Novel bispecific design improves CAR T–cell immunotherapy for childhood leukemia

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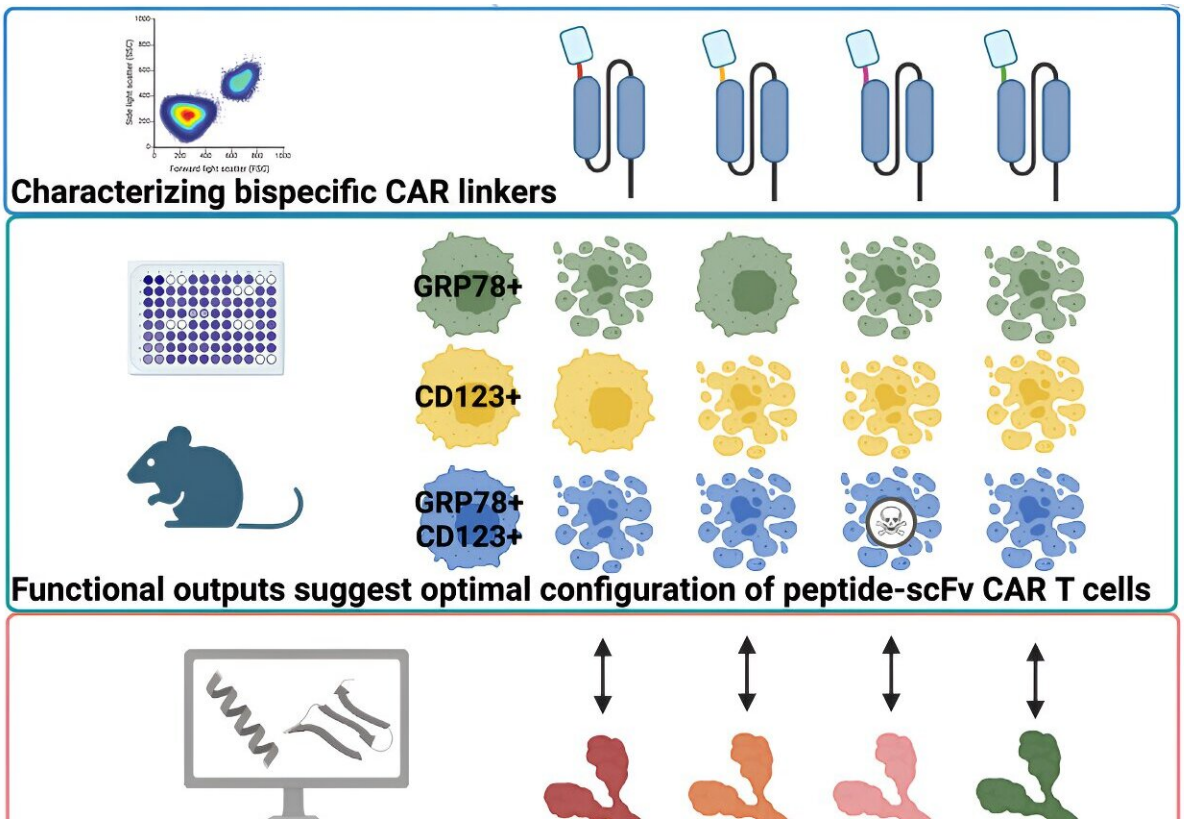

Graphical Abstract. Credit: *Cell Reports Medicine* (2024). DOI: 10.1016/j.xcrm.2024.101422

St. Jude Children's Research Hospital scientists have improved chimeric antigen receptor (CAR) T–cell immunotherapy for acute myeloid

leukemia (AML), demonstrating better efficacy in the lab.

To overcome common problems with CAR T cells, the researchers created an additional means for the therapy to find and eliminate cancer cells, using a small peptide. The study also showed how a computational approach incorporating AlphaFold predicted protein models could help understand how structure impacts antigen recognition and therapy efficacy.

Their findings are published in the journal *Cell Reports Medicine*.

Immunotherapy that reprograms a patient's own immune cells to target a cancer-specific protein, CAR T–cell therapy, has shown success in treating some relapsed leukemias. However, sometimes the treatment is unsuccessful because cancer cells that do not have the targeted protein can still grow, escaping the therapy and causing a relapse. The relapse rate for AML is high, leading to a poor prognosis for the disease overall.

The St. Jude group thought it might be possible to overcome the problem of immune escape in AML models by targeting two different cancer-related proteins instead of just one.

Others have attempted a similar approach but have encountered problems with the structure of the bispecific CAR. The scientists overcame these problems by adding a small peptide to the CAR to serve as the binder for the second targeted protein, then confirmed their results with computational structural analysis of their improved constructs.

"One of the most exciting aspects of the study is that this approach can be widely extrapolated to other tumors," said senior corresponding author Paulina Velasquez, MD, St. Jude Department of Bone Marrow Transplantation and Cellular Therapy. "We focused on leukemia, but

combining bispecific CAR design with computational predictions can be widely extrapolated for other tumors such as solid and brain tumors."

Improving dual targeting by adding a second, small barcode scanner

The CAR the researchers created is a unique design. It is a single molecule, which includes the region of an antibody that binds a specific target (its antigen) and one short peptide that binds a separate target.

"The two different binding domains of the CAR are like having two barcode scanners instead of one, looking for their appropriate barcode, the targeted cancer-related proteins," Velasquez said. "Normally, a CAR has a single barcode scanner. Here, we placed two slightly different barcode scanners on top of each other, and if either one detects an appropriate target barcode, the anti-cancer immunotherapy response activates."

The two binding domains are connected by a linker to allow for the binding of two different cancer-related proteins. This differs greatly from previous dual-targeting approaches in the field, which typically used two full antibody-based binding segments.

"We showed the value in finding creative ways to perform dual-antigen targeting," said first author Jaquelyn Zoine, Ph.D., St. Jude Department of Bone Marrow Transplantation and Cellular Therapy.

"Prior bispecific CAR approaches use two antibody-based single-chain variable fragments, which are physically large molecules and can get in each other's way, sometimes leading to poor or inefficient binding. Our approach instead added a small peptide, enabling our CAR to engage either platform to prevent immune escape."

The dual-targeted CARs performed better than single-targeted CARs in both in vitro and in vivo experiments, demonstrating promise for improving CAR T–cell function.

Untangling two-target constructs' performance with artificial intelligence

"We showed a proof of principle to explain and potentially expand the CAR design repertoire," said co-author M. Madan Babu, Ph.D., FRS, St. Jude Center of Excellence for Data-Driven Discovery director, and the George J Pedersen Endowed Chair in Biological Data Science in the Department of Structural Biology. "But then comes the challenge. How do we know what linkers to choose? How do we know how much physical flexibility is needed?"

Since the physical structure of the targeting molecule and its linker that bridges the two binding domains can cause internal interference that prevents binding to the targets on the cancer cell, identifying what type of linkers were more common ineffective therapies could lead to future improvement. Computational structure predictions and comparing structures with experimental results confirmed to the St. Jude group that shorter, more flexible linkers would work better in their models.

"If we have a rigid linker connecting the barcode scanners, it can only scan a restricted volume on the cancer cell, making it less effective in finding the targets," Babu said. "We found when you have a linker of sufficient flexibility and shorter length so it doesn't fold onto itself, it can scan a much larger volume and is more likely to find the target proteins on the cancer cell. Then you have a more effective pair of barcode scanners that work together."

"We are one of few groups in the world to use AI-based structure

prediction tools for CAR design," said second author Kalyan Immadisetty, St. Jude Department of Bone Marrow Transplantation and Cellular Therapy. Immadisetty confirmed the association between short, flexible linkers and greater anti-cancer efficacy by comparing 3D-modeled structures. This information supported the performance of the CAR in real experimental outputs.

"We were excited that the structural predictions supported our experiments that informed us a short and flexible linker would be the best configuration," Zoine said. "While we performed the experiments, Immadisetty found the structural components correlating almost exactly with what we were showing functionally, even when we switched one of the targeting antibody binding domains. We have now introduced the idea that these AI prediction tools can be extended to other CAR constructs."

"Most importantly, others can now use our computational approach for designing their CARs," Immadisetty said. "And hopefully, it will help them understand the efficacy of their CAR technology and lead to overall improvements for leukemia and other malignancies."

More information: Jaquelyn T. Zoine et al, Peptide-scFv antigen recognition domains effectively confer CAR T cell multiantigen specificity, *Cell Reports Medicine* (2024). DOI: 10.1016/j.xcrm.2024.101422

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