domain involved in T-cell activation were first reported in the early 1990s (“first generation” CAR).6,7 In 1998, a CAR with two intracellular signaling domains, CD3ζ (CD247) and one co-stimulatory molecule CD28, was reported (“second generation” CAR).8 In the study, it was demonstrated that placing the intracellular domain of CD28 in the membrane-proximal region of CD3ζ enhanced IL-2 production from Jurkat (T lymphoblastoma) cells.9 In 2002, CAR-T cells with intracellular domains of CD28 and CD3ζ, generated from human T cells were reported, showing robust proliferation, the production of large amounts of IL-2, and Ag-specific tumoricidal effects.9 In addition, a CAR that used 4-1BB (CD137) instead of CD28 as the intracellular signaling domain was reported in 2004.10 These 4-1BB-based CAR-T cells killed tumor cell lines and primary tumor cells at low effector:target (E:T) ratios in vitro, exhibiting potent anti-tumor activities referred to as “serial killing.”

Currently, the most advanced CAR-T cells in clinical development are the ones targeting CD19, a B-cell differentiation Ag.11-18 Clinical studies with autologous anti-CD19 CAR-T cells have been conducted for some types of relapsed or refractory CD19-positive B-cell hematologic malignancies, and therapeutic achievements have been accumulated. It has been also revealed that lymphodepleting pretreatments with chemotherapeutic agents, such as fludarabine and cyclophosphamide, are critically important in the efficient expansion and proliferation of the infused T cells.19,20 Starting with the first approval of tisagenlecleucel (trade name: Kymriah®) in 2017 for relapsed or refractory acute lymphoblastic leukemia in the USA, several anti-CD19 CAR-T-cell products have been approved in many countries around the world. In addition, anti-BCMA (CD269) CAR-T cells have recently been approved for the treatment of relapsed or refractory multiple myeloma,21 expanding the indications of CAR-T-cell therapy. However, there remain some crucial problems to be overcome in current CAR-T-cell therapies. In particular, CAR-T-cell therapy has demonstrated only a limited efficacy against solid tumors, which comprise the majority of malignant tumors, and is one of the most urgent issues.22-24

WHY IS THE EFFECT OF THE CURRENT CAR-T-CELL THERAPY AGAINST SOLID TUMORS OFTEN LIMITED? ROADBLOCKS FOR CAR-T CELLS IN TUMOR MICROENVIRONMENT

As the enhanced expression of tumor-associated Ags has been identified in solid tumors,25,26 it seemed a feasible approach to generate CAR-T cells against these tumor Ags for the treatment of solid tumors. However, compared with the marked therapeutic efficacies of anti-CD19 or anti-BCMA CAR-T cells for B-cell malignancies, treatments with CAR-T cells against solid tumors have rarely led to the anticipated therapeutic benefits, such as shrinkage of the tumor masses or prolonged survival of the patients,22-24 and unanticipated serious adverse events have also been reported in some cases.27 The limited efficacies of CAR-T cells against solid tumors are due to the unique properties of solid tumors that are critically different from hematologic malignancies.

Difficulty in selecting CAR targets

It is important to select the targets with high coverage, specificity, tolerability, and stability for the safety and efficacy in CAR-T-cell therapies.28 Although CD19 and BCMA are tissue differentiation molecules expressed on normal B lineage cells as well as B-cell malignancies, the success of CAR-T-cell therapy against CD19 and BCMA has been attributed in part to the fact that even if normal B cells are killed by the administered CAR-T cells, B-cell aplasia is clinically manageable by supplemental infusion of γ-globulin.11,13,14,17 For solid tumors, however, the expression of tumor-related target molecules in normal tissues even at low levels may induce intolerable adverse events due to “on-target, off-tumor” toxicities, therefore limiting the applicable targets in CAR-T cells against solid tumors.27

Furthermore, the heterogeneity of Ag expression, which is a common feature of solid tumors, also makes it difficult to select ideal targets of CAR.29,30 Solid tumors usually have three levels of antigenic heterogeneities: (1) heterogeneity among patients, (2) heterogeneity among lesion sites in an individual patient, and (3) heterogeneity within a single lesion site. (1) Heterogeneity among patients refers to the fact that the proportion of tumor cells positive for an Ag varies among patients, even for the same pathological cancer type. (2) Heterogeneity among lesion sites in an individual patient is most typically observed as that among primary and
metastatic sites. (3) Heterogeneity within a single lesion site means the co-existence of Ag-positive and Ag-negative tumor cells within each tumor lesion. In addition, expression levels of tumor Ags are often altered not only spatially but also temporally.29 Furthermore, the therapeutic intervention with CAR-T cells itself can act as a selection pressure for tumor cell survival, resulting in the expansion of tumor cells whose Ags are lost or mutated (Ag escape).31–33 Currently, biopsies are commonly performed to evaluate Ag expression. However, the information obtained by a single biopsy would be insufficient to cover the spatial and temporal changes in Ag expression. It is technically and ethically unacceptable to perform biopsies on many lesions, extensively, or repeatedly over a long period of time to overcome this issue. Therefore, it may be necessary to recognize the inherent limitations in selecting CAR targets based on the tumor Ag information obtained from a single biopsy. It would be essential to establish the novel strategies for selecting CAR targets, such as integration of the information obtained by liquid biopsy and/or the bioinformatics-based antigen prediction.

2.2 | Imunosuppressive microenvironment generated inside the solid tumor tissues

Solid tumors form tissues with microenvironments composed of various cell types, including tumor cells, stromal cells, and infiltrating immune-competent cells.34–36 Those milieus have salient immunosuppressive and immunomodulatory potential to make effector cells, such as T cells, immunologically exhausted37,38 and therefore also massively suppress the potent tumoricidal capacities with which CAR-T cells have been endowed before infusion. Imunosuppressive conditions in the TME could be caused by certain immune cell populations with immunosuppressive activities, such as MDSCs and CD4+CD25+FoxP3+ Tregs, which are enriched in tumor tissues and work to allow tumors to grow efficiently.34,35,39 Actually, tumors often produce large amounts of chemokines that preferentially recruit immunosuppressive cell populations and, importantly, the receptors for those chemokines are rarely expressed on effector T cells.40–42 Furthermore, immunosuppressive humoral mediators, such as IL-10 and TGF-β produced by tumor cells, stromal cells, and certain immune cells, play important roles in the formation and maintenance of an immunosuppressive condition in TME.43–45 In addition, upregulated expression levels of immune checkpoint molecules are one of the most pivotal immune suppressive mechanisms. For instance, tumor cells and tumor-associated macrophages express PD-L1 (CD274)/PD-L2 (CD273). Those immune checkpoint molecules induce poor expansion and short-term persistence of CAR-T cells in TME, disturb the exertion of CAR-T-cell functions, and eventually render CAR-T cells irreversibly exhausted.46–48 Metabolism in TME also affects the anti-tumor functions of effector T cells.49–51 Effector T cells, including CAR-T cells, could compete with tumor cells and other cell types in TME for several nutrients including glutamate and fatty acids. For example, cancer cells, unlike normal cells, produce ATP in the glycolytic system rather than through mitochondrial oxidative phosphorylation, even under aerobic conditions, which is known as the Warburg effect. When glucose is metabolized in the glycolytic system, it is eventually converted to lactate without entering the mitochondria. This is achieved principally by the activation of PI3K/Akt/mTOR, c-myc, and HIF1α and the downregulation of AMPK signaling, leading to the upregulation of GLUT1 and glycolytic enzymes. The glycolytic system produces ATP at a faster rate than oxidative phosphorylation. In contrast, compared with oxidative phosphorylation, which produces 36 molecules of ATP per a molecule of glucose, the glycolytic system, which produces only two molecules of ATP per a molecule of glucose, is very inefficient for ATP production. As a result, cancer cells consume large amounts of glucose. In addition, high oxygen consumption due to rapid proliferation and poor vascularity due to continuous increase in the mass size render the TME hypoxic.49–51 Hypoxia inhibits the differentiation, proliferation, and cytokine production of effector T cells.52

2.3 | Restricted accessibility of CAR-T cells to tumor tissues after the intravenous administration

Access and contact of CAR-T cells to their target tumor cells are crucial steps for exerting the anti-tumor functions and the consequent success of the therapy. For B-cell malignancies, intravenously administered CAR-T cells are expected to efficiently encounter their targets, as tumor cells reside in blood vessels, lymph nodes, and bone marrow, in which a physiological environment favoring recruitment and proliferation of lymphocytes is inherently provided. By contrast, for solid tumors, active migration, penetration, and trafficking into tumor tissues are indispensable for intravenously administered CAR-T cells to access cancer cells, and those steps are tightly regulated by various factors, such as chemokines.40–42 In this regard, solid tumors often lack the production of chemokines that affect the migration of T cells or, conversely, CAR-T cells generally do not express receptors that respond to the chemokines produced by solid tumors, resulting in mismatches between the chemokines from tumors and the receptors on CAR-T cells. Therefore, compared with the conditions in B-cell malignancies, it would be inefficient for CAR-T cells to encounter their targets in solid tumors. It is also known that solid tumors often induce neangiogenesis which has features different from normal vessels.53,54 In the abnormal vasculatures of solid tumors, low expression of adhesion molecules, such as ICAM-1 (CD54) and VCAM-1 (CD106) on tumor ECs prevents effector T cells from adhering to ECs and migrating into the tumor tissues.55–57 In addition, the dense ECM, which is one of the major components of the stroma surrounding solid tumors, acts as a physical barrier that inhibits the access of injected CAR-T cells to the tumor cells.55 ECM is composed of a complex mixture of fibrous proteins, glycoproteins, polysaccharides, and proteoglycans, which are produced by tumor cells and cancer-associated
fibroblasts. It has been reported that there is an inverse correlation between the infiltration and localization of T cells in tumor stroma and the rigidity of the ECM. In other words, T cells have a tendency to accumulate in regions with low fibronectin and collagen density.

3 | DESIGNS FOR THE NEXT GENERATION CAR-T CELLS TO SURMOUNT TME: REMOVING THE ROADBLOCKS ON THE ROAD TO SUCCESS

3.1 | Overcoming Ag heterogeneity and escape

As tumor Ags are often spatiotemporally heterogeneous, as mentioned above, one concept for this issue is to endow CAR-T cells with the capacity to target multiple Ags. One approach based on this concept is bispecific CAR-T cells, on which CARs with two distinct scFvs are expressed. The bispecific CAR-T cells are divided into bicistronic CAR-T cells, which co-express two different CARs, and tandem CAR-T cells, which express a single CAR containing two distinct scFvs tandemly linked (Figure 1A). In murine glioblastoma models, treatment with tandem CAR-T cells targeting IL13Rα2 and HER2 or IL13Rα2 and EphA2 induced improved anti-tumor activity and decreased Ag escape compared with that of the conventional CAR-T cells targeting each single molecule. Another approach is a universal CAR, which typically recognizes a small molecule, for example, biotin and FITC or a peptide (Figure 1B). These universal CAR-T cells can be redirected to recognize cell-surface Ags by the infusion of small molecule-conjugated antibodies or peptide-fused Fabs specific for tumor Ag as adapter molecules. In addition, universal CAR mediated by leucine zipper heterodimerization domains, referred to as split, universal, and programmable (SUPRA) CAR, were also generated (Figure 1B). In the SUPRA CAR system, the CAR has a leucine zipper as the extracellular domain, and tumor Ag-specific scFv fused to a cognate leucine zipper is used as an adapter molecule for the redirection of the CAR-T cells. Universal CAR systems allow diversification of the targets of CAR-T cells using multiple adapter molecules with different targets.

Another concept for overcoming Ag heterogeneity is to collaborate with recipient T cells by inducing epitope spreading. Based on this concept, Flt3L-secreting CAR-T cells were recently developed. Flt3L is a growth factor promoting hematopoietic progenitor of DCs as well as survival and proliferation of DCs in tissues. It was previously reported that repeated injections of Flt3L expanded CD103+ DCs, which include a subset with the capacity of cross-presentation to CD8+ T cells. Actually, combination therapy against an OVA-expressing tumor with Flt3L-secreting CAR-T cells and adjuvants that activate DCs increased CD8+ T cells reactive to OVA in a host. Induction of epitope spreading would be a rational strategy to enhance CAR-T-cell therapy and overcome Ag heterogeneity of solid tumors by inducing anti-cancer responses by polyclonal host CD8+ T cells to Ags other than the target of the CAR.

3.2 | Break through the immunosuppression in TME

As described above, TME contains many immunosuppressive cell populations, and they secrete humoral mediators and express immune checkpoint molecules that support tumor growth. The basic concept for this issue, therefore, is how to make CAR-T cells resistant to the immunosuppressive environment. One approach based on the concept is “switch receptors,” which convert an inhibitory signal to a stimulatory one. Anti-TGF-β CAR which is designed to be a switch receptor consists of an scFv specific to TGF-β and intracellular signaling domains derived from the T-cell co-stimulatory molecules used in conventional CAR (Figure 2A). Actually, it was reported that anti-TGF-β CAR-T cells were strongly activated by soluble TGF-β, suggesting that humoral inhibitory mediators secreted in TME can be converted into cytokines that promote growth and functions of T cells by switch receptors. Another approach is to cancel inhibitory signals from immune checkpoint molecules, by which several types of modified CAR-T cells have been devised. One strategy is to endow CAR-T cells with the capacity to produce scFv to specifically inhibit immune checkpoint functions.

Enhancement of the in vivo anti-tumor efficacies of anti-PD-1 scFv-secreting CAR-T cells was demonstrated using some solid tumor models. Similarly, anti-PD-L1 scFv-producing CAR-T cells were also developed, and the tumor growth inhibitory potential of those CAR-T cells was reported to be upregulated in a clear cell renal cell carcinoma model. Another strategy is to express dominant-negative receptors for PD-1 or switch receptors which consist of the extracellular domain of PD-1 and the transmembrane and cytoplasmic signaling domains of CD28 on CAR-T cells (Figure 2B). Both types of CAR-T cells expressing the receptors that cancel PD-1 signaling were shown to exert the augmented anti-cancer activities in multiple solid tumor models. In addition, it is reasonable to disrupt the expression of immune checkpoint molecules on CAR-T cells by shRNA or CRISPR-Cas9 gene-editing systems (Figure 2C). In this regard, PD-1 knockout CAR-T cells enhanced the clearance of tumors compared with the control CAR-T cells in a xenograft model.
solid tumors. In several murine models, CAR-T cells genetically engineered to express CXCR1 or CXCR2, both of which are IL-8 (CXCL8) receptors, induced significant infiltration into and prolonged persistence in the tumor tissues, resulting in the complete remission of the tumors.\textsuperscript{79–81} CCR2b-expressing CAR-T cells have exhibited an enhanced infiltration into CCL2-producing tumors and potent antitumor activities in xenograft models of neuroblastoma\textsuperscript{82} and malignant pleural mesothelioma.\textsuperscript{83} Although CXCL16 is highly produced in pancreatic cancers, CXCR6, a receptor for CXCL16, is rarely expressed on effector T cells. CAR-T cells modified to express CXCR6 displayed pronounced intratumoral accumulation, sustained antitumor activities, and prolonged murine survivals in orthotopic and PDX models.\textsuperscript{84} The CCL1–CCR8 axis plays an important role in the recruitment of Tregs, which could be a source of TGF-β, into TME.\textsuperscript{85}

To hijack the chemokine axis and to cancel the signal from TGF-β, CAR-T cells co-expressing CCR8 and a dominant-negative form of TGF-β receptor 2 were developed. Actually, these modified CAR-T cells exhibited efficient and sustained infiltration into the tumor and improved therapeutic efficacies in murine pancreatic tumor models.\textsuperscript{86}

Another concept for augmenting the intratumoral accumulation of CAR-T cells is to degrade and “soften” the rigid ECM acting as a physical barrier. Heparanase is an enzyme cleaving heparan sulfate chains of proteoglycans, which is the major component of the ECM.\textsuperscript{87} CAR-T cells engineered to produce heparanase exhibit the capacity to degrade the ECM, which then induced T-cell infiltration and marked anti-tumor activities in xenogenic neuroblastoma and melanoma models\textsuperscript{88} (Figure 3B).
Recently, we developed CAR-T cells that concomitantly produced IL-7, which is a cytokine for stimulating T-cell proliferation and survival, and CCL19, which is a chemokine for regulating chemotaxis of T cells and DCs (7x19 CAR-T cells)\(^9\) (Figure 4). Previous studies have demonstrated that the combination of IL-7 and CCL19 is produced by TRCs in lymph nodes and is important for the formation and maintenance of T-cell and dendritic cell concentrated structures.\(^90,91\)
By conferring the inherent property of this combination on tumor-specific CAR-T cells, we aimed to mimic the function of TRCs and accumulate T cells and DCs within tumor tissues. Treatment with 7×19 CAR-T cells induced dense infiltrations of not only donor T cells but also recipient T cells and DCs within the tumor tissues, leading to potent anti-tumor effects by collaboration between CAR-T cells and recipient immune cells, while it remains unclear whether the recruited DCs actually present tumor antigens to and activate host T cells within the tumor tissue. The salient capacities of 7×19 CAR-T cells themselves to eradicate solid tumors and to induce immunological memory were also displayed in orthotopic and PDX models, in which allogeneic T cells were used as the source of 7×19 CAR-T cells. Interestingly, 7×19 CAR-T cells were suggested to be resistant to immune checkpoint molecules by decreasing the expression levels of those molecules compared with conventional CAR-T cells. Markedly, 7×19 CAR-T cells efficiently induced epitope spreading, resulting in immunological memory even against the tumor cells that did not express the CAR target. While these findings implicate a potential of 7×19 CAR-T cells as a novel therapy for solid tumors, its actual efficacy and safety that need to be evaluated through the results of clinical trials, which are currently awaited.

4 | FUTURE PERSPECTIVE OF CAR-T-CELL THERAPY AGAINST SOLID TUMORS

At present, CAR-T cells have been developed as highly effective agents for B-cell malignancies. In this review, we discuss the hurdles unique to solid tumors that current CAR-T-cell therapy faces and the strategies to solve those issues. To overcome the roadblocks, it would be necessary to establish biomarkers for spatiotemporal information on the cellular and molecular components, on the immunosuppressive environment, and on Ag expression in solid tumor tissues. Based on those types of information, combination therapy
with agents such as immune checkpoint inhibitors or molecular target drugs may be a hopeful approach. In the conventional CAR-T-cell system, the function of the cells has been focused on their ability to directly kill cancer cells. In next generation systems, however, CAR-T cells would be required to act not only as "killers" but also as "deliverers" of molecules regulating and exploiting patient’s inherent immune functions to induce cooperative actions against the tumors, as demonstrated by $7 \times 19$ CAR-T-cell therapy (Figure 4). The time has come for a paradigm shift in CAR-T cells.

ACKNOWLEDGMENTS
We are indebted to Ms. Reiko Ohashi and Ms. Aki Kawai for administrative support.

CONFLICT OF INTEREST
KA has no conflict of interest to declare. KT is shareholder at Noile-Immune Biotech Inc., and received remuneration from Noile-Immune Biotech Inc. KT is a current associate editor of Cancer Science.

REFERENCES
1. Adachi K, Tamada K. Immune checkpoint blockade opens an avenue of cancer immunotherapy with a potent clinical efficacy. Cancer Sci. 2015;106:945-950.
2. Baumeister SH, Freeman GJ, Dranoff G, Sharpe AH. Coinhibitory pathways in immunotherapy for cancer. Annu Rev Immunol. 2016;34:539-573.
3. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. Science. 2018;359:1350-1353.
4. June CH, Sadelain M. Chimeric antigen receptor therapy. N Engl J Med. 2018;379:64-73.
5. June CH, O’Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CART cell immunotherapy for human cancer. Science. 2018;359:1361-1365.
6. Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc Natl Acad Sci USA. 1993;90:720-724.
8. Finney HM, Lawson AD, Bebbington CR, Weir AN. Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. *J Immunol*. 1998;161:2791-2797.

9. Maher J, Brentjens RJ, Gunset G, Rivière I, Sadelain M. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta/CD28 receptor. *Nat Biotechnol*. 2002;20:70-75.

10. Imai C, Mihara K, Andreansky M, et al. Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia. *Leukemia*. 2004;18:676-684.

11. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor- modified T cells in chronic lymphoid leukemia. *N Engl J Med*. 2011;365:725-733.

12. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med*. 2013;368:1509-1518.

13. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med*. 2014;371:1507-1517.

14. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med*. 2019;380:45-56.

15. Locke FL, Neelapu SS, Bartlett NL, et al. Phase 1 results of ZUMA-1: a multicenter study of KTE-C19 anti-CD19 CAR T cell therapy in refractory aggressive lymphoma. *Mol Ther*. 2017;25:285-295.

16. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. 2017;377:2531-2544.

17. Wang M, Munoz J, Goy A, et al. KTE-X19 CAR T-cell therapy in relapsed or refractory mantle-cell lymphoma. *N Engl J Med*. 2020;382:1331-1342.

18. Abramson JS, Palomba ML, Gordon LJ, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSEND NHL 001): a multicentre seamless design study. *Lancet*. 2020;396:839-852.

19. Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science*. 2002;298:850-854.

20. Tan JT, Ernst B, Kieper WC, LeRoy E, Sprent J, Surh CD. Interleukin-12 and IL-15 and IL-7 jointly regulate homeostatic proliferation of memory CD8+ T cells but are not required for memory phenotype CD4+ cells. *J Exp Med*. 2002;195:1523-1532.

21. Munshi NC, Anderson LD Jr, Shah N, et al. Idecabtagene videsel in relapsed and refractory multiple myeloma. *N Engl J Med*. 2021;384:705-716.

22. Newick K, O'Brien S, Moon E, Albeda SM. CAR T cell therapy for solid tumors. *Annu Rev Med*. 2017;68:139-152.

23. Guedan S, Ruella M, June CH. Emerging cellular therapies for cancer. *Annu Rev Immunol*. 2019;37:145-171.

24. Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nat Rev Clin Oncol*. 2020;17:147-167.

25. Morello A, Sadelain M, Adusumilli PS. Mesothelin-targeted CARs: driving T cells to solid tumors. *Cancer Discov*. 2016;6:133-146.

26. Martínez M, Moon EK. CAR T cells for solid tumors: new strategies for finding, infiltrating, and surviving in the tumor microenvironment. *Front Immunol*. 2019:10:128.

27. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing EBV. *Mol Ther*. 2010;18:843-851.

28. Wei J, Han X, Bo J, Han W. Target selection for CAR-T therapy. *J Hematol Oncol*. 2019;12:62.

29. McGranahan N, Swanton C. Clonal heterogeneity and tumor evolution: past, present, and the future. *Cell*. 2017;168:613-628.

30. Kailanygiri S, Altuvia B, Wiebel M, Jamitzky S, Rossig C. Overcoming heterogeneity of antigen expression for effective CAR T cell targeting of cancers. *Cancers (Basel)*. 2020;12:1075.

31. Hegde M, Corder A, Chow KK, et al. Combinational targeting of sets antigen escape and enhances effector functions of adoptively transferred T cells in glioblastoma. *Mol Ther*. 2013;21:2087-2101.

32. O'Rourke DM, Nasrallah MP, Desai A, et al. 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*. 2017;9:eee9894.

33. Majnemer RG, Mackall CL. Tumor antigen escape from CAR T-cell therapy. *Cancer Discov*. 2018;8:1219-1226.

34. Binnewies M, Roberts EW, Kersten K, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med*. 2018;24:541-550.

35. Valkenburg KC, de Groot AE, Pienta KJ. Targeting the tumour stroma to improve cancer therapy. *Nat Rev Clin Oncol*. 2018;15:366-381.

36. Hinshaw DC, Shevde LA. The tumor microenvironment innately modulates cancer progression. *Cancer Res*. 2019;79:4557-4566.

37. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. *J Clin Oncol*. 2015;33:1974-1982.

38. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12:252-264.

39. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med*. 2013;19:1423-1437.

40. Slaney CY, Kershaw MH, Darcy PK. Trafficking of T cells into tumors. *Cancer Res*. 2014;74:7168-7174.

41. Dangaj D, Bruand M, Grimm AJ, et al. Cooperation between constitutive and inducible chemokines enables T cell engraftment and immune attack in solid tumors. *Cancer Cell*. 2019;35:885-900.e810.

42. Ozga AJ, Chow MT, Luster AD. Chemokines and the immune response to cancer. *Immunity*. 2021;54:859-874.

43. Kim R, Emi M, Tanabe K, Arihiro K. Tumor-driven evolution of immune suppressive networks during malignant progression. *Cancer Res*. 2006;66:5527-5536.

44. Massagué J. TGFbeta in cancer. *Cell*. 2008;134:215-230.

45. Mannino MH, Zhu Z, Xiao H, Bai Q, Wakefield MR, Fang Y. The paradoxical role of IL-10 in immunity and cancer. *Cancer Lett*. 2015;367:103-107.

46. Shevach EM. Mechanisms of foxp3+ T regulatory cell-mediated suppression. *Immunity*. 2009;30:636-645.

47. Chen L, Han X. Anti-PD-1/PD-L1 therapy of human cancer: past, present, and future. *J Clin Invest*. 2015;125:3384-3391.

48. Umedu D, Okada N, Sakoda Y, et al. Inhibitory functions of PD-L1 and PD-L2 in the regulation of anti-tumor immunity in murine tumor microenvironment. *Cancer Immunol Immunother*. 2019;68:201-211.

49. Hong M, Clubb JD, Chen YY. Engineering CAR-T cells for next-generation cancer therapy. *Cancer Cell*. 2020;38:473-488.

50. Shen L, Xiao Y, Tian J, Lu Z. Remodeling metabolic fitness: strategies for improving the efficacy of chimeric antigen receptor T cell therapy. *Cancer Lett*. 2022;529:139-152.

51. Gao TA, Chen YY. Engineering next-generation CAR-T cells: overcoming tumor hypoxia and metabolism. *Annu Rev Chem Biomol Eng*. 2021;13:193-216.

52. Nakagawa Y, Negishi Y, Shimizu M, Takahashi M, Ichikawa M, Takahashi H. Effects of extracellular pH and hypoxia on the function and development of antigen-specific cytotoxic T lymphocytes. *Immunol Lett*. 2015;167:72-86.

53. Risau W. Mechanisms of angiogenesis. *Nature*. 1997;386:671-674.

54. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature*. 2000;407:249-257.

55. Griffioen AW, Damen CA, Martinotti S, Blijham GH, Groenewegen G. Endothelial intercellular adhesion molecule-1 expression is suppressed in human malignancies: the role of angiogenic factors. *Cancer Res*. 1996;56:1111-1117.
56. Dirkx AE, Oude Egbrink MG, Kuipers MJ, et al. Tumor angiogenesis modulates leukocyte- vessel wall interactions in vivo by reducing endothelial adhesion molecule expression. Cancer Res. 2003;63:2322-2329.

57. Lohr J, Ratliff T, Huppertz A, et al. Effector T-cell infiltration positively impacts survival of glioblastoma patients and is impaired by tumor-derived TGF-β. *Clin Cancer Res*. 2011;17:4296-4308.

58. Theocharis AD, Skandalis SS, Gialeli C, Karamanos NK. Extracellular matrix structure. *Adv Drug Deliv Rev*. 2016;97:4-27.

59. Salmon H, Franciszkieczak K, Damotte D, et al. Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. *J Clin Invest*. 2012;122:899-910.

60. van der Schans JJ, van de Donk N, Mutis T. Dual targeting to over-expressing HER2 and IL13Rα2 for enhanced glioblastoma therapy. *Adv Ther Oncolytics*. 2022;24:729-741.

61. Hegde M, Mukherjee M, Grada Z, et al. Tandem CAR T cells targeting HER2 and IL13Rα2 mitigate tumor antigen escape. *J Clin Invest*. 2016;126:3036-3052.

62. Muhammad N, Wang R, Li W, et al. A novel TanCAR targeting IL13Rα2 and EphA2 for enhanced glioblastoma therapy. *Mol Ther Oncolytics*. 2022;24:729-741.

63. Urbanska K, Lanitis E, Poussin M, et al. A universal strategy for adoptive immunotherapy of cancer through use of a novel T-cell antigen receptor. *Cancer Res*. 2012;72:1844-1852.

64. Tamada K, Geng D, Sakoda Y, et al. Redirecting gene-modified T cells toward various cancer types using tagged antibodies. *Clin Cancer Res*. 2012;18:6436-6445.

65. Ma JS, Kim JY, Kazane SA, et al. Versatile strategy for controlling the specificity and activity of engineered T cells. *Proc Natl Acad Sci U S A*. 2016;113:E450-E458.

66. Rodgers DT, Mazagova M, Hampton EN, et al. Switch-mediated activation and retargeting of CAR-T cells for B-cell malignancies. *Proc Natl Acad Sci USA*. 2016;113:E459-E468.

67. Cho JH, Collins JJ, Wong WW. Universal chimeric antigen receptors for multiplexed and logical control of T cell responses. *Cell* 2018;173:1426-1438.

68. Brossart P. The role of antigen spreading in the efficacy of immunotherapies. *Clin Cancer Res*. 2020;26:4442-4447.

69. Lai J, Mardiana S, House IG, et al. Adoptive cellular therapy with T cells expressing the dendritic cell growth factor Flt3L drives epitope spreading and antitumor immunity. *Nat Immunol*. 2020;21:914-926.

70. Mayer CT, Ghorbani P, Nandan A, et al. Selective and efficient generation of functional Batf3-dependent CD103+ dendritic cells from mouse bone marrow. *Blood*. 2014;124:3081-3091.

71. Salmon H, Idoyaga J, Rahman A, et al. Expansion and activation of CD103+ dendritic cell progenitors at the tumor site enhances tumor responses to therapeutic PD-L1 and BRAF inhibition. *Immunity*. 2016;44:924-938.

72. Chang ZL, Lorenzini MH, Chen X, Tran U, Bangayan NJ, Chen YY. Rewiring T-cell responses to soluble factors with chimeric antigen receptors. *Nat Chem Biol*. 2018;14:317-324.

73. Rafiq S, Yokuu OO, Jackson HJ, et al. Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy in vivo. *Nat Biotechnol*. 2018;36:847-856.

74. Nakajima M, Sakoda Y, Adachi K, Nagano H, Tamada K. Improved survival of chimeric antigen receptor-engineered T (CAR-T) and tumor-specific T cells caused by anti-programmed cell death protein 1 single-chain variable fragment-producing CAR-T cells. *Cancer Sci*. 2019;110:3079-3088.

75. Suarez ER, Chang de K, Sun J, et al. Chimeric antigen receptor T cells secreting anti-PD-L1 antibodies more effectively regress renal cell carcinoma in a humanized mouse model. *Oncotarget*. 2016;7:34341-34355.

76. Cherkassky L, Morello A, Villena-Vargas J, et al. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. *J Clin Invest*. 2016;126:3130-3144.

77. Liu X, Ranganathan R, Jiang S, et al. A chimeric switch-receptor targeting PD1 augments the efficacy of second-generation CAR T cells in advanced solid tumors. *Cancer Res*. 2016;76:1578-1590.

78. Rupp LJ, Schumann K, Roybal KT, et al. CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells. *Sci Rep*. 2017;7:737.

79. Jin L, Tao H, Karachi A, et al. CXCR1- or CXCR2-modified CAR T cells co-opt IL-8 for maximal antitumor efficacy in solid tumors. *Nat Commun*. 2019;10:4016.

80. Whilling LM, Halim L, Draper B, et al. CAR T-cells targeting the integrin αvβ6 and co-expressing the chemokine receptor CXCR2 demonstrate enhanced homing and efficacy against several solid malignancies. *Cancers (Basel)*. 2019;11:674.

81. Liu G, Rui W, Zheng H, et al. CXCR2-modified CAR-T cells have enhanced trafficking ability that improves treatment of hepatocellular carcinoma. *Eur J Immunol*. 2020;50:712-724.

82. Craddock JA, Lu A, Bear A, et al. Enhanced tumor trafficking of GD2 chimeric antigen receptor T cells by expression of the chemokine receptor CCR2b. *J Immunother*. 2010;33:780-788.

83. Moon EK, Carpenito C, Sun J, et al. Expression of a functional CCR2 receptor enhances tumor localization and tumor eradication by targeted human T cells expressing a mesothelin-specific chimeric antibody receptor. *Clin Cancer Res*. 2011;17:4719-4730.

84. Lesch S, Blumenberg V, Stoiber S, et al. T cells armed with C-X-C chemokine receptor type 6 enhance adoptive cell therapy for pancreatic tumors. *Nat Biomed Eng*. 2021;5:1246-1260.

85. Whiteside TL. Regulatory T cell subsets in human cancer: are they regulating for or against tumor progression? *Cancer Immunol Immunother*. 2014;63:67-72.

86. Cadilha BL, Benmebarek MR, Dorman K, et al. Combined tumor-directed recruitment and protection from immune suppression enable CAR T cell efficacy in solid tumors. *Sci Adv*. 2021;7:eabi5781.

87. Coombe DR, Gandhi NS. Heparanases: a challenging cancer drug target. *Front Oncol*. 2019;9:1316.

88. Caruana I, Savoldi B, Hoyos V, et al. Heparanase promotes tumor infiltration and antitumor activity of CAR-directed T lymphocytes. *Nat Med*. 2015;21:524-529.

89. Adachi K, Kano Y, Nagai T, Okuyama N, Sakoda Y, Tamada K. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. *Nat Biotechnol*. 2018;36:346-351.

90. Luther SA, Bidgol A, Hargreaves DC, et al. Differing activities of homoeostatic chemokines CCL19, CCL21, and CXCL12 in lymphocyte and dendritic cell recruitment and lymphoid neogenesis. *J Immunol*. 2002;169:424-433.

91. Link A, Vogt TK, Favre S, et al. Fibroblastic reticular cells in lymph node regulate the homeostasis of naive T cells. *Nat Immunol*. 2007;8:1255-1265.

92. Goto S, Sakoda Y, Adachi K, et al. Enhanced anti-tumor efficacy of IL-7/CCL19-producing human CAR-T cells in orthotopic and patient-derived xenograft tumor models. *Cancer Immunol Immunother*. 2021;70:2503-2515.