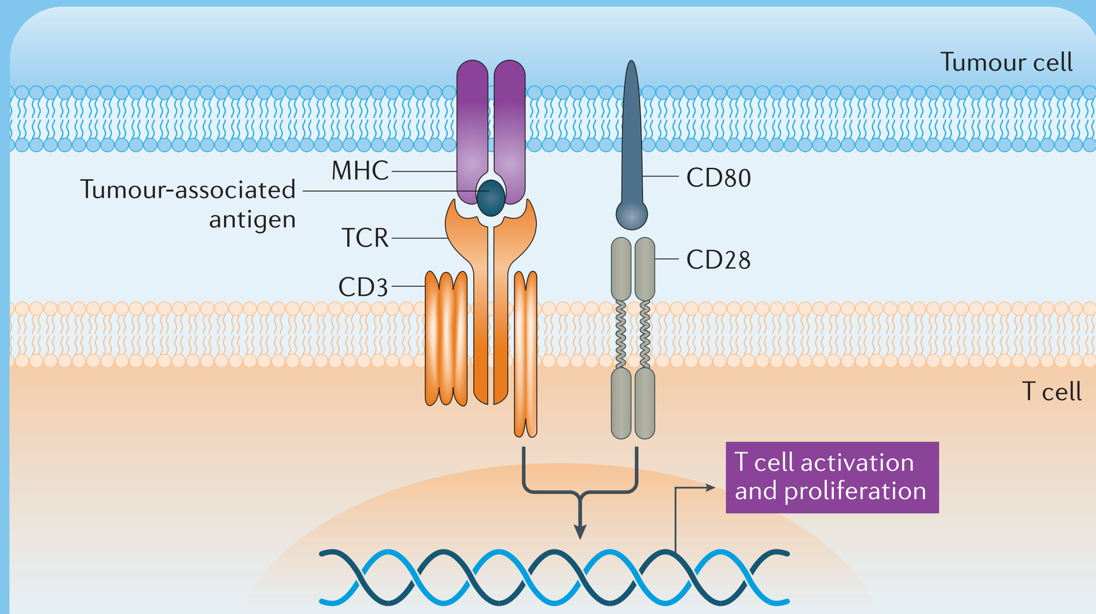
nature REVIEWS DRUG DISCOVERY

CAR T cells in cancer therapy

Michel Sadelain and Isabelle Rivière

CARs are synthetic receptors that reprogramme T cells. Their signalling domain enables the CAR T cell to activate effector functions and expand upon recognition of antigens on cancer cells. Cell surface antigens are recognized through the CAR external domain, which most often consists of a single chain linking the variable fraction of immunoglobulins (scFv). CAR T cells thus engage their target antigen independently of HLAs, in contrast to the physiological TCR. T cells that are genetically engineered to express a CAR expand in the cancer patient and thus become targeted 'living drugs', programmed to eliminate cancer cells. CAR T cells that target CD19, a cell surface molecule expressed in most leukaemias and lymphomas, have shown remarkable results in patients with relapsed, chemorefractory B cell malignancies, especially ALL. The first CAR therapies to obtain FDA approval in 2017 are indicated for refractory childhood ALL and adult NHL. The CD19 paradigm serves as the model for other therapies based on engineered T cells, which are in principle applicable to a wide range of cancers including solid tumours. There remain, however, multiple challenges to overcome, including immunosuppressive tumour microenvironments, immune evasion (antigen escape) and severe toxic effects (cytokine release syndrome and neurotoxicity). Further advances in CAR design, genetic engineering, the isolation or derivation of optimal T cells, and cell manufacturing, will broaden the applicability of T cell-based therapies for cancer and eventually infectious and autoimmune diseases.



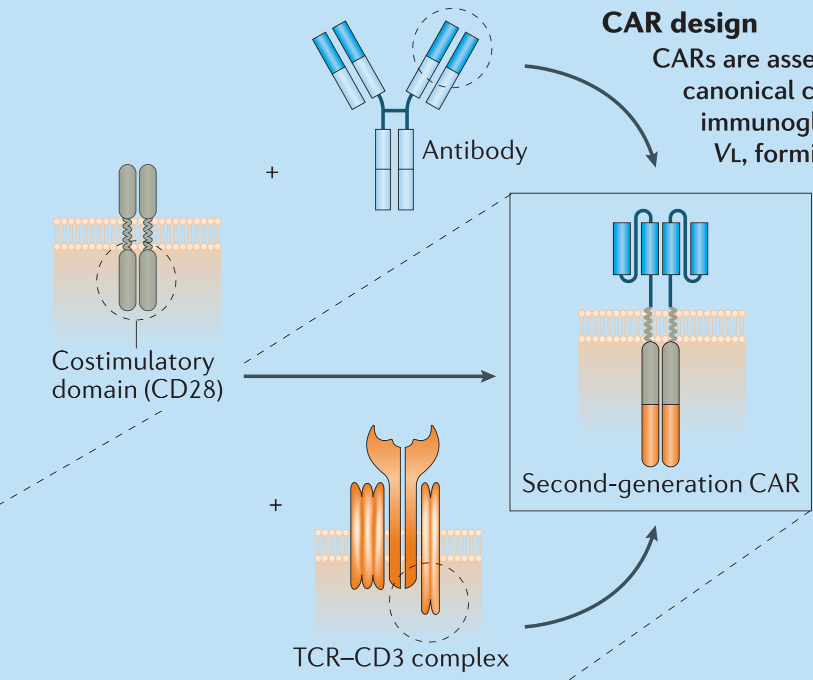
Recognition of tumour peptides by T cells

The physiological recognition of tumour antigens by T cells is normally mediated by the TCR–CD3 complex. TCRs are made up of α- and β-chains, which associate with the γ-, δ-, ε- and ζ-chains of the CD3 complex. When the TCR encounters a processed tumour antigen peptide displayed on the MHC (known as HLA in humans) of the tumour cell, a cascade of intracellular signalling occurs, which results in T cell expansion and the release of cytokines and cytotoxic compounds from T cells, promoting tumour control. The TCR–CD3 complex alone is insufficient to direct productive T cell responses, which require the concomitant engagement of costimulatory receptors such as CD28 to sustain T cell expansion, function and persistence.

CAR T cell manufacturing

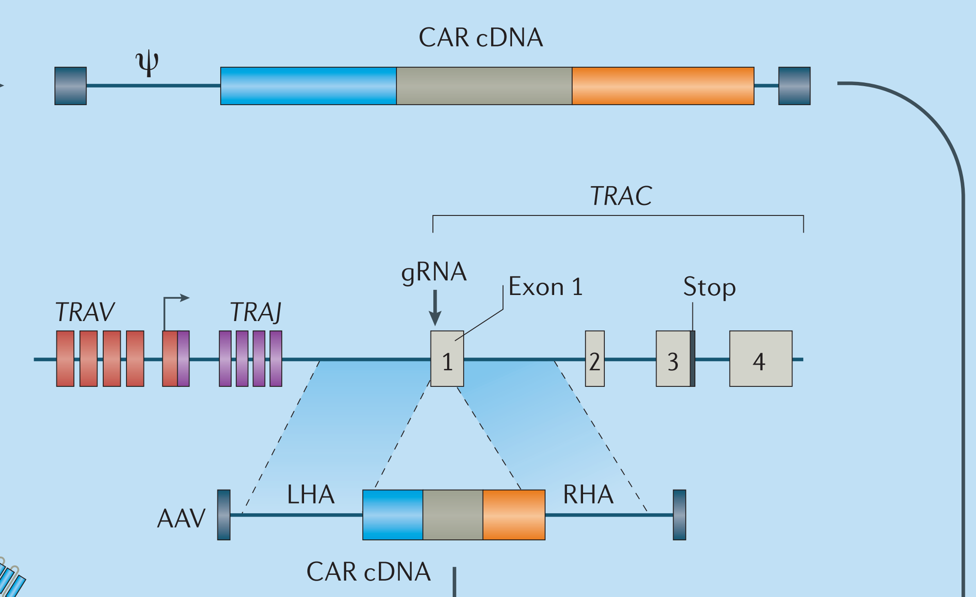
CAR design

CARs are assembled by combining three canonical components borrowed from immunoglobulin genes (such as V_H and V_L, forming the antigen-binding scFv), the TCR–CD3 complex (such as CD3ζ) and costimulatory receptors (such as CD28).



T cell engineering

CAR gene transfer may use randomly integrating vectors (gRV and LV and DNA transposons, shown at the top) or targeted nucleases (meganucleases, zinc-finger nucleases, TALENs or CRISPR–Cas9, shown at the bottom). In the latter case, the donor DNA encodes the CAR cDNA, flanked by a LHA and a RHA to mediate homologous recombination around the site, targeted by the guide RNA (gRNA). In the example below, the CAR cDNA is targeted to the *TRAC* locus[37].

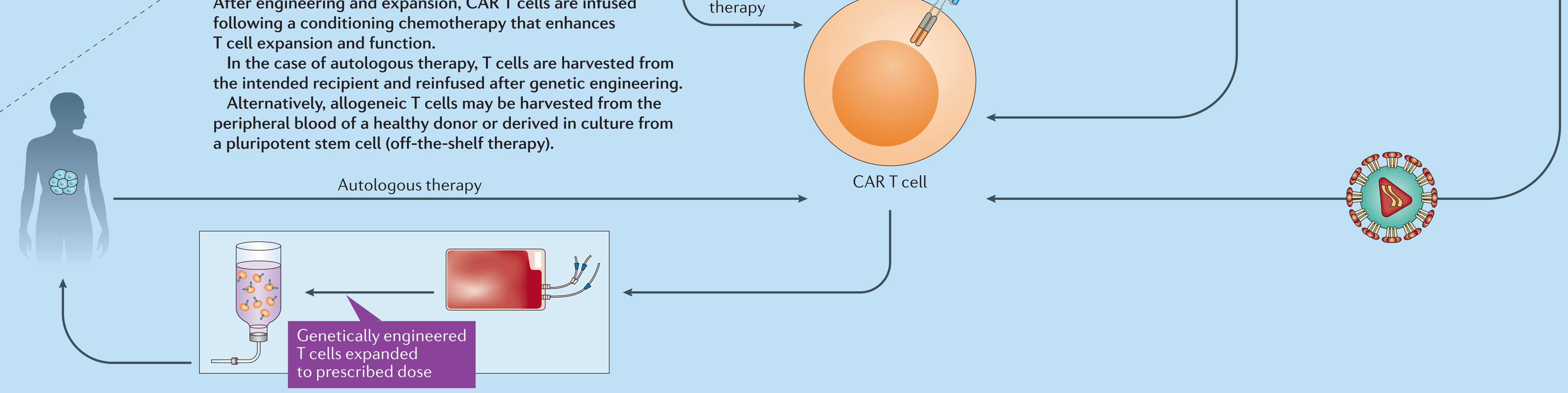


CAR T cell therapy

After engineering and expansion, CAR T cells are infused following a conditioning chemotherapy that enhances T cell expansion and function.

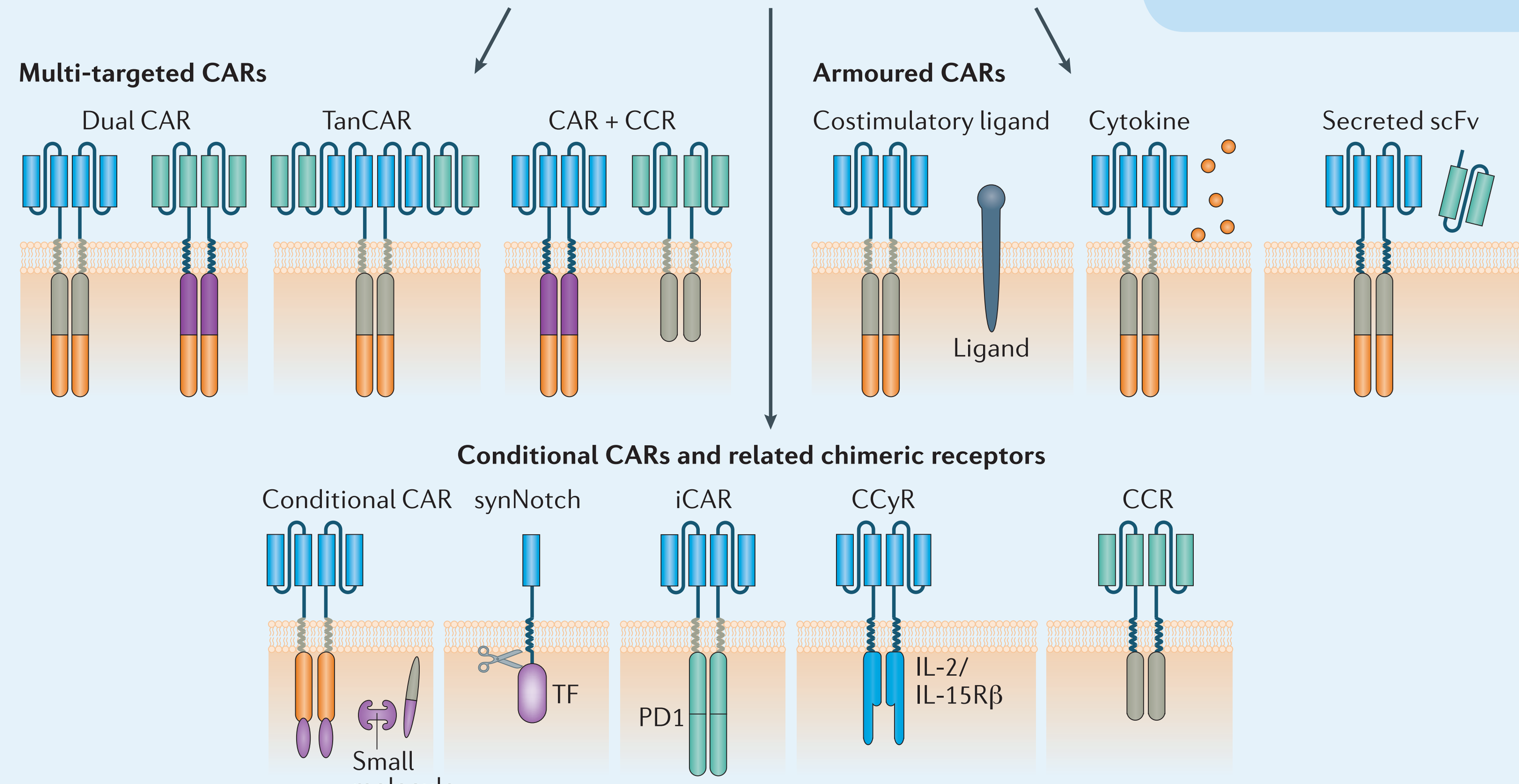
In the case of autologous therapy, T cells are harvested from the intended recipient and reinfused after genetic engineering.

Alternatively, allogeneic T cells may be harvested from the peripheral blood of a healthy donor or derived in culture from a pluripotent stem cell (off-the-shelf therapy).



Prototypic second-generation CARs

CD28 CAR	Structure	Function	4-1BB CAR
	scFv	Antigen binding: • Epitope • Affinity	
	EC and TM	Structure: • Affects binding and function	
	CD28 or 4-1BB	Costimulation: • Effector function • Persistence • Metabolism	
	CD3	Activation: • Function trigger • Proliferation	



Types of chimeric antigen receptor

Unlike the endogenous natural TCR, CARs target cell surface antigens independently of MHC and reprogramme T cell function. The latter is achieved by combining elements of the CD3 complex and costimulatory domains to amplify T cell activation and sustain effector and metabolic functions that enhance the antitumour activity and persistence of the engineered cells. The most studied CARs, and the first to reach FDA approval, are 'second-generation' CARs that encompass the signalling domains of either CD28[1] or 4-1BB[2] (top panel).

Novel CAR constructs are being developed to address the limitations of these prototypic designs, including their off-tumour cytotoxic activity and the risk of antigen escape. The three panels exemplify new directions in CAR design. Multiple antigen targeting (using multi-targeted CARs, middle left panel) is used to address the challenges of antigen escape, which arises as a consequence of tumour heterogeneity, and of antigen loss or downregulation. Multi-targeted T cells may express two CARs with different signalling domains[3,4] (dual CAR, left), a bispecific CAR[5,6] that can recognize two antigens (such as TanCAR), or a CAR and a chimeric costimulatory receptor (CCR)[7], in which CCRs provide antigen-dependent costimulation without initiating T cell activation. CAR T cells can also be 'armed' with additional features (middle right panel) to increase their intrinsic (sustained effector function, persistence and trafficking) or extrinsic (action on the tumour microenvironment) functions. Examples include expression of a costimulatory ligand (for auto- and trans-costimulation)[8], a cytokine (such as IL-12)[9] or secreted scFv designed to block or stimulate environmental factors[10]. CARs may be expressed conditionally (lower panel) to enable titrable, reversible or temporally controllable CAR activity. Controlled CAR expression can be achieved with small molecules[11] (conditional CAR) or a synthetic Notch-like receptor that induces secondary CAR expression following Notch cleavage[12] (synNotch). Regulatory CAR-like structures may be inhibitory[13] (iCAR) or activating such as chimeric cytokine receptors[14] (CCyR) and CCRs[15]. New therapeutic strategies based on T cell engineering using these various synthetic receptors will emerge to increase the breadth, efficacy and safety of CAR therapy.

Clinical response rates to CD19 CAR T cell therapy

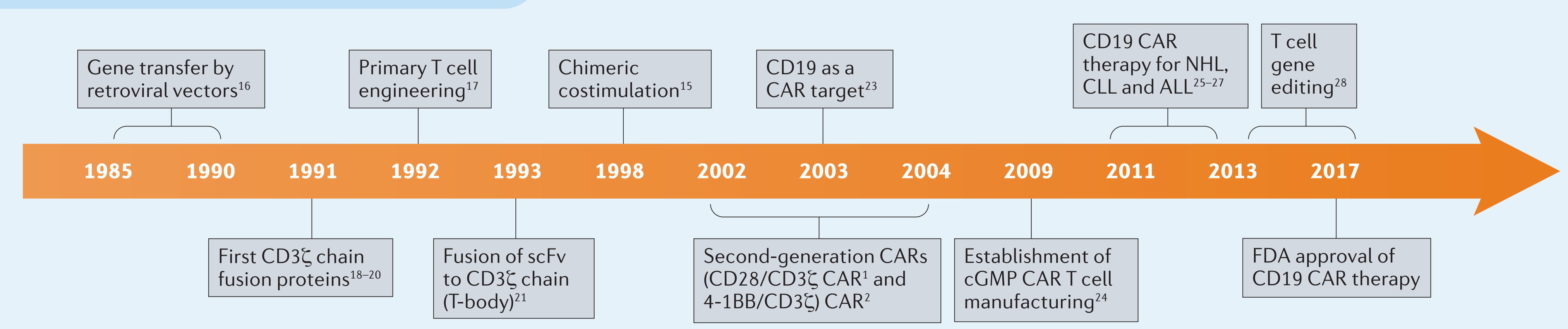
Disease	CAR	Vector type	n	Cond.	T cells	CR rate (%)	Refs
Adult ALL	CD28	gRV	16	CY	Autologous	88	30
Paediatric ALL	4-1BB	LV	25	CF	Autologous	90	31
Paediatric ALL	CD28	gRV	21	CY	Autologous	68	32
Adult ALL	CD28	gRV	53	CY	Autologous	83	33
Paediatric ALL	4-1BB	LV	2	Post-T	Allogeneic	100	29
NHL and CLL	CD28	gRV	15	CF	Autologous	53	34
B-mix	CD28	gRV	20	Post-T	Allogeneic	30	35
NHL	4-1BB	LV	32	CY or CF	1:1 CD4+/CD8+	79	36

Clinical responses to CD19 CAR therapy in patients with relapsed, chemorefractory B cell malignancies. These trials make use of different CAR signalling elements (CD28 or 4-1BB), different vector types (gRV or LV) and different conditioning regimens (cond.).

Examples of CAR T cell therapies in development

Target antigen	Indications	Status
CD19 (first-in-modality candidate)	Relapsed or refractory B-ALL, relapsed or refractory aggressive NHL, CLL, B cell lymphoma and DLBCL	Approved* (tisagenlecleucel-t for B-ALL and axicabtagene ciloleucel for NHL); multiple next-generation CD19 CARs in phase I
CD22 (rescue target in CD19-negative ALL)	ALL	Phase I
BCMA	Multiple myeloma	Phase I
Mesothelin	Mesothelioma and lung, breast, ovary and pancreas tumours	Phase I
IL-13Ra2	Glioblastoma	Phase I
EGFRvIII (tumour-restricted EGFR splice variant)	Glioblastoma	Phase I
MUC16 (IL-12 secreting CAR T cell)	Ovarian cancer	Phase I
PSCA (rimiducid-activated 'on switch')	Various solid tumours	Phase I
GPC3	Hepatocellular carcinoma	Phase I

This table features only a sample of CAR T cell therapies under evaluation in clinical trials. There are at present more than 250 CAR T cell trials listed on the ClinicalTrials.Gov website, mostly in the USA and China. For more information visit https://clinicaltrials.gov/.
*Approved by the FDA, under review in other regions, including Europe.



The advent of retroviral vectors[16] opened a path for primary T cell engineering[17]. The first CD3ζ-chain fusions[18-20] were rendered antigen-specific by incorporation of an scFv, termed a T-body[21], and were later renamed as first-generation CARs[22]. These ζ-chain fusions, however, provide limited signalling in primary T cells, resulting in rapid loss of function or apoptosis. The effectiveness of chimeric CD28 receptors, which provide functional costimulation in primary T cells[15], led to a novel, dual-signalling design that enabled human peripheral blood T cells to expand and retain their function upon repeated exposure to antigen[1]. Other second-generation CARs[22] emerged, incorporating different costimulatory domains such as 4-1BB[2].

CD19, a B cell surface molecule, was identified as an effective target in B cell malignancies[23] and became the target of choice for the first trials of second-generation CARs conducted at the Memorial Sloan Kettering Cancer Center, CHOP and the National Cancer Institute. The establishment of cGMP methods for autologous CAR T cell manufacturing[24] paved the way for clinical studies, which all proved to be successful in early studies in NHL[25], CLL[26] and ALL[27]. The first CD19 CAR therapies were approved by the FDA in 2017 for the treatment of relapsed or refractory paediatric B cell ALL (tisagenlecleucel-t) and NHL (axicabtagene ciloleucel). T cell gene editing[28] has been used to disrupt the TCR in allogeneic CD19 CAR therapy[29].

Abbreviations

AAV, adeno-associated virus; ALL, acute lymphoblastic leukaemia; B-ALL, B cell acute lymphoblastic leukaemia; BCMA, B cell maturation antigen; CAR, chimeric antigen receptor; CF, cyclophosphamide + fludarabine; cGMP, current good manufacturing practice; CHOP, Children's Hospital of Philadelphia; CLL, chronic lymphocytic leukaemia; CR rate, complete remission rate; CY, cyclophosphamide; DLBCL, diffuse large B cell lymphoma; EC, extracellular domain; EGFRvIII, epidermal growth factor receptor variant III; GPC3, glypican 3; gRV, γ-retroviral vector; HLA, human leukocyte antigen; IL-13Ra2, interleukin-13 receptor α2; IL-15Rβ, interleukin-15 receptor β; LHA, left homology arm; MHC, major histocompatibility complex; LV, lentiviral vector; MUC16, mucin 16; NHL, non-Hodgkin lymphoma; PD1, programmed cell death protein 1; PSCA, prostate stem cell antigen; Post-T, post-transplant; RHA, right homology arm; TALEN, transcription activator-like effector nuclease; TCR, T cell receptor; TF, transcription factor; TM, transmembrane domain; TRAC, TCRα constant region; TRAV, TCRα variable region; TRAJ, TCRα joining region.

References

1. Maher, J. et al. *Nat. Biotechnol.* **20**, 70–75 (2002).
2. Imai, C. et al. *Leukemia* **4**, 676–84 (2004).
3. Duong, C. P. et al. *Immunotherapy* **3**, 33–48 (2011).
4. Wilkie, S. et al. *J. Clin. Immunol.* **32**, 1059–1070 (2012).
5. Hegde, M. et al. *J. Clin. Invest.* **126**, 3036–3052 (2016).
6. Zah, E. *Cancer Immunol. Res.* **4**, 639–641 (2016).
7. Kloss, C. C. Nat. *Biotechnol.* **31**, 71–75 (2013).
8. Stephan, M. T. et al. *Nat. Med.* **13**, 1440–1449 (2007).
9. Chinnasamy, D. et al. *Clin. Cancer Res.* **18**, 1672–1683 (2012).
10. Jackson, H. et al. *The New York Academy of Sciences* (2016).
11. Wu, C. Y. et al. *Science* **350**, aab4077 (2015).
12. Roybal, K. T. et al. *Cell* **164**, 770–779 (2016).
13. Fedorov, V. D. et al. *Sci. Transl Med.* **5**, 215ra172 (2013).
14. Wilkie S. et al. *J. Biol. Chem.* **285**, 25538–25544 (2010).
15. Krause, A. et al. *J. Exp. Med.* **188**, 619–626 (1998).
16. Miller, A. D. *Hum. Gene Ther.* **1**, 5–14 (1990).
17. Sadelain, M. & Mulligan, R. C. in *8th International Congress of Immunology* (ed International Congress of Immunology) (Springer-Verlag, 1992).
18. Irving, B. A. & Weiss, A. *Cell* **64**, 891–901 (1991).
19. Romeo, C. & Seed, B. *Cell* **64**, 1037–1046 (1991).
20. Letourneur, F. & Klausner, R. D. *Proc. Natl Acad. Sci. USA* **88**, 8905–8909 (1991).
21. Eshhar, Z. et al. *Proc. Natl Acad. Sci. USA* **90**, 720–724 (1993).
22. Sadelain, M. et al. *Curr. Opin. Immunol.* **21**, 215–223 (2009).
23. Brentjens, R. J. et al. *Nat. Med.* **9**, 279–286 (2003).
24. Hollyman, D. et al. *J. Immunother.* **32**, 169–80 (2009).
25. Kochenderfer, J. N. et al. *Blood* **116**, 4099–4102 (2010).
26. Kalos, M. et al. *Sci. Transl Med.* **3**, 95ra73 (2011).
27. Brentjens, R. J. et al. *Sci. Transl Med.* **5**, 177ra138 (2013).
28. Poirot, L. et al. *Cancer Res.* **75**, 3853–3864 (2015).
29. Qasim, W. et al. *Sci. Transl Med.* **9**, (2017).
30. Davila, M. L. et al. *Sci. Transl Med.* **6**, 224ra225 (2014).
31. Maude, S. L. et al. *N. Engl. J. Med.* **371**, 1507–1517 (2014).
32. Lee, D. W. et al. *Lancet* **385**, 517–528 (2015).
33. Park, et al. *N. Engl. J. Med.* (in the press).
34. Kochenderfer, J. N. et al. *J. Clin. Oncol.* **33**, 540–549 (2015).
35. Brudno, J. N. et al. *J. Clin. Oncol.* **34**, 1112–1121 (2016).
36. Turtle, C. J. et al. *J. Clin. Invest.* **126**, 2123–2138 (2016).
37. Eyquem, J. et al. *Nature* **543**, 113–117 (2017).

Affiliations

Michel Sadelain is at the Center for Cell Engineering and Immunology Program, Sloan Kettering Institute, New York, New York 10065, USA.
Isabelle Rivière is at the Center for Cell Engineering and Molecular Pharmacology Program, Sloan Kettering Institute, New York, New York 10065, USA.

Competing interests statement

M.S and I.R. declare that they receive research support from Juno Therapeutics.

The poster content is peer reviewed, editorially independent and the sole responsibility of Macmillan Publishers Limited. Edited by M. Teresa Villanueva; copyedited by Stephanie Wills; designed by Susanne Harris.