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IMMUNOTHERAPY

Start your engineering — CARs take to the *TRAC*

T cells genetically engineered to express chimeric antigen receptors (CARs) that recognize tumour-associated antigens are promising anticancer agents. In particular, anti-CD19 CAR T cells can induce complete remission in patients with B-cell malignancies. To date, randomly integrating vectors have typically been used to insert CAR genes into the T-cell genome; however, this approach can result in variegated transgene expression, transcriptional silencing and, potentially, serious adverse effects (oncogenic transformation). New data demonstrate that targeted integration of the CAR gene into a T-cell receptor (TCR) locus using precision genome editing might overcome the challenges associated with random vector integration and enhance anticancer activity.

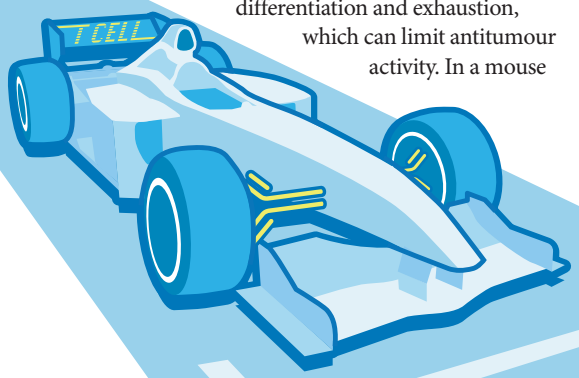
Michel Sadelain and co-workers used the revolutionary CRISPR/Cas9 system to efficiently insert an anti-CD19 CAR construct specifically within the T-cell receptor α constant (*TRAC*) locus. This process simultaneously disrupts expression of the native TCR and places the CAR gene under dynamic control of the *TRAC* promoter. Using blood samples from multiple donors, this protocol enabled reproducible production of 50×10^6 T cells with extremely uniform CAR expression; retroviral transfection resulted in variegated CAR expression at a median twofold higher level.

CAR T cells can undergo terminal differentiation and exhaustion, which can limit antitumour activity. In a mouse model of pre-B-cell acute lymphoid leukaemia, compared with retroviral-CAR T cells, *TRAC*-CAR T cells showed greater persistence in the bone marrow and tumours, were much less likely to undergo terminal differentiation or display markers of exhaustion, and significantly improved tumour responses and survival. "We found that the level of CAR expression and the dynamic response of the CAR following antigen recognition are critical in determining whether T-cell exhaustion will occur rapidly," Sadelain explains. Indeed, further elegant experiments revealed that highly regulated CAR expression and recycling, which results in reduced tonic signalling, underlies the advantages of *TRAC*-CAR T cells.

"In addition to providing scientific insights into how CARs function to reprogramme T cells, these findings represent another milestone in the engineering of T cells for medical purposes," Sadelain opines. "It should be noted that because T cells engineered at the *TRAC* locus are devoid of their own TCR, they cannot engage in graft-versus-host responses; thus, in addition to generating more potent T cells for autologous application, this approach will also facilitate the safe use of CAR T cells in the post-transplant setting."

Notably, the researchers have scaled up their new manufacturing schema, and are converting it into a Current Good Manufacturing Practice (CGMP) process suitable for clinical implementation. Sadelain concludes, "improvements in CAR designs, cell-manufacturing processes, and genetic engineering approaches, including the approach described in this study, will result in more effective, safer, and cheaper T-cell therapies."

David Killock



ORIGINAL ARTICLE Eyquem, J. *et al.* Targeting a CAR to the *TRAC* locus with CRISPR/Cas9 enhances tumour rejection. *Nature* http://dx.doi.org/10.1038/nature21405 (2017)
FURTHER READING Jackson, H. J. *et al.* Driving CAR T-cells forward. *Nat. Rev. Clin. Oncol.* **13**, 370–383 (2016)