

# A brief history of CAR-T cells: from laboratory to the bedside

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**Abstract**

Chimeric antigen receptors (CARs) are genetically engineered receptors that provide specific properties to an immune effector cell and these receptors gain the specificity of a monoclonal antibody targeted against specific tumor cells. T cells with engineered CARs acquire potent immunological properties and redirect the immune system in order to eliminate malignant cells. First-engineered T cells with chimeric molecule (CAR-T cells) were developed in 1989–1993 by Israeli immunologists Zelig Eshhar and Gideon Gross. The first clinical application of CAR-T cells was done in the University of Pennsylvania and Children's Hospital in Philadelphia by the immunologist Carl June and hematologist David Porter to patients with chronic lymphocytic leukemia in 2011 and together with the pediatrician Stephan Grupp to patients with acute lymphoblastic leukemia (ALL) in 2012. The US Food and Drug Administration Agency (FDA) in 2017 and the European Medicines Agency (EMA) in 2018 have licensed two products of CAR-T cells: tisagenlecleucel for the use in children and young adults up to 25 years of age with B-cell ALL who do not respond to treatment or have relapsed two or more times and tisagenlecleucel and axicabtagene ciloleucel for the use in adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL). Current progress in CAR technology includes the use in other hematological malignancies, solid tumors, the use of dual CAR-T cells and chimeric antigen receptor natural killer cells (CAR-NK cells).

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chimeric antigen receptors, CAR-T cells, tisagenlecleucel, axicabtagene ciloleucel, CAR-NK cells

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## Chimeric antigen receptor

Chimeric antigen receptors (CARs) are genetically engineered receptors that provide specific properties to an immune effector cell (e.g., lymphocyte T-cell). These receptors gain the specificity of a monoclonal antibody targeted against specific tumor cells. The coding sequence is transferred to the receptor by viral (retroviral and lentiviral) vectors. The term “chimeric” indicates different sources of composing parts of the receptor. T cells with engineered CARs acquire potent immunological properties and by redirecting the immune system in order to eliminate malignant cells act as a living drug, expanding in the patient and ensuring long-term antitumor memory [1, 2, 3].

CAR-T cells can be either autologous or allogenic. Autologous CAR-T cells are derived via leukapheresis from the blood of the patient. Allogenic CAR-T cells are derived from the blood of a healthy donor. CARs as recombinant receptors transferred in various T-cell subsets provide in a restricted manner specific antigen binding in a non-major histocompatibility complex (non-MHC) [3]. Theoretically, CAR-T cells can be engineered to target virtually any tumor-associated antigen and, in general, any other antigen, e.g., fungal antigen [4].

## Four generations of CARs

First engineered T-cell with chimeric molecule was developed in 1989–1993 by Israeli immunologists Zelig Eshhar and Gideon Gross in the Department of Chemical Immunology of Weizmann Institute of Science in Rehovot, Israel (Tab. I). These first-generation CARs were not yet clinically effective [5, 6, 7].

Over next thirty years, CARs were immunologically and technologically modernized and sophisticated as first, second, third, and currently fourth generation, depending on their composition. The second-generation CARs had improved antitumor activity of T cells: improved T-cell proliferation, resistance to apoptosis, cytokine secretion, and *in vivo* persistence. The third-generation CARs, in comparison with second-generation CARs, had improved effector functions and *in vivo* persistence [8, 9].

Fourth-generation CARs, the so-called TRUCKs (CAR redirected T cells that deliver a transgenic product to the targeted tumor tissue) or armored CARs, present further enhancement in antitumoral potency, cytokine activity, and costimulatory ligands and enzymes that can degrade the extracellular matrix in solid tumors [9, 10]. Further enhancement in the safety of CAR-T cell therapy can be obtained with the so-called smart T cells, which is under investigation [11, 12, 13].

Finally, new engineering modalities, based on the use of targeted nucleases like clustered regularly interspaced short palindromic repeats (CRISPR), may further enhance the efficacy and safety of CAR-T cells [14].

## First clinical application

Clinical application of CAR-T cells became available through the dedicated team in the University of Pennsylvania and Children's Hospital in Philadelphia. Carl June was the immunologist who led the development of CAR-T cell technology, striving with many difficulties. Together with David Porter and Stephan Grupp, he administered CAR-T cells to patients with chronic lymphocytic leukemia (CLL) in 2011 [1] and ALL in 2012 [2].

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The real progress was achieved with the first therapy in child with ALL: a 7-year-old Emily Whitehead with relapsed and refractory ALL, who was recommended to hospice, became the first child to receive CART-19 in 2012. She experienced all severe complications of this therapy, but she was finally cured. As a result, her survival helped reenergize a line of research that was near the failure [15].

## Current status

The US Food and Drug Administration Agency (FDA) and the European Medicines Agency (EMA) have licensed two products of CAR-T cells: tisagenlecleucel for the use in children and young adults up to 25 years of age with B-cell ALL that does not respond to treatment or has relapsed two or more times, and tisagenlecleucel and axicabtagene ciloleucel for the use in adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) (Tab. II). Several other products are at different stages of research and progress toward registration (Tab. III).

## Further progress and worldwide distribution

Owing to emergence of relapse of ALL with cells that do not express CD19 in mechanisms of a phenomenon known as antigen escape, the Phase I study with the use of CAR-T cells with dual targeting of CD19/CD22 has demonstrated good efficacy, safety, and tolerability, thus opening possibility to trials studying bispecific targeting to circumvent CD19 down-regulation [17, 23, 24].

An option to increase the potential of CAR-T cells is its enhancement by combining CAR-T cells with a monoclonal antibody targeting the human programmed death receptor 1 (anti-PD1) performed so far in the prostate cancer model [25].

As of December 2019, there were 284 ongoing clinical trials (www.clinicaltrials.gov) happening globally involving CAR-T cells, mostly in the USA (130) and China (124), most of them in hematological malignancies (ALL, non-Hodgkin lymphoma, multiple myeloma), targeting mostly CD19 and also CD20, CD22, and BCMA, almost exclusively CAR-T cells, but in 10 cases also CAR-NK [16] (majority in China). Currently, at least 24 countries use CAR-T cell technology in therapy [26].

**Table I. History of development of CAR effector cells**

Year	Achievement
1989	Generation of effector T cells expressing chimeric T-cell receptor [5, 6]
1993	First-generation CAR-T cells, not clinically effective [7]
2002	First effective CAR-T cells against prostate cancer antigen in the laboratory
2003	Second-generation CARs: CD19-directed CAR-T cells can kill leukemia cells in mouse
2009	CD19 CAR-T cells used in relapsed/refractory leukemia
2011	CD19 CAR-T cells in patients with chronic lymphocytic leukemia [1]
2013	CD19 CAR-T cells in pediatric acute lymphoblastic leukemia [2]
2013	Science magazine announced cancer immunotherapy as "Breakthrough of the Year"
2014	Third-generation CAR-T cells: inducible caspase-9 suicide gene system as a "safety switch" to limit on-target, off-tumor toxicities [8]
2015	Fourth-generation CARs, the so-called TRUCKs (CAR redirected T cells that deliver a transgenic product to the targeted tumor tissue) or armored CARs, which produce other molecules built and being tested for ovarian cancer [10]
2015	Concept of CAR-NK cells [16]
2017	Clustered regularly interspaced short palindromic repeats (CRISPR) used to optimize CAR placement in T cells [14]
2017	FDA approves CD19-CAR-T cells for relapsed/refractory acute lymphoblastic leukemia in children and young adults
2017	FDA approves CD19-CAR-T cells for relapsed/refractory DLBCL in adults
2018	EMA approves CD19-CAR-T cells for relapsed/refractory acute lymphoblastic leukemia in children and young adults and for relapsed/refractory DLBCL in adults
2019	Dual CD19/CD22 CAR-T cells in acute lymphoblastic leukemia in children and adults [17]

CAR – chimeric antigen receptor; CRISPR – clustered regularly interspaced short palindromic repeats; FDA – US Food and Drug Administration Agency; EMA – European Medicines Agency; DLBCL – diffuse large B-cell lymphoma.

**Table II. Approval of CAR-T cell technology for clinical use**

Date	Authorities	CAR-T cell	Study	Indications
August 30, 2017	US Food and Drug Administration	Tisagenlecleucel (Kymriah, Novartis)	ELIANA, Maude et al. [18]	Children and young adults up to 25 years of age with B-cell ALL who do not respond to treatment or have relapsed two or more times
October 18, 2017	US Food and Drug Administration	Axicabtagene ciloleucel (Yescarta, Gilead-Kite)	ZUMA-1, Neelapu et al. [19]	Adult patients with relapsed or refractory DLBCL
May 1, 2018	US Food and Drug Administration	Tisagenlecleucel (Kymriah, Novartis)	JULIET, Schuster et al. [20]	Adult patients with relapsed or refractory DLBCL, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma
June 28, 2018	European Medicines Agency	Tisagenlecleucel (Kymriah, Novartis)	ELIANA, Maude et al. [18]	Children and young adults up to 25 years of age with B-cell ALL who do not respond to treatment or have relapsed two or more times
June 28, 2018	European Medicines Agency	Axicabtagene ciloleucel (Yescarta, Gilead-Kite)	ZUMA-1, Neelapu et al. [19]	Adult patients with relapsed or refractory DLBCL

CAR – chimeric antigen receptor; ALL – acute lymphoblastic leukemia; DLBCL – diffuse large B-cell lymphoma.

**Table III. Advanced research in CAR-T cell application**

CAR-T cell	Study	Indications	Comments
Lisocabtagene maraleucel (Lisocel, Bristol-Myers Squibb/Celgene Company)	TRANSCEND NHL 001 trial, Abramson et al. [21]	Adult patients with relapsed or refractory (R/R) DLBCL after at least two prior therapies	Application to FDA (December 18, 2019)
Idecabtagene vicleucel (CT053, CARsgen Therapeutics)	KarMMA	Multiple myeloma	The FDA has given (September 5, 2019) orphan drug status to CT053 (anti-B-cell maturation antigen (anti-BCMA)), an investigational chimeric antigen receptor T-cell therapy for the treatment of multiple myeloma
Anti-BCMA CAR-T cell bb2121 therapy (Bluebird Bio and Celgene)	CRB-401 trial, Raje et al. [22]	Relapsed or refractory multiple myeloma	The product was granted breakthrough therapy designation by the FDA in November 2017 and will thus receive expedited review by the agency. It has also been fast-tracked in Europe
Axicabtagene ciloleucel (Yescarta, Gilead-Kite)	ZUMA-3 (adult) and ZUMA-4 (pediatric and adolescents 2–21 years) 1. Shah BD et al. ASCO 2019. Abstract 7006 2. Lee DW et al. ESMO 2017. Abstract 1008PD	ALL in children and adults	Registration in progress

CAR – chimeric antigen receptor; NHL – non-Hodgkin lymphoma; DLBCL – diffuse large B-cell lymphoma; FDA – US Food and Drug Administration Agency; ALL – acute lymphoblastic leukemia.

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### Authors' contributions

JS – the only author.

### Conflict of interest

The author was the participant of Novartis Advisory Board, has received lecture fee, and participated in meetings organized by Gilead.

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### Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

### References

- Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med* 2011;365:725–33.
- Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 2013;368:1509–18.
- Heiblig M, Elhamri M, Michallet M, Thomas X. Adoptive immunotherapy for acute leukemia: New insights in chimeric antigen receptors. *World J Stem Cells* 2015;7:1022–38.
- Seif M, Einsele H, Loffler J. CAR T cells beyond cancer: hope for immunomodulatory therapy of infectious diseases. *Front Immunol* 2019;10:2711.
- Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc Natl Acad Sci U S A* 1989;86:10024–8.
- Gross G, Gorochov G, Waks T, Eshhar Z. Generation of effector T cells expressing chimeric T cell receptor with antibody type-specificity. *Transplant Proc* 1989;21:127–30.
- Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc Natl Acad Sci U S A* 1993;90:720–4.
- Gargett T, Brown MP. The inducible caspase-9 suicide gene system as a “safety switch” to limit on-target, off-tumor toxicities of chimeric antigen receptor T cells. *Front Pharmacol* 2014;5:235.
- Hartmann J, Schussler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells-challenges and opportunities in translating innovative treatment concepts. *EMBO Mol Med* 2017;9:1183–97.
- Chmielewski M, Abken H. TRUCKS: the fourth generation of CARs. *Expert Opin Biol Ther* 2015;15:1145–54.
- Zhang E, Xu H. A new insight in chimeric antigen receptor-engineered T cells for cancer immunotherapy. *J Hematol Oncol* 2017;10:1.
- Cho JH, Collins JJ, Wong WW. Universal Chimeric Antigen Receptors

- for Multiplexed and Logical Control of T Cell Responses. *Cell* 2018;173:1426–38.
- [13] Sachdeva M, Busser BW, Temburni S, et al. Repurposing endogenous immune pathways to tailor and control chimeric antigen receptor T cell functionality. *Nat Commun* 2019;10:5100.
- [14] Ren J, Zhao Y. Advancing chimeric antigen receptor T cell therapy with CRISPR/Cas9. *Protein Cell* 2017;8:634–43.
- [15] Rosenbaum L. Tragedy, perseverance, and chance – the story of CAR-T therapy. *N Engl J Med* 2017;377:1313–5.
- [16] Rezvani K, Rouse RH. The application of natural killer cell immunotherapy for the treatment of cancer. *Front Immunol* 2015;6:578.
- [17] Schultz LM, Muffly LS, Spiegel JY, et al. Phase I trial using CD19/CD22 bispecific CAR T cells in pediatric and adult acute lymphoblastic leukemia (ALL). *Blood* 2019;134 (Suppl\_1):abstract 744.
- [18] Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med* 2018;378:439–48.
- [19] Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med* 2017;377:2531–44.
- [20] Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med* 2019;380:45–56.
- [21] Abramson JS, Palomba ML, Gordon LI, et al. Pivotal safety and efficacy results from Transcend NHL 001, a multicenter Phase 1 study of lisocabtagene maraleucel (liso-cel) in relapsed/refractory (R/R) large B cell lymphomas. *Blood* 2019;134 (Suppl\_1):abstract 241.
- [22] Raje N, Berdeja J, Lin Y, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. *N Engl J Med* 2019;380:1726–37.
- [23] Schultz LM, Majzner R, Davis KL, Mackall C. New developments in immunotherapy for pediatric solid tumors. *Curr Opin Pediatr* 2018;30:30–9.
- [24] Schultz L, Mackall C. Driving CART cell translation forward. *Sci Transl Med* 2019;11(481).
- [25] Serganova I, Moroz E, Cohen I, et al. Enhancement of PSMA-directed CAR adoptive immunotherapy by PD-1/PD-L1 Blockade. *Mol Ther Oncolytics* 2017;4:41–54.
- [26] Tang J, Hubbard-Lucey VM, Pearce L, O'Donnell-Tormey J, Shalabi A. The global landscape of cancer cell therapy. *Nat Rev Drug Discov* 2018;17:465–6.