CAR-T WORKFLOW: CUSTOM SOLUTIONS

Adoptive cell transfer holds great promise in many areas of medicine, including the treatment of neurological disorders, inflammatory or autoimmune diseases, and viral infections such as HIV/AIDS. In the field of cancer therapeutics, novel immuno-oncology approaches take advantage of our powerful immune system by reprogramming immune cells (cytotoxic T cells, natural killer cells, or macrophages), creating an army of soldiers to seek and destroy the tumor cells [1]. The three kinds of adoptive cell transfer strategies developed to treat cancer are Tumor-Infiltrating Lymphocytes (TILs), T Cell Receptor TCR-T cells, and Chimeric Antigen Receptor CAR-T cells, which have shown spectacular efficacy [2].

Five approved CAR-T cell therapeutics target proteins CD19 and BCMA, both expressed in B lymphocytes. They are used in patients with relapsed/refractory hematological cancer whose disease has progressed despite previous treatment.

Brand name	KYMRIAH™	YESCARTA™	TECARTUS™	BREYANZI®	ABECMA®
Full name	**tisagenlecleucel**	**axicabtagene ciloleucel**	**brexucabtagene autoleucel**	**lisocabtagene maraleucel**	**idecabtagene vicleucel**
Development name	CTL019 CART-19	KTE-C19 Axi-cel	KTE-X19	JCAR017	ide-cel bb2121
Target	CD19	CD19	CD19	CD19	BCMA
Year approved	2017	2017	2020	2021	2021
Indications	- ALL - DLBCL	- DLBCL - Follicular lymphoma - Primary mediastinal large BCL	Mantle cell lymphoma	- DLBCL - Follicular lymphoma - Primary mediastinal large BCL - High-grade BCL	Multiple Myeloma
Reference	[kymriah]	[yescarta]	[tecartus]	[breyanzi]	[abecma]

Acute lymphoblastic leukemia (ALL); diffuse large B-cell lymphoma (DLBCL); B-cell lymphoma (BCL)

KYMRIAH™ was the first CAR-T cell therapy to be approved by the FDA. It targets CD19 and uses the 4-1BB costimulatory domain in its CAR construct to improve T cell activation. ABECMA® was the first FDA-approved CAR-T cell therapy targeting the B-cell maturation antigen (BCMA). Over 900 CAR-T cell clinical trials are ongoing as of mid-2021.

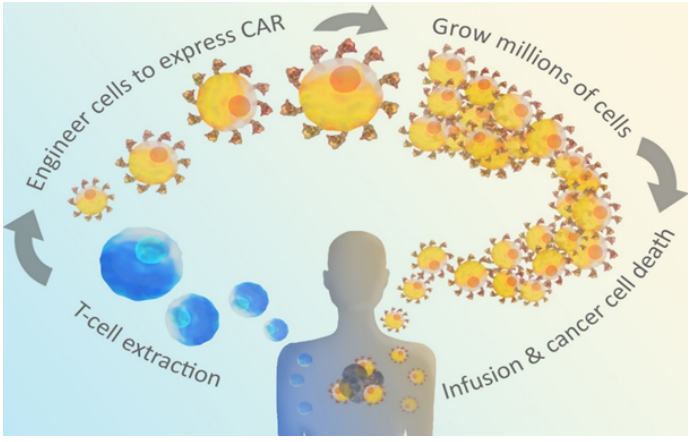
Despite initial success and a clear transformative potential, challenges abound. CAR-T technology is currently used to treat only a small fraction of human cancers. Ideal therapeutic targets are rare; while an antigen may be highly expressed in tumor cells, it may not be entirely absent from other tissues, leading to harmful side effects.

In addition, clinical trials have not been nearly as successful with solid tumors as with hematopoietic cancers, owing to the complexity of solid tumor biology. For example, high tumor cell heterogeneity favors escape and treatment resistance. Structural impediments to immune cell infiltration, abnormal vascularization, a hypoxic milieu, and immune suppression induced by the tumor microenvironment are some of the hurdles slowing progress. Further research efforts are warranted, not only to discover new therapeutic options but also to improve patients' lives by decreasing the toxicity and cost of existing treatments.

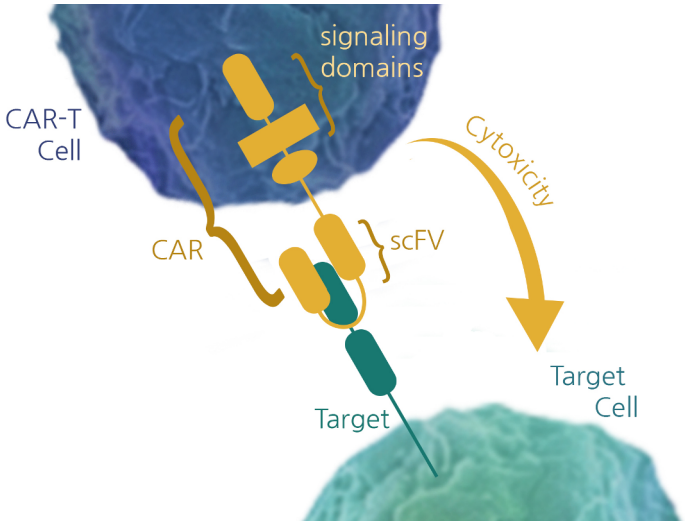
What is CAR-T cell therapy?

CAR-T cell therapy uses genetically modified cytotoxic T cells to fight tumor cells. From the patient's standpoint, the process involves several steps.

- **Collection**: White blood cells are collected from the patient's blood via leukapheresis.
- **Engineering**: The collected cells are sent to the laboratory, where the patient's T cells are genetically engineered to express a chimeric antigen receptor (CAR) on their surface, typically by transduction using a viral vector.
- **Multiplication**: The genetically modified T cells are expanded to increase the number of stable CAR-positive cells.
- **Infusion**: The patient receives conditioning chemotherapy to improve the ability of the T cells to multiply. A few days later the CAR-T cells are infused back into their bloodstream.



Engineered CAR-T cells must be manufactured according to an extremely rigorous process (extensively reviewed in [3]). From the drug discovery standpoint, the critical step of T cell engineering is very complex and takes years of careful design and optimization before reaching the clinic. Clearly, the therapeutic target must be extensively validated. An ideal target is a cell surface protein that is highly expressed on tumor cells but not on normal cells or in other tissues. In some circumstances, such as CD19 in B cell lymphomas, low-level expression in normal B cells is deemed acceptable because CD19 is not found in other tissue types and the side effects from destroying healthy B cells can be managed.

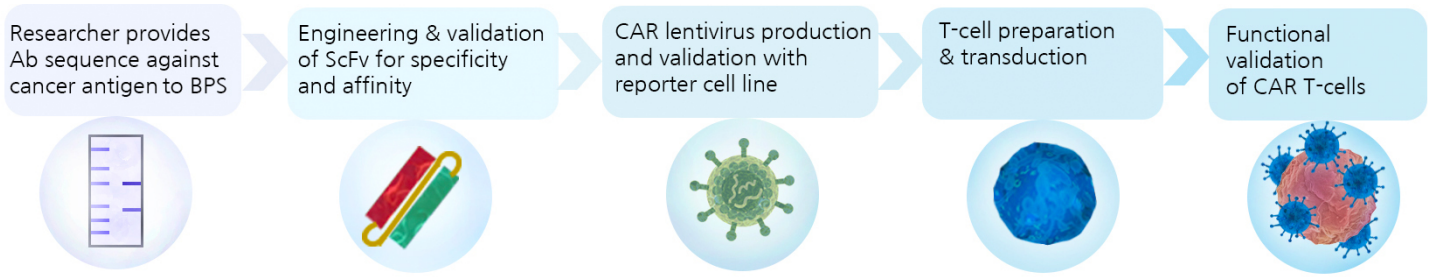


Chimeric Antigen Receptor

A Chimeric Antigen Receptor (CAR) is a transmembrane protein that contains an antibody portion (single-chain fragment variable, or ScFv) expressed on the surface of the T cell and an intracellular signaling portion to harness the effector function of the T cell, tethered by a transmembrane "linker" domain capable of signal transmission. The ScFv is designed to specifically recognize the target, such as CD19 or BCMA. Binding of the ScFv portion to the target antigen activates the signaling domain, ultimately resulting in the release of cytotoxins that kill the tumor cell.

At the experimental level, multiple steps are involved in the design of the CAR construct, and the stable genetic modification of the cells requires thorough validation and optimization.

The functional characterization of the resulting CAR-T cell is especially critical. A typical workflow includes iterations of the following:



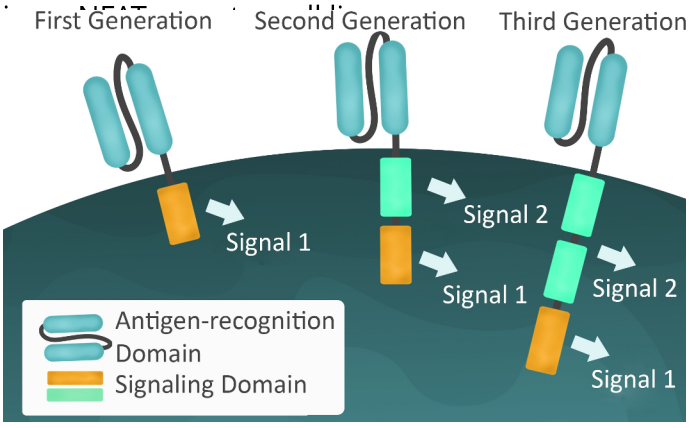
I. Screening, characterization and sequencing of highly specific monoclonal antibodies against the cancer antigen of interest
II. Engineering and validation of various ScFv-containing CAR constructs for specificity and activity
III. Lentivirus optimization and production at high titer for transduction of the CAR construct
IV. Isolation, activation and expansion of primary T cells
V. Transduction of the activated primary T cells with the CAR-encoding lentivirus
VI. Functional validation of the engineered CAR-T cells

BPS Bioscience offers custom CAR-T cell development services addressing multiple components of the CAR-T cell workflow (CAR-T services). This includes designing and constructing the lentivector for CAR transduction, verification of CAR expression, and functional validation of the resulting CAR-T cells.

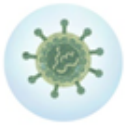
Validation of SvFc and CAR construct

Lentivectors can be constructed with various ScFv-CARs including mono-ScFv, dual ScFv and tandem ScFv. Optimization and validation of the ScFv antibody portion is performed by demonstrating target binding and specificity and by measuring the affinity for the target, for example using interferometry. Binding of the ScFv to the target can be further assessed in ELISA or in a cell-based assay using one of our 200 antigen-expressing cells. Specificity can be evaluated by comparing binding to several cell lines or by using KO cells.

The transmembrane linker domain, which needs to efficiently transmit the signal across the plasma membrane, can also be optimized. Various combinations of CAR signaling domains can be evaluated, and newer generation constructs typically contain up to three signaling units. The quality of the lentivirus construct is established by verifying CAR expression using flow cytometry and by preliminary measurement of CAR activity after transduction of the construct



Lentivirus Production and Initial Validation

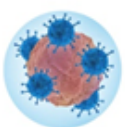


Once the CAR construct has been built, BPS scientists focus on the production of high-quality lentiviral particles, optimization of T cell transduction, and stable expression. Primary T cells are notoriously difficult to transfect and lentiviral vectors are more efficient than most other methods. Lentiviruses (a subclass of retroviruses) have a safer integration site profile than gamma-retroviral vectors which makes them more suitable for clinical use. New generation HIV-based lentiviruses commonly used in CAR-T cell therapy are pseudotyped by replacing the HIV envelope protein with Vesicular stomatitis virus G protein (VSV-G), which binds to the human LDL receptor (low-density lipoprotein receptor) on a broad range of cell types. Separate plasmids containing the CAR construct and all the necessary components for virus production are transfected into packaging cells such as HEK293. The viral particles produced by the packaging cells are replication-deficient and do not allow for expression of the HIV genes, therefore providing a safe and effective method of transducing the T cells.

T-Cell preparation for lentivirus transduction

This step requires the isolation, activation, and expansion of primary T cells from healthy donors or patients before transduction with the CAR lentivirus. The expression of CAR is verified by flow cytometry before establishment of the stable cells.

Functional validation of CAR activity in co-culture assays



In this final step, CAR activation in the presence of the antigen is assessed by measuring IFN-γ (Interferon-γ) production by the CAR-T cells. Production of other cytokines can also be measured if desired using one of BPS Bioscience's cytokine assay kits. Cytotoxicity towards cancer cells is measured using co-culture assays. First, reporter cells are generated by stable transfection of a reporter gene, such as luciferase, in cells that overexpress the target of interest. These target cells are cultured together with the effector CAR-T cells. Killing of the target cells is quantified by measuring luciferase activity, which is directly proportional to the number of live tumor cells.

Conclusion

The design and engineering of CAR-T cells includes extensive validation and optimization at various steps of the process, which in turn requires the development of many types of assays. This can be time and resource consuming, therefore the availability of pre-validated tools and assay kits can significantly accelerate research efforts. BPS Bioscience's CAR T-cell development services can be applied to any of the following areas:

- Design and optimization of CAR constructs
- Transduction of CAR-T cells and validation of CAR expression
- Compound and antibody screening using CAR-T cells or CAR/NFAT reporter cells
- Comparison of intracellular co-stimulatory and activation domains
- Study of CAR-T signaling in the tumor microenvironment
- Investigation of CAR-T donor variations

Bibliography

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