

to cross the activation barrier ( $\Delta G$ ) for the excited MC state to relax into the ground state decreases, thereby shortening the MC lifetime from 55 ns to roughly 1 ns (see the figure). When the MC-state energy increases further in a family of cobalt(III) tris(2,2'-bipyridine)  $\{[\text{Co}(\text{bpy})_3]^{3+}\}$  compounds, the MC lifetime elongates to 6 ns in the best case, because of the build-up of a new activation barrier that must be overcome so that the MC excited state can relax to the ground state. The rate of the MC-state relaxation (the inverse of the MC-state lifetime) as a function of the MC-state energy, follows a bell-shaped curve, analogously to the energy dependence of electron transfer rates in Marcus theory (6, 7). In the “normal” regime, which is encountered by iron(II) compounds, relaxation rates increase with increasing excited state energy, whereas in the “inverted” zone, which is encountered by cobalt(III) compounds, relaxation decay rates decrease with increasing energy.

The excited states of cobalt(III) compounds are very strong oxidants, with potentials approaching 1.7 V compared with those of saturated calomel electrodes (SCEs), which is roughly 0.5 V more oxidizing than some of the benchmark iridium(III) photo-oxidants that are widely used in synthetic photochemistry (8). This makes them attractive for photocatalysis and enables new photoreactivity, including, for example, the oxidative coupling of aryl amides and aryl boronic acids, as demonstrated by Chan *et al.* This reaction type, which is of interest, for example, for the synthesis of medicinal agents, had previously proved very difficult to carry on with some reactants.

The work reported by Chan *et al.* is conceptually groundbreaking, yet some challenges remain for future research. The amount of visible light absorbed by the cobalt(III) compounds is low, and a substantial portion of the excitation energy is lost between light absorption and photocatalysis. It seems evident that after decades of research focused on iron(II), its neighboring metal elements in the periodic table with identical electron configurations might deserve more attention (9). ■

## REFERENCES AND NOTES

1. A. Y. Chan *et al.*, *Science* **382**, 191 (2023).
2. M. D. Woodhouse, J. K. McCusker, *J. Am. Chem. Soc.* **142**, 16229 (2020).
3. L. H. M. de Groot *et al.*, *J. Am. Chem. Soc.* **145**, 9369 (2023).
4. S. Kauffhold *et al.*, *J. Am. Chem. Soc.* **143**, 1307 (2021).
5. J. T. Yarranton, J. K. McCusker, *J. Am. Chem. Soc.* **144**, 12488 (2022).
6. R. A. Marcus, N. Sutin, *Biochim. Biophys. Acta Rev. Bioenerg.* **811**, 265 (1985).
7. G. L. Closs, J. R. Miller, *Science* **240**, 440 (1988).
8. C. K. Prier *et al.*, *Chem. Rev.* **113**, 5322 (2013).
9. N. Sinha, O. S. Wenger, *J. Am. Chem. Soc.* **145**, 4903 (2023).

10.1126/science.adk5923

## IMMUNOTHERAPY

# Engineered bacteria guide T cells to tumors

T cells and bacteria are engineered to work together to find and destroy tumor cells

By Eric M. Bressler and Wilson W. Wong

Since the US Food and Drug Administration (FDA) approved the first chimeric antigen receptor (CAR)-T cell product for acute lymphoblastic leukemia (ALL), the promise of similar success against nonhematologic cancers has fueled CAR-T cell development. CAR-T cells are a patient's own T cells engineered to express a synthetic CAR that enables native T cell signaling upon recognition of a chosen target antigen (1). Despite five additional CAR-T cell approvals for hematologic malignancies, efficacy against solid tumors remains elusive. On page 211 of this issue, Vincent *et al.* (2) report engineering bacteria to infiltrate solid tumors and produce antigens that selectively attach to extracellular matrix (ECM) proteins in the tumor microenvironment (TME); they also release a chemokine. The antigen and chemokine payloads recruit CAR-T cells into the tumors, resulting in reduction in tumor volume in mouse models of leukemia, colorectal cancer, and breast cancer. The

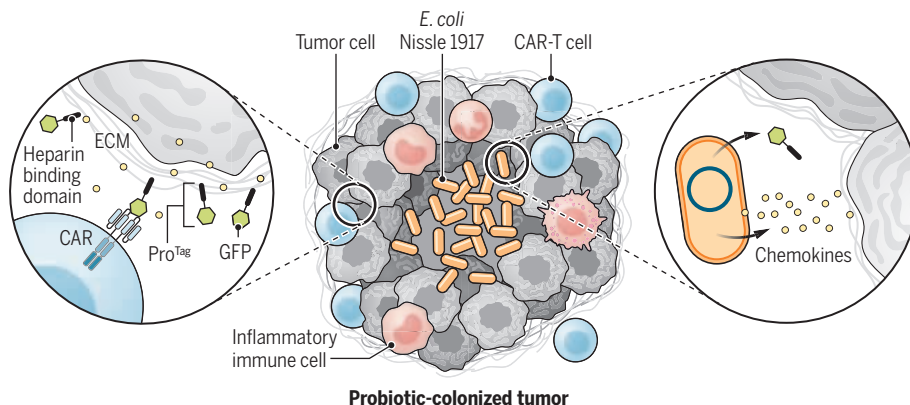
platform promises an antigen-agnostic targeting strategy with substantial customization potential.

Hematologic malignancies lend themselves to CAR-T cell therapy because tumor cells express ubiquitous targets (e.g., CD19); associated on-target, off-tumor toxicity is amenable to clinical intervention; and T cells efficiently traffic to tumors and malignant cells in circulation. However, solid tumors express heterogeneous and nonspecific antigens, and they are poorly infiltrated by T cells. Thus, on-target, off-tumor toxicity, wherein CAR-T cells attack the targeted antigen on healthy tissue, lead to potentially fatal effects in patients treated with CAR-T cells (3). Furthermore, heterogeneous expression of antigens tempers overall efficacy and enables continued proliferation of cancer cells that do not express the targeted antigen (4). Dense stroma and poor vascularization further inhibit T cell entry into solid tumors (1).

Strategies to overcome these challenges include incorporating genetic circuitry (e.g., logic-gated CAR-T cells) (5), switchable adapter proteins (6, 7), and targeted biomaterial enhancements (8). Vincent *et*

## Combining two immunotherapies

The probiotic-guided chimeric antigen receptor (CAR)-T cell (ProCAR) platform enables antigen tagging of tumors, adjuvant immune stimulation, and enhanced T cell trafficking to tumors. Engineered attenuated *Escherichia coli* Nissle 1917 release Pro<sup>Tag</sup> proteins into the extracellular space during intratumoral colonization. Pro<sup>Tag</sup> comprises a heparin binding domain to bind the extracellular matrix (ECM), and thereby “tag” tumors, and green fluorescent protein (GFP), which CAR-T cells are engineered to recognize. The *E. coli* Nissle 1917 could also be programmed to produce chemokines to drive CAR-T cell infiltration into solid tumors and inflammation.



*al.*'s probiotic-guided CAR-T cell (ProCAR) platform showcases the utility of engineered bacteria as a new enhancement to CAR-T cell therapy. Probiotic therapy for cancer involves intravenous or oral administration of attenuated bacterial strains to enable tumor colonization and stimulate immune system activity against tumors. Clinical trials have demonstrated safety, though not efficacy, for patients with substantial solid tumor burden (9, 10).

Combining probiotic therapy with CAR-T cell therapy, Vincent *et al.* used *Escherichia coli* Nissle 1917 engineered to release an immunogenic payload throughout the tumor upon colonizing the TME (see the figure). Therapeutic bacterial tumor colonization traditionally occurs through the natural proclivity of engineered bacteria for the immune-privileged necrotic tumor core after intravenous or intratumoral injection, and attenuation strategies such as chromosomal deletion and lipopolysaccharide (LPS) modification enable greater than 10,000-fold tumor accumulation without significant growth in healthy tissue (11). The released "Pro<sup>Tag</sup>" antigen comprises green fluorescent protein (GFP) linked to the heparin binding domain (HBD) of placenta growth factor 2 (PGF-2), which enables efficient binding to tumor ECM through HBD and bio-orthogonal antigen recognition by CAR-T cells through GFP. Thus, tumors colonized with the bacteria will be coated in Pro<sup>Tag</sup>, making them vulnerable targets to circulating CAR-T cells targeting GFP.

Vincent *et al.* tested the ProCAR platform in both humanized and immunocompetent mouse models of leukemia, colorectal cancer, and breast cancer. ProCAR reduced tumor volume compared to mock treatment and treatment with probiotics that express nonfunctional Pro<sup>Tag</sup> in all models. They demonstrated enhanced survival in a model of aggressive leukemia. Moreover, T cell tumor infiltration and tumor clearance were enhanced in the ProCombo system in which the bacteria also released an activating mutant chemokine, C-X-C motif chemokine 16 (CXCL16<sup>K42A</sup>). In particular, the success of ProCombo suggests the potential for greater engineering complexity of the probiotic, which could enable optimization and modification without increasing the complexity of treatment administration.

Additionally, Vincent *et al.* demonstrate key safety features of the ProCAR system in mice. Pro<sup>Tag</sup> binding was specific for CAR-T cells, so immune cell targeting and attendant treatment failure are unlikely to occur. Furthermore, both bacteria and Pro<sup>Tag</sup>

localized to the tumor, rather than healthy tissue. Thus, both bacterial blood growth (bacteremia) and on-target, off-tumor toxicity may be avoidable with careful probiotic attenuation and protocol optimization. Studies demonstrating T cell response to bacterial adjuvants in vitro and in vivo suggest a high potential for this strategy in immunologically "cold" tumors, which escape immune detection through low expression density of neoantigens.

Translation of the ProCAR system to the clinic will depend on scalability to larger tumors and attenuation of bacterial strains for safety. Vincent *et al.* note the possibility of tumor regions that could be untagged or may not be colonized by bacteria; such areas could be substantial in bulky tumors. Human tumors that are 2 cm in diameter, a lower threshold for the size of advanced tumors of a variety of cancer types in humans, are 20- to 40-fold larger than the mouse tumors in this study (12, 13). Future studies will require an investigation of the diffusion distance of Pro<sup>Tag</sup> through progressively larger tumors. Pro<sup>Tag</sup> production must be tailored to balance the intratumoral bacterial load, subsequent immune response, and Pro<sup>Tag</sup> density within the tumor. Additionally, humans mount a far more robust immune response against bacteria and the endotoxins that they produce than do mice. A phase I study of intravenous administration of attenuated *Salmonella typhimurium* remains the strongest evidence that tumor colonization with intravenous probiotics is safely achievable. However, dose-limiting bacteremia and other toxicities were observed at high doses (8). Thus, there is precedent to indicate that ProCAR could be safely achieved in humans with bacterial attenuation and efficacy at low bacterial dosage. The study of Vincent *et al.* is an important proof-of-concept for a potential approach to treating heterogeneous, immunologically cold, and poorly infiltrated solid tumors. ■

#### REFERENCES AND NOTES

1. S. Rafiq, C. S. Hackett, R. J. Brentjens, *Nat. Rev. Clin. Oncol.* **17**, 147 (2020).
2. R. L. Vincent *et al.*, *Science* **382**, 211 (2023).
3. R. A. Morgan *et al.*, *Mol. Ther.* **18**, 843 (2010).
4. C. L. Flügge *et al.*, *Nat. Rev. Clin. Oncol.* **20**, 49 (2023).
5. K. T. Roybal *et al.*, *Cell* **164**, 770 (2016).
6. J. H. Cho, J. J. Collins, W. W. Wong, *Cell* **173**, 1426 (2018).
7. J. S. Y. Ma *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **113**, E450 (2016).
8. X. Huang *et al.*, *Nat. Nanotechnol.* **16**, 214 (2021).
9. J. F. Toso *et al.*, *J. Clin. Oncol.* **20**, 142 (2002).
10. T. J. Gniadek *et al.*, *J. Immunother.* **43**, 217 (2020).
11. C. R. Gurbatri, N. Arpaia, T. Danino, *Science* **378**, 858 (2022).
12. H. G. Welch, P. C. Prorok, A. J. O'Malley, B. S. Kramer, *N. Engl. J. Med.* **375**, 1438 (2016).
13. W. D. Travis *et al.*, *J. Thorac. Oncol.* **11**, 1204 (2016).

#### ACKNOWLEDGMENTS

E. M. B. is a paid consultant of Ionic Pharmaceuticals, LLC. W. W. W. owns shares in Senti Biosciences.

Biomedical Engineering and Biological Design Center, Boston University, Boston, MA, USA. Email: emb1@bu.edu; wilwong@bu.edu

#### CELL BIOLOGY

## A signaling lipid drives synapse formation

Phosphatidylinositol 3,5-bisphosphate enables transport of proteins to synaptic sites

By Pilar Rivero-Ríos and Lois S. Weisman

**B**rain function relies on the accurate formation of synaptic contacts, and alterations in this process are implicated in disease, including some psychiatric and neurodevelopmental disorders. Synapse formation requires the synthesis and transport of pre-synaptic proteins from the soma (which contains the nucleus) to the axon terminal. The identity and regulation of the transport organelle or organelles that are responsible for the assembly of presynaptic sites is not yet clear. On page 223 of this issue, Rizalar *et al.* (1) define the ultrastructure of these organelles and show that they are precursor vesicles that transport several types of presynaptic proteins at once. They also show that the signaling lipid phosphatidylinositol 3,5-bisphosphate [PI(3,5)P<sub>2</sub>] plays a key role in their transport along the axon by binding to kinesin family member 1A (KIF1A), which is a microtubule-dependent motor protein. Further investigation may aid the development of therapies for disorders that are characterized by defective synapses.

During neuronal development, presynaptic proteins such as synaptic vesicle proteins, active zone proteins (which provide the site for docking and release of synaptic vesicles), P/Q-type calcium channels (which are involved in presynaptic vesicle release) and adhesion molecules (for example, neuroligin-1), are produced in the soma and transported along the axon in precursor vesicles (PVs) to nascent presynaptic sites. Two models for this transport have been proposed. In one, multiple types of vesicles act as carriers, each transporting a different class of synaptic proteins. In the other model, multiple types of synaptic proteins are transported by the same carrier or cluster of carriers. To distin-

Downloaded from https://www.science.org at Columbia University on October 12, 2023



## Engineered bacteria guide T cells to tumors

Eric M. Bressler and Wilson W. Wong

*Science* **382** (6667), . DOI: 10.1126/science.adk6098

### View the article online

<https://www.science.org/doi/10.1126/science.adk6098>

### Permissions

<https://www.science.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of service](#)

---

*Science* (ISSN 1095-9203) is published by the American Association for the Advancement of Science. 1200 New York Avenue NW, Washington, DC 20005. The title *Science* is a registered trademark of AAAS.

Copyright © 2023 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works