Adoptive T cell therapies for solid tumors: T(I)ME is of the essence

Nour Amwas,[a,b] Chu-Hsuan Chiu,[b] Diana Gumber,[a,b] and Leo D. Wang[a,b,c,*]

[a]Irell and Manella Graduate School of Biological Sciences, City of Hope National Medical Center, Duarte, CA, United States
[b]Department of Immunooncology, City of Hope National Medical Center, Duarte, CA, United States
[c]Department of Pediatrics, City of Hope Children's Cancer Center, Duarte, CA, United States

eBioMedicine
2024;109: 105449
Published Online xxx
https://doi.org/10.1016/j.ebiom.2024.105449

Immunotherapy has revolutionized the treatment of many cancers. In particular, adoptive T cell therapies (ATTs) including tumor-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) T cell therapy, and chimeric antigen receptor (CAR) T cell therapy have all been used to affect durable and robust clinical responses in patients.[1] However, "immune desert" tumors, such as brain tumors, pose distinct challenges to ATT. On the tumor side, these challenges include antigen heterogeneity and antigen loss or escape. Poor immune cell trafficking, exhaustion, and hypofunction are central challenges on the immune cell side. Different ATTs address each of these challenges differently; for instance, TIL therapy typically consists of T cells that react with an array of tumor antigens, helping to combat antigen heterogeneity. In contrast, TCR T cell therapy is more responsive to tumors with low antigen density due to the exquisite sensitivity of TCR signaling. To capitalize on the strengths of each modality, recent work in the CAR T field seeks to develop an ATT sensitive to low levels of target antigen while also responsive to diverse tumor antigens.[2] Engineering CAR T cells that can promote immune responses against a broad array of tumor-associated antigens promises to transform the field of adoptive cellular therapy (ACT).

Current strategies

Approaches to overcoming antigen loss or escape include multi-antigen targeting CARs, which have been tested in clinical trials using anti-CD19/CD20 (NCT04007029) and HER2/IL13RA2/EGFR/B7H3 (NCT05768880) CAR T cells. Alternatively, combining virus-specific T (VST) cells and CAR technologies has been shown to broaden immune responses. For instance, transducing Epstein–Barr Virus (EBV) specific T cells with a CD30 CAR targets lymphomas that express CD30 or EBV antigens (NCT04288726). Similarly, cytomegalovirus (CMV)-pp65-specific T cells have been used to manufacture CD19 CAR T cells. These cells can be expanded *in vivo* through stimulation with a CMV triplex vaccine, currently being tested in clinical trials (NCT05801913, NCT05432635). Additionally, vaccinating CAR T cells with the CAR antigen itself has been shown to boost endogenous antitumor responses in preclinical glioblastoma models.[3]

Other approaches focus on increasing CAR T cell trafficking into the tumor microenvironment (TME) through the expression of chemokine receptors such as CCR6 and CXCR2.[4] Conversely, oncolytic adenoviruses have been used to induce tumor cells to express homing chemokines such as CXCL11 to recruit antitumor immune cells and promote a local proinflammatory milieu.[2] To further address the physical barriers within the TME, CAR T cells have been engineered to express heparanase to degrade heparan sulfate proteoglycans in the extracellular matrix.[4]

To combat the profoundly immunosuppressive TME found in immune desert tumors, recent studies have focused on CAR T cells that carry additional transgenes to improve CAR T cell function or alter the TME. One strategy aims to arm CAR T cells with cytokine signals to enhance T cell response, often with modifications to spatially or temporally restrict cytokine expression.[5] Another strategy counteracts inhibitory signaling by either inducing the secretion of immune checkpoint blockade antibodies or deleting inhibitory receptors such as TGFβ receptor II or PD-1 (NCT03089203, NCT03545815).[5]

These next-generation strategies allow for the further enhancement of CAR T cell function, persistence, and infiltration. However, despite these advances, clinical cures have remained elusive in the treatment of solid tumors. As such, ACT strategies have emerged that aim to improve anti-tumor function and the durability of the immune response by engaging endogenous immune cells.

Future directions

As a robust endogenous anti-tumor response has been shown to improve response to ATT, many novel strategies for improving ATT use cellular therapies to communicate directly with endogenous immune cells. For example, a recent study utilizes CAR T cells that secrete T cell-engaging antibody molecules to recruit endogenous T cells and redirect them against the tumor (NCT05660369). Alternatively, CAR T cells have been

*Corresponding author. Departments of Pediatrics and Immunooncology, City of Hope, Duarte, CA 91010, United States.
E-mail address: leo.wang@coh.org (L.D. Wang).

engineered to secrete exosomes containing RNA RN7SL1, a molecule that restricts maturation of myeloid-derived suppressor cells, inhibits secretion of TGFβ from myeloid cells, augments expression of costimulatory molecules on dendritic cells (DC), and promotes the migration of endogenous immune cells in solid tumor mouse models.[2] CAR T cells have also been engineered to augment epitope spreading by secreting FMS-like tyrosine kinase 3 ligand (Flt3L) or amphiphilic polyethylene glycol (PEG)-lipid 'amph-ligand' conjugates that can coat DC in draining lymph nodes (dLN) with antigenic ligands, in effect priming T cells to expand *in vivo* and improve their function and efficacy.[6]

Whereas amph-ligand vaccines rely on immune cell trafficking to dLNs for priming and expansion, an exciting parallel strategy seeks to nucleate tertiary lymphoid structures (TLSes) within the TME. TLSes are ectopic lymphoid aggregates that form in non-lymphoid tissue at the site of chronic inflammation, including within solid tumors. Resembling secondary lymphoid organs in both appearance and function, TLSes direct a local antigen-specific immune response. The presence of TLSes in some solid tumors has been correlated with improved survival and response to immunotherapy in patients. Preclinical models demonstrate that TLSes can be induced to drive robust antitumor responses characterized by increased infiltration of tumor-reactive T cells, production of tumor-reactive antibodies, maturation of DCs, and the formation of memory T and B cells.[7] Current strategies for nucleating TLSes include administration of chemokines and cytokines, vessel targeted peptide-LIGHT fusion proteins, antibodies, or cancer vaccines.[7] These strategies hold promise for promoting ATT and endogenous immune cell crosstalk while also underscoring the complexity of TLS biology.

Epigenetic modifiers (EM) are utilized during CAR T cell manufacturing to resist exhaustion and improve metabolic fitness, proliferation and persistence. Importantly, EMs influence both immune cells and non-immune cells, and therefore hold great potential in combination with CAR T therapy. For example, Azacitidine (Aza), a DNA methyltransferase inhibitor (DNMTi), induces M1 macrophage polarization and DC maturation, and reduces the number of myeloid-derived suppressor cells (MDSCs). Additionally, Aza increases antigen presentation by MHC class I on tumor cells.[8] Similarly, augmented antigen presentation is observed with inhibitors of histone methyltransferases-EZH2, histone demethylase-LSD1, or histone deacetylase-HDAC, which facilitate epitope spreading and improve anti-tumor response. Preclinical models demonstrate that combining EMs and CAR T cells improves T cell persistence and anti-tumor activity as well as increased infiltrating cytotoxic T lymphocytes and TILs.[9,10] Combining these epigenetic strategies with CAR T cell therapy in solid tumor clinical trials may be a promising avenue for further study.

ATTs, including CAR T cell therapy, face significant challenges when targeting solid tumors. Tumor associated antigen loss or escape, poor immune trafficking, and immune cell hypofunction present complex and mutually reinforcing mechanisms of resistance in the immunosuppressive tumor microenvironment. Overcoming these barriers will require CAR T cells to resist this environment and promote a robust and broad antitumor response by engaging the endogenous immune system. Understanding the crosstalk between adoptively transferred T cells, the immune system, and the tumor microenvironment will be essential for improving the clinical efficacy of CAR T cells in solid tumors.

Contributors

This Commentary was conceived of by LDW and NA. NA, DG, and CHC wrote the first draft. All authors edited and approved the final manuscript.

Declaration of interests

The authors have no conflicts of interest relevant to the above work. LDW serves on the SAB of Autolomous, Ltd, consults for Gerson Lehrman Group, and has provided expert witness services.

Acknowledgements

LDW acknowledges support from CIRM, the V Foundation, the Hyundai Hope on Wheels Foundation, the Marcus Foundation, the Pediatric Cancer Research Foundation, the Panda Cares Foundation, and the Turtle Pond Foundation. DG acknowledges support from CIRM.

References

1 Mitra A, Barua A, Huang L, Ganguly S, Feng Q, He B. From bench to bedside: the history and progress of CAR T cell therapy. *Front Immunol*. 2023;14:1188049.
2 Zhang P, Zhang G, Wan X. Challenges and new technologies in adoptive cell therapy. *J Hematol Oncol*. 2023;16(1):97.
3 Ma L, Hostetler A, Morgan DM, et al. Vaccine-boosted CAR T crosstalk with host immunity to reject tumors with antigen heterogeneity. *Cell*. 2023;186(15):3148–3165.e20.
4 Lefler DS, Manobianco SA, Bashir B. Immunotherapy resistance in solid tumors: mechanisms and potential solutions. *Cancer Biol Ther*. 2024;25(1):2315655.
5 Tang L, Pan S, Wei X, Xu X, Wei Q. Arming CAR-T cells with cytokines and more: innovations in the fourth-generation CAR-T development. *Mol Ther*. 2023;31(11):3146–3162.
6 Zhang AQ, Hostetler A, Chen LE, et al. Universal redirection of CAR T cells against solid tumours via membrane-inserted ligands for the CAR. *Nat Biomed Eng*. 2023;7(9):1113–1128.
7 Khanal S, Wieland A, Gunderson AJ. Mechanisms of tertiary lymphoid structure formation: cooperation between inflammation and antigenicity. *Front Immunol*. 2023;14:1267654.
8 Zhong F, Lin Y, Zhao L, Yang C, Ye Y, Shen Z. Reshaping the tumour immune microenvironment in solid tumours via tumour cell and immune cell DNA methylation: from mechanisms to therapeutics. *Br J Cancer*. 2023;129(1):24–37.
9 Xia L, Liu JY, Zheng ZZ, et al. BRD4 inhibition boosts the therapeutic effects of epidermal growth factor receptor-targeted chimeric antigen receptor T cells in glioblastoma. *Mol Ther*. 2021;29(10):3011–3026.
10 Yang J, Xu J, Wang W, Zhang B, Yu X, Shi S. Epigenetic regulation in the tumor microenvironment: molecular mechanisms and therapeutic targets. *Signal Transduct Target Ther*. 2023;8(1):210.