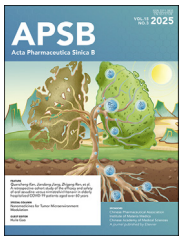
Acta Pharmaceutica Sinica B 2025;15(3):1703−1705

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



HIGHLIGHT

Engineered bacteria assist CAR-cell immunotherapy

KEY WORDS

Chimeric antigen receptor immunotherapy;
Engineered bacteria;
Cancer immunotherapy;
Cytotoxicity effects;
Antigen recognition;
Solid tumor;
Tumor microenvironment;
Challenge

Adoptive cell therapy with chimeric antigen receptor (CAR) immunotherapy has demonstrated remarkable potential for hematologic malignancy while encountering challenges in extending the responsive list to solid tumors. The major hurdles include a lack of tumor-specific targets, inefficient trafficking, and tumor infiltration of the immune effector cells, along with their dysfunction and exhaustion in immune-suppressive tumor microenvironment (TME)[1]. Bacteria-mediated cancer immunotherapies (BCITs) have made remarkable progress in cancer immunotherapy over the past two decades. Parts of bacterial strains have already undergone phase 1 to 2 clinical trials, such as *Escherichia coli*, *Salmonella*, and *Listeria*[2]. Vincent's group[3] and Yang's group[4] have shed some light on integrating BCITs with CAR immunotherapy for further developing approach to cancer (Fig. 1).

CAR-cell therapy includes two key processes, antigen recognition and the cytotoxic activity of effector immune cells. For antigen recognition, target selection is essential. However, in solid tumors, heterogeneous antigen and antigen escape pose significant obstacles, not only reducing the targeting efficacy but also increasing the risk of off-tumor toxicity. CD19 and BCMA were two targets approved by the US Food and Drug Administration (FDA), while CD19-targeted CAR-T cells initially show high response rates in relapsed acute lymphoblastic leukemia, many patients experience recurrence due to CD19 loss. Similarly, the downregulation of BCMA in multiple myeloma complicates outcomes[5]. Current strategies often employ dual-antigen or multi-antigen targeting to enhance the breadth and specificity of CAR-cells, which has limitations of cell engineering complexity, difficulties in target selection, and potential crosstalk between targets. The other focus is the cytotoxicity of CAR-cells, hindered by the complex TME structure once they reach the tumor site. Current studies have shown limited tumor infiltration of intravenous CAR-cells in solid tumors, due to more accessible targets in the peripheral circulation of hematologic malignancies[6]. Developing armored CAR-cells secreting immunostimulatory cytokines is one strategy, that aims to modulate TME for better CAR-cells survival and the recruitment of endogenous immune cells.

Bacterial exhibits characteristic of tumor colonization due to the immune privilege, nutrient-rich, and hypoxic environment of tumor core[2], which manage to actively penetrate deep into the tumor through permeable blood vessels within the TME by chemotaxis released from dying cancer cells. The TME, as the "soil", determines the colonization and growth of the "seeds" of both tumor and bacterial. Additionally, immunogenic bacteria can directly activate immune cells, and the bacteria-derived metabolite or surface molecules can modulate immune cell functions and alter the metabolic characteristics of TME, potentially enhancing antitumor efficacy. Nowadays, live bacteria can be engineered and repurposed as immunotherapeutic agents or drug delivery vehicles with genetic manipulations. And various early-phase clinical trials have shown engineered bacteria is feasible and safe[2]. Enabling bacterial surface modification and drug production transforms bacteria into multifunctional warriors with significant application potential.

Vincent's group[3] has leveraged probiotics to address the first challenge of antigen recognition in CAR-cell therapy. They have constructed a probiotic-guided CAR-T cells (ProCARs) system, which engineered probiotic strain *E. coli* Nissle 1917 (EcN) to synthesize Pro^{tag}, releasing *in situ* and labeling the tumor cells as

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2025.01.020

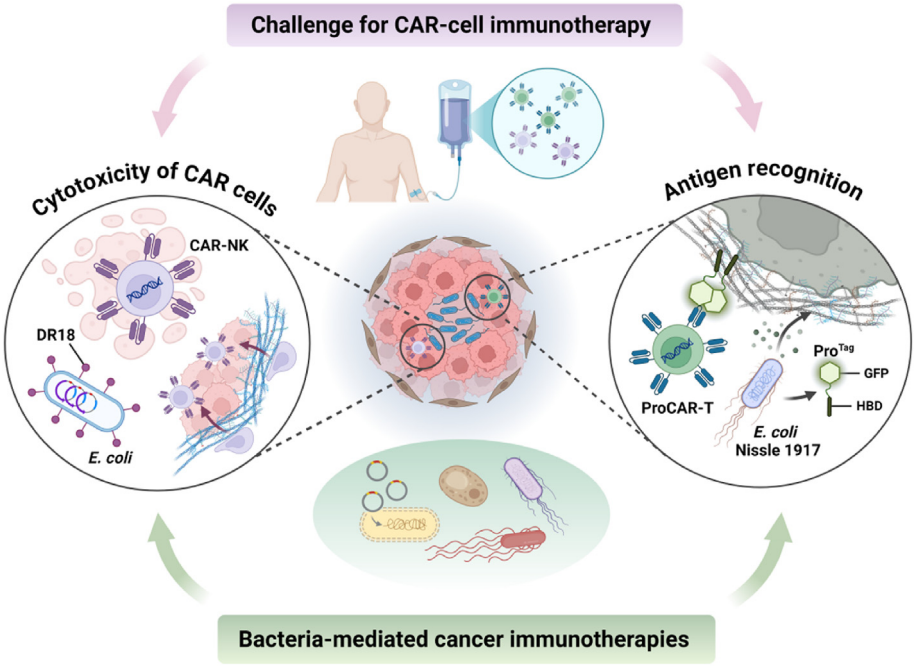


Figure 1 Challenges and innovations in CAR-cell immunotherapy using bacteria-mediated approaches.

CAR-T targets. The two terminals of Pro^{tag} have been designed for heparin binding domain (HBD) marking the tumor cells and surrounding stroma, and superfolder green fluorescent protein for CAR-T recognition. HBD is widely shared with cell surfaces and extracellular matrix as developmental biomarkers, which circumvents the antigen escape of non-developmental biomarkers mutations, though poses a potential risk for on-target off-tumor effects, the tumor-homing ability of the bacteria provides safety support. Additionally, EcN is engineered to co-release the human chemokine CXCL16, aimed at recruiting more CAR-T cells to the tumor site. In their previous study[7], engineered bacteria combine the expression of CXCL16 with CCL20 to recruit innate and adaptive immune cells, augmenting the overall anti-tumor immune response.

Regarding the other challenge, enhancing the cytotoxic activity of effector CAR-cells, Yang's group[4] has developed an engineered non-pathogenic *E. coli* K-12 DH5α to display murine decoy-resistant IL18 mutein (DR18) on bacterial surface, which induces CD8$^+$ T cell and natural killer (NK) cell responses, heat the TME, and further enhance CAR-NK cells therapy through serving as a "tumor GPS" increasing infiltration and prolong half-life in the TME due to its continued biosynthesis by the live bacteria. Advancing beyond Vincent's design of bacteria that secretes CXCL16, Yang's group[4] has applied surface display of immune-activating cytokines on the bacterial outer membrane, which results in significantly enhanced efficacy, due to the higher effective concentration, enabled by two-dimensional rather than three-dimensional mobility.

Building on the above promising research, further implementation is warranted. Firstly, the increased mechanical stress of solid tumors is one significant influence on bacteria diffusion efficiency, which leads to a denser and stiffer stroma, particularly evident in pancreatic cancer, liver cancer, and prostate cancer. Genetic engineering to modify bacterial envelope rigidity or expressing stress-related genes may be one viable strategy while balancing potential metastasis risks due to stromal alterations is essential. Secondly, patients with different types and stages of cancer exhibit significant variations in tumor size, which can also affect bacterial colonization efficiency. Vincent's group[3] notes that antitumor immunity in treated tumors can subsequently sufficiently direct responses against uncolonized tumors or "untagged" tumor areas. However, the applicability of this result to cancer patients remains uncertain because the volume of human solid tumors is 20−40 times larger than that of murine tumors[8]. Thirdly, bacteria as the live microorganism possess inherent immunogenicity, which has dual actions of an adjuvant for immunotherapy or cause cytokine storm and sepsis. Hence, clinical patients undergoing immunosuppressive cytotoxic therapies, and with artificial implants such as artificial joints/heart valves may be at an elevated risk of infection when administered live bacteria systemically[6]. Hence, balancing the bacterial load necessary for localized tumor treatment with the clearance of bacteria from healthy tissues to maintain homeostasis is essential. Nanotechnology and synthetic biology strategies may offer approaches for bacterial attenuation and delivery.

Overall, Vincent's group[3] and Yang's group[4] leveraged the advantages of BCITs to propose a novel strategy for improving tumor recognition and CAR-cell cytotoxic efficacy, expanding the application of CAR-cell therapy in solid tumors, which represents a significant validation study in the field of living therapies.

Acknowledgments

This work was supported by grants from the National Key R&D Program of China (2022YFB3808100 and 2021YFA0909900, Xiao Zhao), the National Natural Science Foundation of China (32222045 and 32171384, Xiao Zhao), High Level Chinese Medical Hospital Promotion Project (HLCMHPP2023001, HLCMHPP2023097, and HLCMHPP2023085, Jie Li). The figure is created in BioRender. Shao, T. (2024) https://BioRender.com/m65k464.

Author contributions

Tianyu Shao, Manman Xu, and Xiao Zhao wrote the paper. Jie Li and Xiao Zhao conceived and supervised the project.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Pan K, Farrukh H, Chittepu V, Xu H, Pan CX, Zhu Z. CAR race to cancer immunotherapy: from CAR T, CAR NK to CAR macrophage therapy. *J Exp Clin Cancer Res* 2022;**41**:119.
2. Kwon SY, Thi-Thu Ngo H, Son J, Hong Y, Min JJ. Exploiting bacteria for cancer immunotherapy. *Nat Rev Clin Oncol* 2024;**21**:569−89.
3. Vincent RL, Gurbatri CR, Li F, Vardoshvili A, Coker C, Im J, et al. Probiotic-guided CAR-T cells for solid tumor targeting. *Science* 2023; **382**:211−8.
4. Yang S, Sheffer M, Kaplan IE, Wang Z, Tarannum M, Dinh K, et al. Non-pathogenic *E. coli* displaying decoy-resistant IL18 mutein boosts anti-tumor and CAR NK cell responses. *Nat Biotechnol* 2024;**7**:1−13.
5. Albelda SM. CAR T cell therapy for patients with solid tumours: key lessons to learn and unlearn. *Nat Rev Clin Oncol* 2024;**21**:47−66.
6. Hahn J, Ding S, Im J, Harimoto T, Leong KW, Danino T. Bacterial therapies at the interface of synthetic biology and nanomedicine. *Nat Rev Bioeng* 2024;**2**:120−35.
7. Savage TM, Vincent RL, Rae SS, Huang LH, Ahn A, Pu K, et al. Chemokines expressed by engineered bacteria recruit and orchestrate antitumor immunity. *Sci Adv* 2023;**9**:eadc9436.
8. Travis WD, Asamura H, Bankier AA, Beasley MB, Detterbeck F, Flieder DB, et al. The IASLC Lung Cancer Staging Project: proposals for coding t categories for subsolid nodules and assessment of tumor size in part-solid tumors in the forthcoming eighth edition of the TNM classification of lung cancer. *J Thorac Oncol* 2016;**11**: 1204−23.

Tianyu Shao[a], Manman Xu[a], Jie Li[a,*], Xiao Zhao[b,*]

[a]*Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing 100053, China*

[b]*CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety & CAS Center for Excellence in Nanoscience, National Center for Nanoscience and Technology of China, Beijing 100190, China*

*Corresponding authors.

E-mail addresses: qfm2020jieli@yeah.net (Jie Li), zhaox@nanoctr.cn (Xiao Zhao)

Received 27 November 2024

Received in revised form 8 December 2024

Accepted 9 December 2024