
Biocompare
The Buyer's Guide for Life Scientists
Advances in CAR-T Cell Therapy Research

Contents

Lentivirus Research Tools

for CAR-T Research

Ready-to-use lentiviral products for multiple applications:

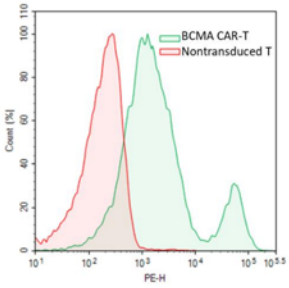
- CAR expression
- Stable cell line generation
- Protein expression or protein knockout
- Detection method for biochemical and reporter assays (GFP, luciferase)
- Screen for neutralizing antibodies

Advantages

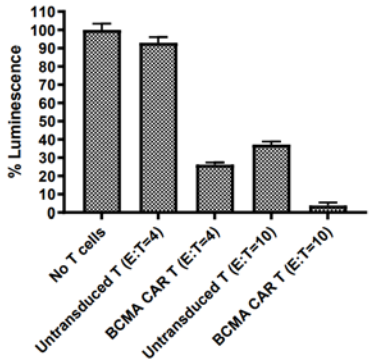
- Ideal for transducing actively dividing and non-dividing cells, as well as primary cells or stem cells
- Low immunogenicity, low toxicity
- Inserted DNA can be up to 10 kb
- Extremely high transduction efficiencies

Learn more at:
bpsbioscience.com/product-types/lentivirus

Anti-BCMA CAR Lentivirus, #78603



Expression of anti-BCMA CAR in T cells transduced with anti-BCMA CAR lentivirus.



Luciferase-based cytotoxicity assay using anti-BCMA CAR T cells and Firefly Luciferase-RPMI 8226 targets cells.

BPS Bioscience

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CAR-T Workflow: Custom Solutions

Assay kits and prevalidated tools can help accelerate research efforts.

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 immunooncology 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.

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

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)

FDA-approved CAR-T cell therapy targeting the B-cell maturation antigen (BCMA). Over 900 CAR-T cell clinical trials are ongoing as of 2022.

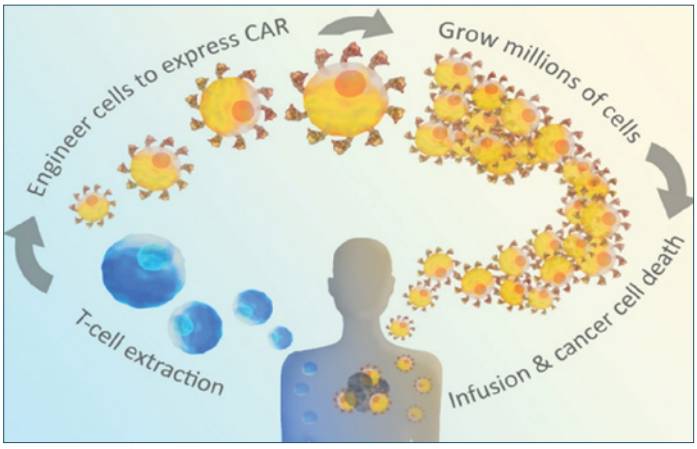
Despite initial success and a clear transformative potential, challenges abound. CAR-T technology is currently used to treat only a small fraction of human cancers. Ideal therapeutic targets are rare; while an antigen may be highly expressed in tumor cells, it may not be entirely absent from other tissues,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

How does CAR-T cell therapy work?

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

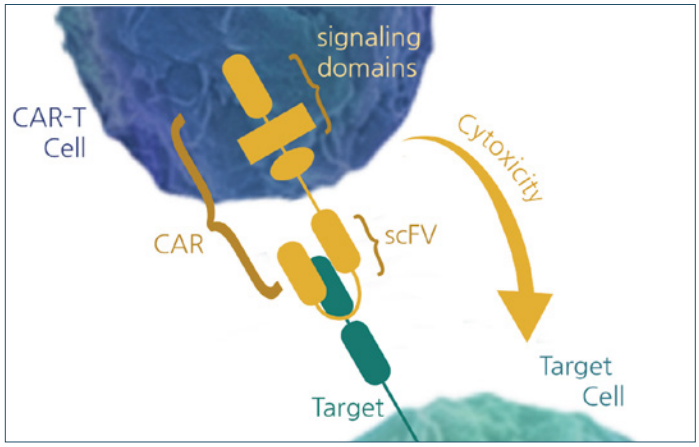
- **Collection:** White blood cells are collected from the patient's blood via leukapheresis.
- **Engineering:** The collected cells are sent to the laboratory, where the patient's T cells are genetically engineered to express a chimeric antigen receptor (CAR) on their surface, typically by transduction 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. Furthermore, 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

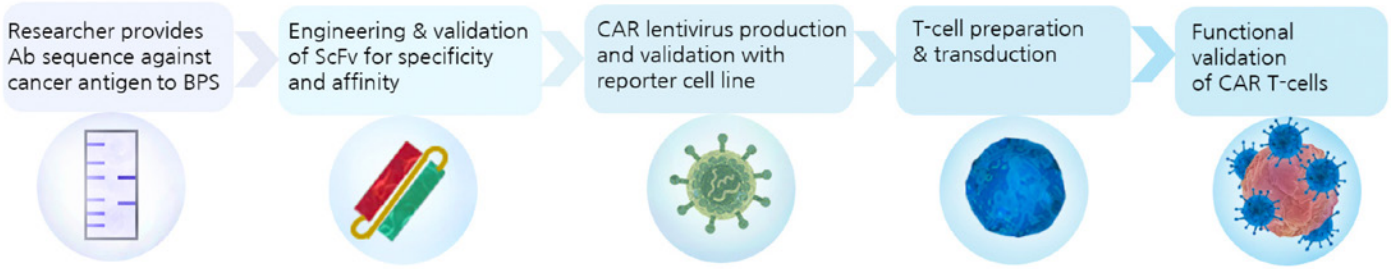
A Chimeric Antigen Receptor (CAR) is a transmembrane protein that contains an antibody portion (single-chain fragment variable, or ScFv) expressed



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 steps shown in the figure below:



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

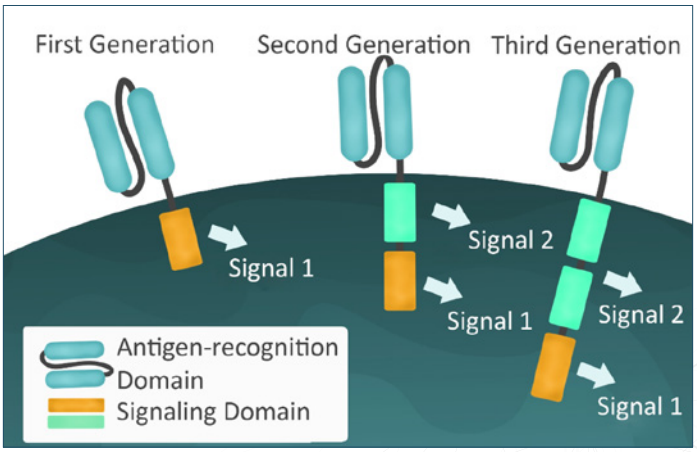
VI. Functional validation of the engineered CAR-T cells

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 ScFv 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

1. Guedan S, Ruella M, June CH. Emerging Cellular Therapies for Cancer. Annu Rev Immunol. (2019) 37: 145-171. PMID: 30526160. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7399614/

2. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CART cell immunotherapy for human cancer. Science. (2018) 359: 1361-1365. PMID: 29567707. https://pubmed.ncbi.nlm.nih.gov/29567707/

3. Vormittag P, Gunn R, Ghorashian S, Veraitch FS. A guide to manufacturing CART cell therapies. Curr Opin Biotechnol. (2018) 53: 164-181. PMID: 29462761. https://pubmed.ncbi.nlm.nih.gov/29462761/

Evaluation of CAR Expression and Antigen Binding

Essential steps in the production of CAR-T cells

Chimeric Antigen Receptors (CAR) are transmembrane receptors engineered to redirect the function of cytotoxic T cells. A CAR construct contains an extracellular antibody fragment ScFv (single-chain fragment variable) that recognizes a chosen antigen independently of HLA (Human Leukocyte Antigen), a transmembrane domain, and a cytoplasmic signaling module typically containing a CD3ζ domain and two co-activator/signaling modules. Target antigens are rationally chosen based on a selective expression pattern and high level of expression in tumor cells. Ideally, the target is expressed only in tumor cells, although in practice very few targets display such selectivity. The target must also be expressed on the surface of the tumor cell in order to be accessible for T cell recognition.

CD19 and BCMA (B Cell Maturation Antigen) are well-validated targets expressed in B lymphoma and multiple myeloma cells, respectively, which were used to generate the first series of FDA-approved CAR-T cell therapies. These have shown remarkable efficacy, although relapse to treatment occurs frequently owing to antigen escape. This is likely due to the selection of pre-existing antigen-negative tumor cells or the emergence of new cell populations. Further research is needed to identify new targets that may be used in bi-specific targeting or in sequential lines of treatment to decrease the likelihood of antigen escape. Ongoing research also aims at targeting solid tumors, which have not been very amenable to CAR-T cell therapy so far.

Targets under investigation for the treatment of hematopoietic cancers include CD22, CD33, CD37, CD38, CD123 and CD30 [1]. The alternatively spliced variant of the EGFR (Epidermal Growth Factor Receptor) termed EGFRvIII, and the interleukin-13 receptor α2 are being investigated for the treatment of glioblastoma [2, 3]. Additional promising targets in solid tumors include HER2 for sarcoma and the follicle stimulating hormone receptor for ovarian cancer, among others [4].

Once an antigen has been chosen, engineering of the CAR construct is initiated by screening and characterization of the extracellular antibody portion. High specificity for the target is required.

It is also particularly important to demonstrate CAR expression in T cells as well as recognition of the target in intact cells.

CAR expression is often evaluated by flow cytometry using the labeled target protein or anti-Fab antibodies to detect the extracellular domain of the CAR construct. Purified recombinant proteins, labeled or unlabeled, and containing either an N-terminal or a C-terminal tag are very desirable because they offer low-background, high-specificity staining options (Figure 1).

For example (Figure 2), recombinant human CD38 containing a C-terminal His-Tag was affinity purified to obtain an active HiP™ protein, indicating a high purity protein (≥90%) and less than 10% aggregation as measured by gel filtration.

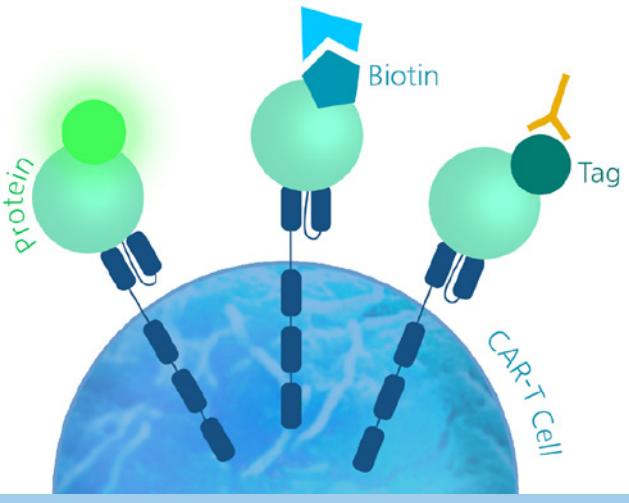


Figure 1: Protein binding to CAR

Antigen-expressing cell lines

Cells overexpressing an antigen of interest are useful for the screening or characterization of antibodies and CAR constructs in a biologically relevant model. In contrast to human primary cells, immortalized cell lines are easy to maintain and expand, do not have the variability of primary cells, and are well suited for some of the optimization steps involved in the successful design of a CAR-T cell. In addition, they do not carry the same risks of containing human pathogens inherent to the use of donated blood cells. Thus, recombinant cell lines simplify and standardize target recognition experiments.

CD19 is present in B cell lymphocytes through almost all phases of B cell maturation and is a common target for CARs recognizing B cell lymphomas. Another common target, BCMA is a cell surface receptor preferentially expressed on mature B-lymphocytes. It is a highly attractive target for immunotherapy because of its low expression levels in non-malignant tissue and almost universal expression on multiple myeloma cells. Clonal stable CHO cell lines were engineered to constitutively express BCMA, as confirmed

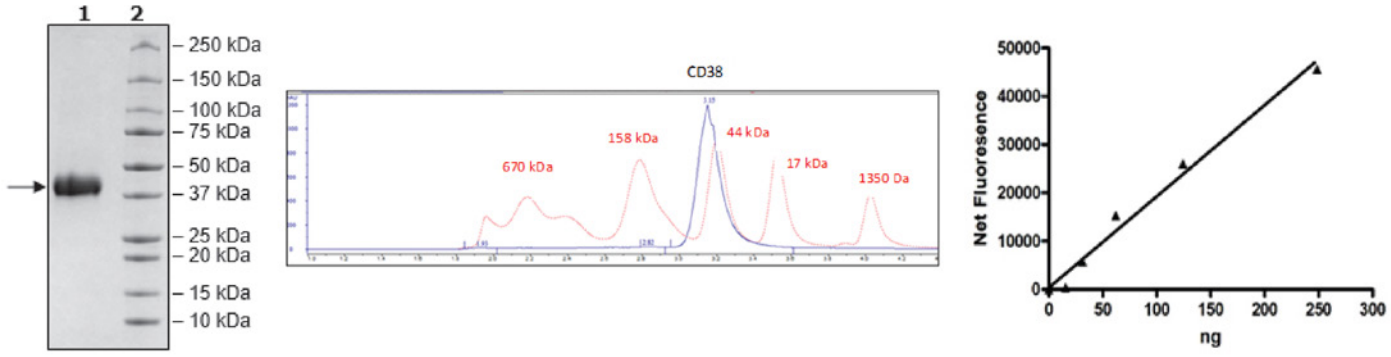


Figure 2: CD38, His-Tag (Human), HiP™ (#71277). Left panel: 4-20% SDS-PAGE Coomassie staining. Middle panel: gel filtration trace of the purified protein. Right panel: a representative result of CD38 activity obtained using the fluorescent CD38 Inhibitor Screening Assay Kit, Cyclase Activity (#71275) with CD38 titrated from 16.5ng/ul to 250ng/ul.

by flow cytometry (Figure 3). Each cell line was selected for low, medium or high levels of expression to mimic cancer cells displaying various BCMA expression levels.

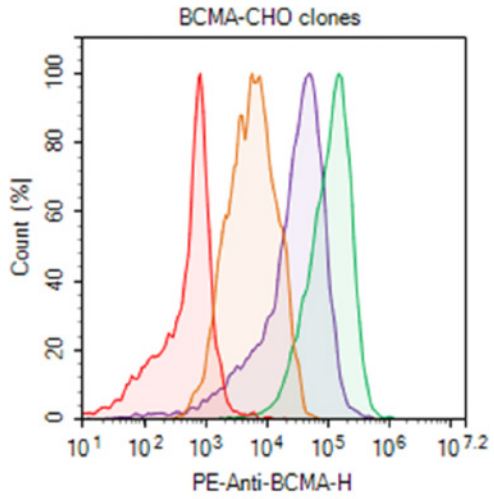


Figure 3: BCMA-CHO Cell Lines (#79500). Expression of BCMA was validated by flow cytometry using PE-conjugated anti-BCMA antibody (BioLegend #357504). High, medium, and low expression shown in green, purple and brown, respectively. Parental CHO cells: red.

Of note, BCMA-overexpressing CHO cells were used to validate a new monoclonal antibody for CAR-T cell therapy in a study by Berahovich et al. [5].

Dual expression cell lines

To address the issue of resistance to therapy resulting from loss of antigen expression in the tumor cells, bi-functional antibodies and bi-functional CAR-T cells are being developed such as anti-CD20-CD19 or anti-CD20-BCMA bispecific CAR-T cells.

CD20 (MS4A1) is expressed on the surface of B cells and is present in more than 90% of patients with B cell lymphoma. This antigen has been a highly relevant target for immunotherapy since the 1997 approval of Rituximab (Rituxan), a chimeric monoclonal anti-CD20 antibody now classified by the World Health Organization as an "Essential Medicine". Additional monoclonal antibodies against CD20 have been approved or are being tested in clinical trials for the treatment of cancer as well as autoimmune diseases.

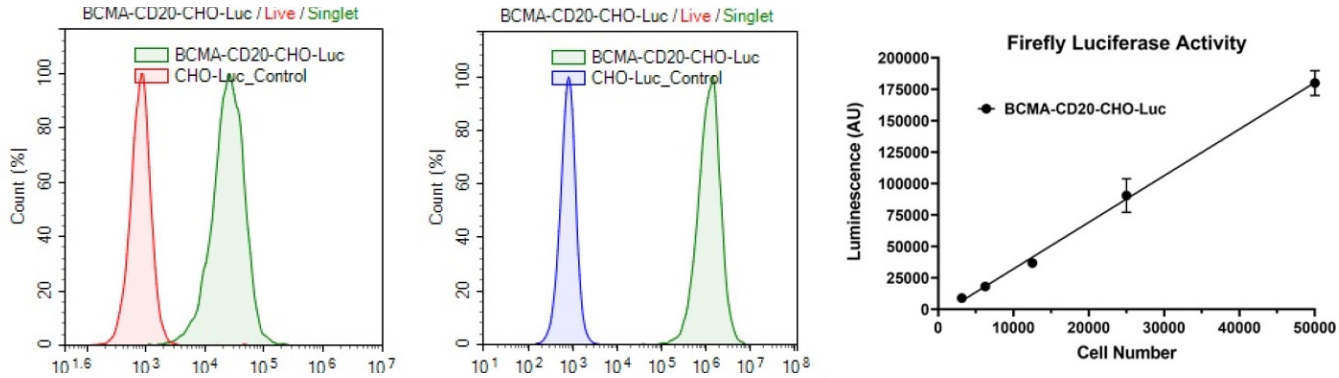


Figure 4: Expression of CD20, BCMA and luciferase in the BCMA / CD20 / Firefly Luciferase CHO Cell Line (#78185). The cells were treated with PE-conjugated anti-human BCMA antibody (BioLegend, #357503; left panel) or PE-conjugated anti-human CD20 antibody (BioLegend, #302346; middle panel) and analyzed by flow cytometry to detect surface expression of the antigens. CHO luciferase cells were used as control. Cells were seeded in a 96-well plate at increasing densities (right panel). After four hours, luciferase activity was measured using the ONE-Step™ luciferase assay system (#60690).

The BCMA / CD20 / Firefly Luciferase CHO Cell Line was developed specifically to study anti-BCMA/anti-CD20 bi-specific antibodies or bi-specific CAR-T cells. This cell line contains both human CD20 and human BCMA, in addition to the firefly luciferase reporter. The expression of BCMA and CD20 was validated by flow cytometry, while it was demonstrated that luciferase activity is directly proportional to the number of cells (Figure 4).

Tumor cell killing assays and cytokine production assays can be performed following co-culture of these antigen-presenting cells with BCMA/CD20-specific CAR-T cells.

Case study

The expression of cell surface glycoprotein CD37 is restricted to cells of the immune system, with high expression observed in mature B cells and lowest expression in T cells and myeloid cells. It is also expressed in B cell non-Hodgkin Lymphomas, in chronic lymphocytic leukemia, and in some cutaneous and peripheral T cell lymphomas. Anti-CD37 CAR-T cells displayed antigen-specific activation, cytokine production and cytotoxic activity in models of B and T cell lymphomas *in vitro* and *in vivo*. They have substantial activity against lymphoid lineages without significant cytotoxicity against T cells. Anti-CD37 CAR-T cells therefore represent novel therapeutic agents for the treatment of CD37-expressing lymphoid malignancies.

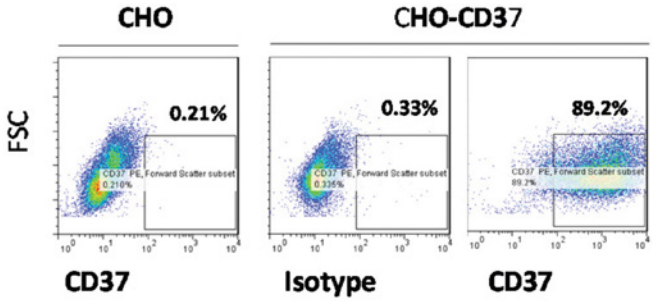


Figure 5: Anti-CD37 antibody binding to CD37-expressing cells. Recognition of human CD37 in a cellular context was assessed by flow cytometry using the new anti-CD37 antibody incubated with intact CHO-CD37 (#79607) or with control CHO cells. Figure reproduced in part and without modification under license CC BY 4.0. https://doi.org/10.3390/cancers13050981

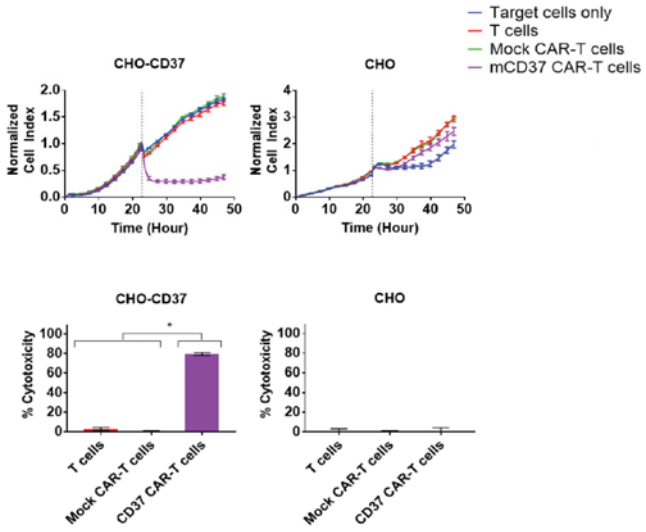


Figure 6: Real-time cytotoxicity assay. Adherent target cells (CHO-CD37 or CHO) were seeded into 96-well E-plates and monitored overnight with the impedance-based real-time cell analysis xCELLigence system (Acea Biosciences). Effector CAR-T cells or non-transduced T cells were added the next day. The cells in the E-plates were monitored in real-time for another 24–48 hours, and impedance was plotted over time. Cytotoxicity was calculated as (impedance of target cells without effector cells - impedance of target cells with effector cells) x 100 / impedance of target cells without effector cells. Figure reproduced in part and without modification, under license CC BY 4.0. https://doi.org/10.3390/cancers13050981

In their recent publication, V. Golubovskaya et al. used CD37-CHO cells for the validation of novel, humanized, bi-specific CD37-CD19 CAR-T cells, developed for the treatment of lymphoma [6]. A mouse monoclonal anti-CD37 antibody (clone 2B8D12F2D4) was generated with high binding affinity (KD = 1.6 nM). The antibody was able to bind recombinant CHO cells constitutively expressing human CD37 but not control CHO cells, which do not express measurable levels of CD37 (Figure 5).

The anti-CD37 CAR-T cells killed target cells CD37-CHO, but not the CD37-negative control cell line (Figure 6). Of note, these CAR-T cells also killed CD19 positive CD19-HeLa cells but not parental HeLa cells (not shown). The authors went on to show that the bi-specific humanized CD37-CD19 CAR-T cells inhibited the growth of Raji xenograft tumors and increased survival in a mouse model [6].

Bibliography

1. Guedan S, Ruella M, June CH. Emerging Cellular Therapies for Cancer. Annu Rev Immunol. (2019) 37: 145-171. PMID: 30526160. https://pubmed.ncbi.nlm.nih.gov/30526160/

2. O'Rourke DM, Nasrallah MP, Desai A, et al. A single dose of peripherally infused EGFRvIII-directed CART cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Transl Med. (2017) 9: eaaa0984. PMID: 28724573. https://pubmed.ncbi.nlm.nih.gov/28724573/

3. Brown CE, Alizadeh D, Starr R, et al. Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy. N Eng l JMed. (2016) 375: 2561-2569. PMID: 28029927. https://pubmed.ncbi.nlm.nih.gov/28029927/

4. Freitag F, Maucher M, Riester Z, Hudecek M. New targets and technologies for CAR-T cells. Curr Opin Oncol. (2020) 32: 510-517. PMID: 32657796. https://pubmed.ncbi.nlm. nih.gov/32657796/

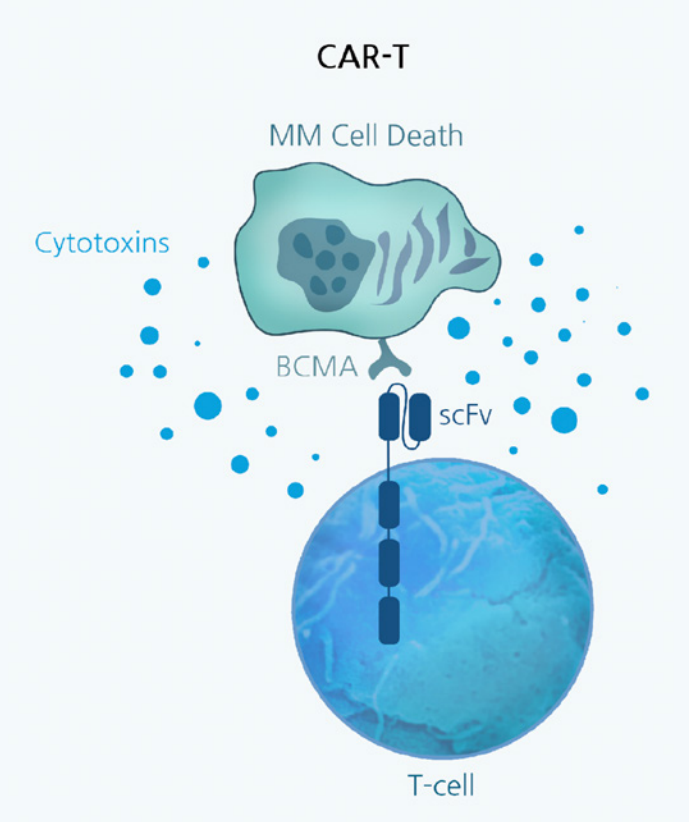
5. Berahovich R, Zhou H, Xu S, Wei Y, Guan J, Guan J, Harto H, Fu S, Yang K, Zhu S, Li L, Wu L, Golubovskaya V. CAR-T Cells Based on Novel BCMA Monoclonal Antibody Block Multiple Myeloma Cell Growth. Cancers, Basel. (2018) 10: 323. PMID: 30208593. https://pubmed.ncbi.nlm.nih.gov/30208593/

6. Golubovskaya V, Zhou H, Li F, Valentine M, Sun J, Berahovich R, Xu S, Quintanilla M, Ma MC, Sienkiewicz J, Huang Y,Wu L. Novel CD37, Humanized CD37 and Bi-Specific Humanized CD37-CD19 CAR-T Cells Specifically Target Lymphoma. Cancers, Basel. (2021) 13: 981. PMID: 33652767. https://doi.org/10.3390/cancers13050981.

Functional Validation of CAR-T Cells

Perhaps the most important step in the CAR-T cell development process.

The ultimate function of a CAR-T cell in cancer immunotherapy is to kill the tumor cells. Once activated, cytotoxic T cells trigger the apoptosis of the target cells, although it is suspected that other death mechanisms also take place since cell death can still happen when the tumor cell apoptotic pathway is disabled. Mechanistically, activation of the T cell is initiated by the binding of the antibody ScFv portion of the CAR to the antigen present on the surface of the tumor cells. The intracellular signaling portion of CAR activates several signaling pathways, culminating in activation of transcription factors such as NFAT (Nuclear Factor of Activated T Cell), increased gene transcription and release of death ligands FasL (Fas Ligand) and TRAIL (TNF-related apoptosis-inducing ligand). These bind to specific death receptors present on the tumor cells to activate programmed cell death. In addition, the granule exocytosis pathway of the T cell is engaged, leading to the outpouring of pore-forming protein perforin and serine proteases of the granzyme family into the immunological synapse, directly contributing to the lysis of the tumor cells (reviewed in [1]).



Apart from sending death signals, T cells produce TNF-α (Tumor Necrosis Factor-α) and IFN-γ (Interferon-γ) upon activation, *in vitro* and *in vivo*, suggesting that both TNF-α and IFN-γ release may be a prerequisite to their function. IFN-γ is a cytokine primarily secreted by activated T cells and natural killer (NK) cells; it plays an important role in innate and adaptive immunity. In the clinic, patients infused with anti-CD19 CAR-T cells displayed increases in serum levels of IFN-γ

and TNF. Peripheral blood T cells from these patients also produced TNF and IFN-γ ex vivo in a CD19-dependent fashion, indicating that the CAR-T cells were the cause of this cytokine release [2].

Functional characterization is arguably one of the most important steps of the CAR-T cell development process. In addition to ensuring that the CAR is correctly activated, functional assays should demonstrate whether tumor cell death is achieved, since this is the ultimate purpose of the CAR-T cell. Experimentally, CAR-T cells are placed in culture with the target tumor cells expressing the antigen of interest, for example CD19 or BCMA. Cytokines released by the T cells in the culture medium are quantified, and tumor cell viability is assessed, for example using a reporter cell system.

CAR NFAT-luciferase Reporter Jurkat Cells

The anti-CD19 and the anti-BCMA NFAT-luciferase Jurkat cells are used to optimize or test antigen-presenting cells, optimize co-culture assay conditions, and ensure that proper T cell activation is taking place in a particular assay.

A firefly luciferase reporter, expressed under the control of a promoter containing an NFAT response element, monitors the activation of the CD3 signaling domain downstream of CAR, which in turn activates transcription factor NFAT when the cells are in contact with antigen-presenting cells. In addition to the conditional luciferase, the anti-BCMA CAR/NFAT-luciferase reporter Jurkat cell line stably expresses an anti-BCMA CAR construct. When these recombinant Jurkat cells are added to BCMA-presenting cells the NFAT transcription factor is activated and induces luciferase expression, which can be quantified as shown in Figure 1.

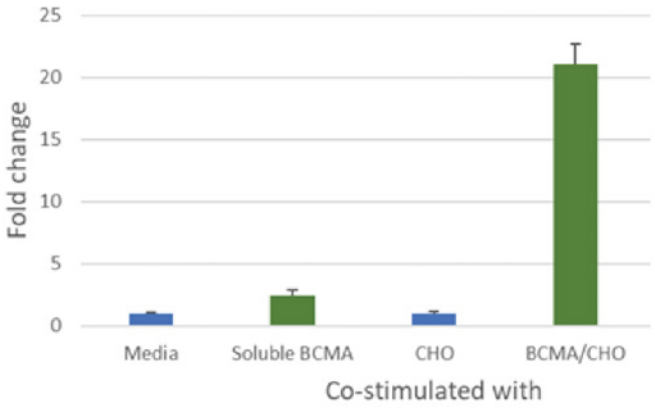


Figure 1: BCMA-dependent activation of anti-BCMA CAR NFAT-luciferase Jurkat cells. The anti-BCMA CAR NFAT luciferase cells (#79694) were cultivated alone, in the presence of soluble BCMA, in the presence of control CHO cells, or in the presence of BCMA-presenting CHO cells at a ratio of 10 to 1 (effector to target). Luciferase activity was measured using the ONE-Step™ luciferase assay system (#60690).

The Jurkat cells were not activated when cultivated alone or with control CHO cells not expressing BCMA. The addition of a soluble form of BCMA resulted in a 4-fold increase in luciferase activity, whereas the presence of BCMA-presenting CHO cells induced a 23-fold increase in luciferase activity.

Interferon- γ ELISA

Since IFN-γ has been accepted as an indicator of T cell activation, measuring its release in response to target recognition is routinely performed as part of CAR-T cells functional validation. The IFN-γ Colorimetric ELISA Detection Kit quantifies human IFN-γ in cell culture medium.

Assay principle: An anti-IFN-γ capture antibody is coated on a 96-well plate. The test samples presumed to contain IFN-γ are incubated on the coated plate. The plate is washed, and an anti-IFN-γ detection antibody is added. After washing, the plate is

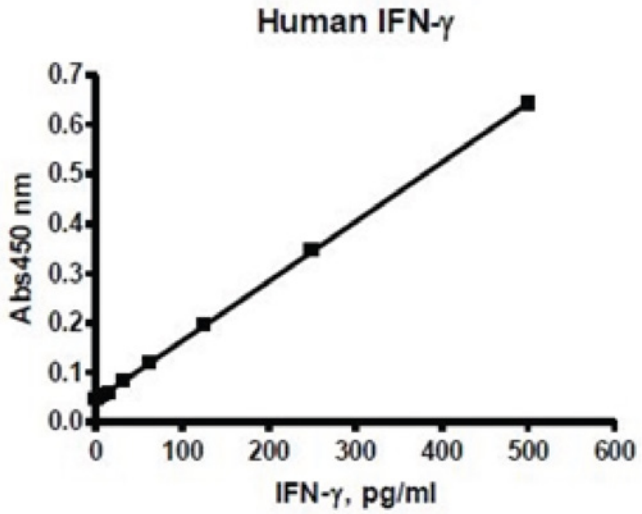


Figure 2: Quantification of human IFN-γ. The Colorimetric Human IFN-γ Detection Kit (#79777) was validated by incubating increasing amounts of purified IFN-γ (2 pg/ml–500 pg/ml) onto the plate coated with the capture antibody. Colorimetric signal was acquired using a UV/Vis spectrophotometer.

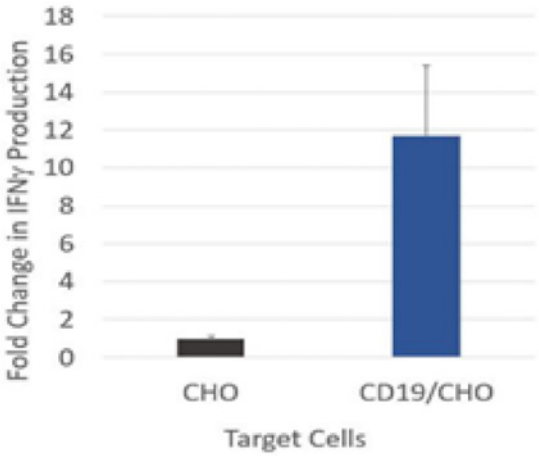


Figure 3: IFN-γ production by anti-CD19 CAR-T cells in co-culture. CAR-T cells transduced with an anti-CD19 CAR lentivirus were cultivated with CD19-expressing CHO cells (#79561) or parental control CHO cells, at a ratio of 10 to 1 for 16 hours. The amount of secreted IFN-γ was measured using the Colorimetric Human IFN-γ Detection Kit (#79777). Background absorbance from medium-only wells was subtracted from all measurements. Results are expressed relative to the amount of IFN-γ detected in the co-culture of CAR-T cells and parental CHO cells (set at 1).

treated with streptavidin-HRP followed by addition of a colorimetric HRP substrate. The assay is linear between at least 2 pg/ml and 500 pg/ml, as shown in Figure 2. This assay kit is useful to measure the production of IFN-γ by CAR-T cells cultivated together with antigen-presenting cells. If the CAR-T cells are properly activated, they will release cytokines in the culture medium, which can be sampled.

An example of IFN-γ measurement in a co-culture assay is shown in Figure 3.

In this experiment, primary T cells from donors were stably transduced with an anti-CD19 CAR lentivirus. Twelve days later, the CAR-T cells were placed in culture with CD19-expressing CHO cells or with parental control CHO cells. The amount of secreted IFN-γ was measured using the Colorimetric Human IFN-γ ELISA Detection Kit. The anti-CD19 CAR-T cells cultivated with CD19-expressing CHO cells produced 12 times more IFN-γ than anti-CD19 CAR-T cells cultivated with control CHO cells, indicating that the T cells were properly activated by presentation of CD19 cells.

CAR-T Cytotoxicity Assays

The most relevant functional assay to assess CAR-T function is to measure cytotoxicity towards target tumor cells. This is best performed using co-culture assays in which the CAR-T cells are cultivated together with antigen-presenting tumor cells, and the number of live tumor cells is quantified. This can be made difficult by the presence of the T cells. A simple solution is to add a reporter into the tumor cells that allows detection of the tumor cells without interference from the T cells. Thus, target tumor cells

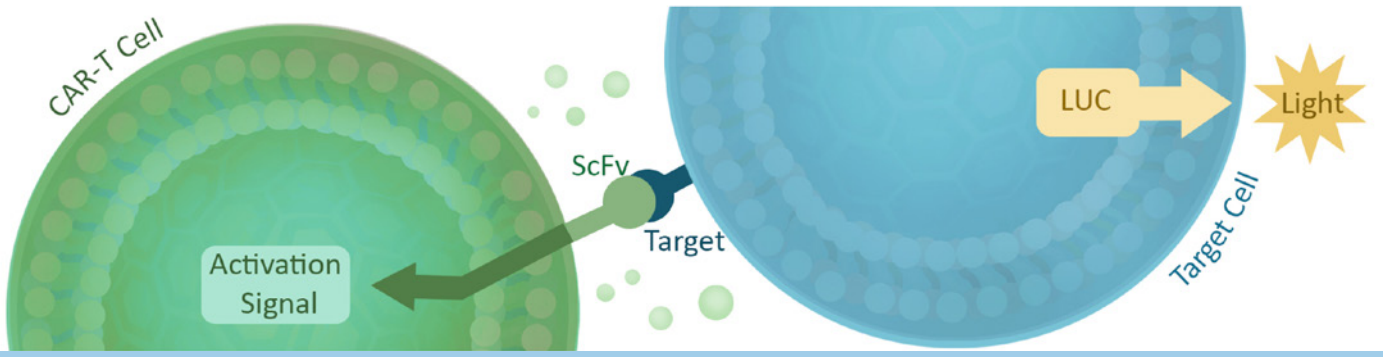


Figure 4: Illustration of a co-culture assay using luciferase-positive target cells

are engineered to express eGFP (Green Fluorescent Protein), which can be quantified by fluorescence. Alternatively, tumor-specific expression of firefly luciferase allows sensitive and robust quantification of luciferase activity, which is directly proportional to the number of live cells present in the cell culture.

During the development of co-culture assays, a known CAR-T cell system is useful to optimize assay conditions and to use as a positive control or internal control (standard) in subsequent experiments.

To assess CD19-directed activity of CAR-T cells expressing the anti-CD19 CAR construct, the CAR-T cells were added to a culture of CD19 Luciferase CHO cell line. As shown in Figure 5, presentation of CD19 by the CHO cells resulted in activation of the anti-CD19 CAR-T cells and killing of the CHO cells as indicated by the loss in luciferase activity. As expected, control T cells not expressing the CAR construct did not kill the CD19 CHO luciferase cells.

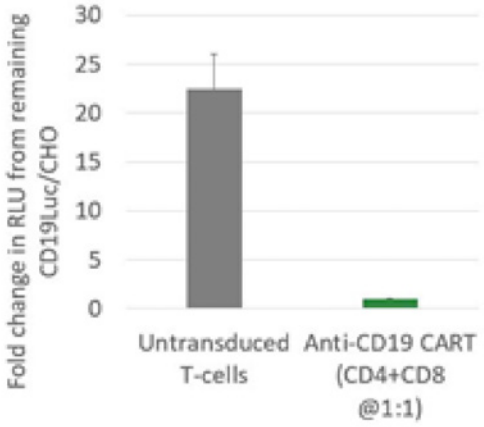


Figure 5: Anti-CD19 cytotoxicity assay. Primary T cells from donors were transduced with an anti-CD19 CAR lentivirus and expanded under conditions of antibiotic selection for twelve days. The resulting anti-CD19 CAR-T cells were cultivated with CD19-expressing CHO luciferase cells (#79714) at a 10 to 1 ratio for 16 hours. Non-transduced T cells were used as negative control. Luciferase activity was measured using the ONE-Step luciferase assay system (#60690).

Bibliography

1. Martínez-Lostao L, Anel A, Pardo J. How Do Cytotoxic Lymphocytes Kill Cancer Cells? *Clin Cancer Res.* (2015) 21: 5047-5056. PMID: 26567364. https://pubmed.ncbi.nlm.nih.gov/26567364/

2. Kochenderfer JN, Dudley ME, Feldman SA, *et al.* B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood* (2012) 119: 2709-2720. PMID: 22160384. https://pubmed.ncbi.nlm.nih.gov/22160384/

Tools for Research on Anti-CD19

CD19 is an ideal target for cancer immunotherapy.

The CD19 molecule (also known as Cluster of Differentiation 19, B-lymphocyte antigen CD19, B-Lymphocyte Surface Antigen B4, or CVID3) belongs to the family of immunoglobulins. It is a glycoprotein of 95 kDa expressed at the surface of B cell lymphocytes through almost all phases of B cell maturation from earliest stages to terminal differentiation, for which it is strictly required [1]. Thus, mutations in the CD19 gene cause severe immunodeficiency syndromes associated with impaired antibody production, such as CVID3 disease (common variable immunodeficiency 3) [2]. CD19 may also be involved in several autoimmune diseases.

CD19 contains two extracellular immunoglobulin- like domains termed C2, a single span hydrophobic transmembrane domain and a large C-terminal cytoplasmic domain containing several conserved tyrosine phosphorylation sites. No specific ligand has been identified; however, it is known that the protein forms a complex with CD21 and CD81. Activation of this complex has been shown to facilitate B cell activation by lowering the signaling threshold by several orders of magnitude [3]. Engagement of the BCR (B Cell antigen Receptor) by a membrane-bound antigen recruits tyrosine kinases SYK and LYN, which in turn phosphorylate the cytoplasmic domain of CD19 to serve as a docking site for SH2-containing signaling proteins (Figure 1).

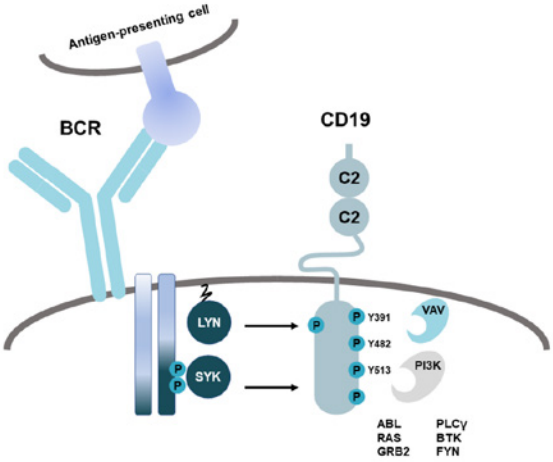


Figure 1: CD19 signaling

The majority of B cell malignancies, notably B cell lymphomas, acute lymphoblastic leukemia (ALL),

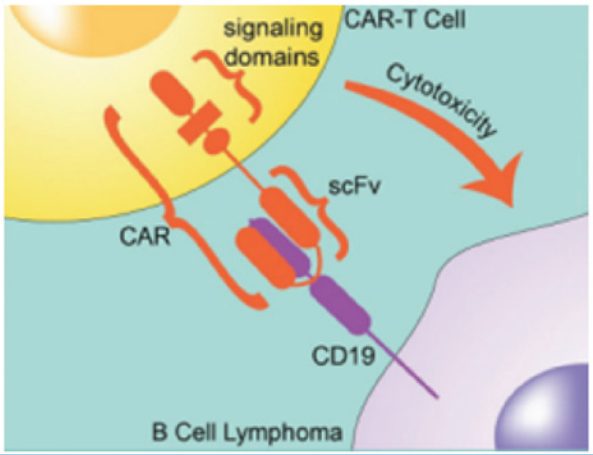


Figure 2: Illustration of the structure of an anti-CD19 CAR (CD19 ScFv-CD8-4-1BB-CD3ζ).

and chronic lymphocytic leukemia (CLL), express normal to high levels of CD19. Since it is a marker for B cells, CD19 has been used to detect leukemia and lymphoma [4]. It is also a nearly ideal target for cancer immunotherapy. Blinatumomab, a CD19/CD3 bi-specific T cell engager (BiTE) has remarkable anti-tumor efficacy [5]. It has been approved for relapsed/refractory B-precursor ALL. In addition, CD19 is the target of the first CAR-T cell therapy, approved 30 years after the concept of a chimeric antigen receptor (CAR) was proposed [6].

CD19-presenting cells

CD19-presenting cells such as the CD19 CHO (Chinese Hamster Ovary) Recombinant Cell Lines are particularly useful to screen, characterize and validate antibodies against CD19, or to study the binding of CAR constructs. Cells expressing CD19 can also be used to activate CAR-T cells in various experimental settings.

Clonal stable CHO cell lines were generated to constitutively express full length human CD19, as confirmed by flow cytometry (Figure 3). Each cell line was selected for low, medium or high levels of CD19 expression to model tumor cells. Cells with low expression of CD19 may be particularly useful to assess the potential efficacy of a new CAR construct against tumor cells expressing only low levels of CD19.

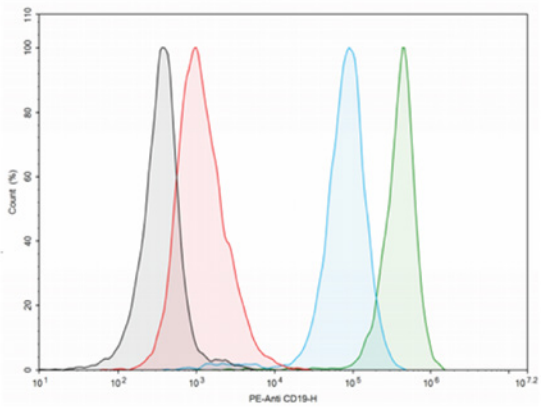


Figure 3: Expression of CD19 in CD19-CHO Recombinant Cell Lines. (#79561). The cells were incubated with PE-conjugated anti-human CD19 antibody (BioLegend) and analyzed by flow cytometry to detect surface expression of CD19. Green, blue and red: high, medium and low expression, respectively. Black: parental CHO cells.

CD19 reporter cells

CD19 Luciferase CHO Recombinant Cells constitutively express human CD19, as demonstrated by flow cytometry (Figure 4). The cells also stably express firefly luciferase, so that luciferase activity is directly proportional to the number of cells. This cell

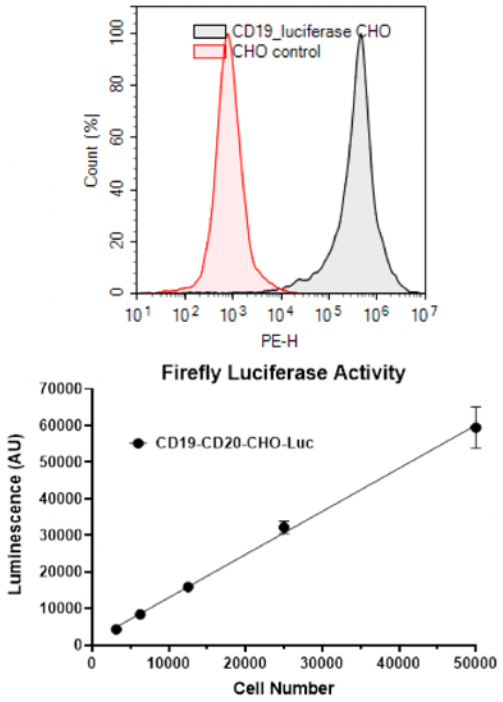
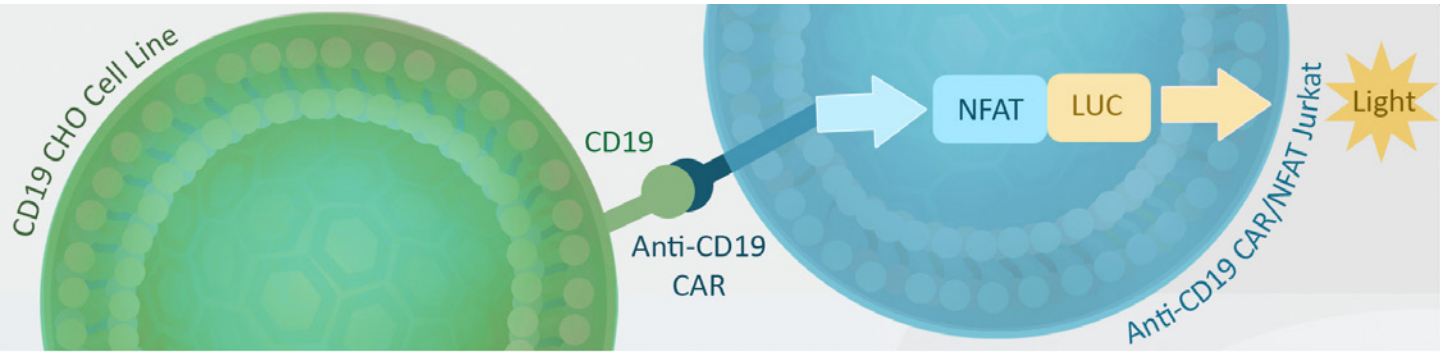


Figure 4: CD19 expression and luciferase activity in reporter cells. Left panel: CD19 luciferase cells were incubated with PE-conjugated anti-human CD19 antibody (BioLegend) and analyzed by flow cytometry (left panel). Parental CHO-K1 cells: red. CD19 Luciferase CHO cells #79714: black. Right panel: Increasing numbers of CD19/CD20 luciferase cells were seeded in a 96-well plate. Four hours later, luciferase activity was measured using the ONE-Step™ luciferase assay system (#60690).

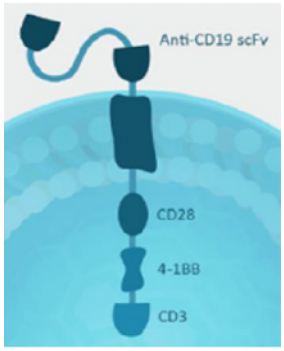


line offers a simple way to quantify changes in cell viability, which is particularly useful in co-culture experiments in which the cytotoxicity of T cells is determined against the CD19-presenting cells. Additional reporter cells were designed to co-express other targets of interest together with CD19, including CD20 (#78186), CD38 (#78149) and BCMA (#78030). These cells are used for the characterization of bi-specific antibodies and functional co-culture studies.

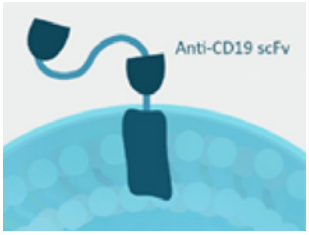
Anti-CD19 CAR Luciferase Reporter Jurkat Cells

Jurkat cells are immortalized human T lymphocytes established from a patient with leukemia. A firefly luciferase reporter, expressed under the control of a promoter containing an NFAT response element, monitors the activation of transcription factor NFAT (Nuclear factor of activated T cells). This cell line has been validated for response to NFAT agonists, an anti-CD3 antibody, and the CD3/CD19 bispecific antibody blinatumomab in co-culture with CD19-positive Raji cells. The anti-CD19 CAR/NFAT-luciferase reporter Jurkat cell line (#79853) is a double transfectant cell line that can be used as a surrogate T cell to develop assays for CAR-T cell optimization, and as a positive control in co-culture assays. It is a valuable tool during the generation of CD19-expressing target cells.

This cell line displays constitutive expression of an anti-CD19 CAR in which the anti-CD19 ScFv is linked to the CD28 transmembrane and costimulatory domains, the 4-1BB costimulatory domain and the CD3ζ signaling domain. The CAR is activated when added to cells expressing CD19, ultimately leading to the stimulation of transcription factor NFAT which in turn induces luciferase expression.



The anti-CD19 CAR-negative/NFAT luciferase reporter Jurkat cell line is used as negative control in co-culture experiments with CD19-presenting cells. They express an anti-CD19 ScFv linked to the CD28 transmembrane domain but lacking the T cell activation components.



Although the cells contain the NFAT responsive luciferase reporter, the short CAR protein is incapable of inducing luciferase expression following binding to CD19.

Both cell lines contain the anti-CD19 ScFv, demonstrated by flow cytometry. The functional validation (Figure 5) shows that the anti-CD19 CAR Jurkat cell line was activated when co-cultivated with CD19 CHO cells, indicated by a 10-fold increase in luciferase activity, but not when co-cultivated with parental CHO cells. On the other hand, the luciferase

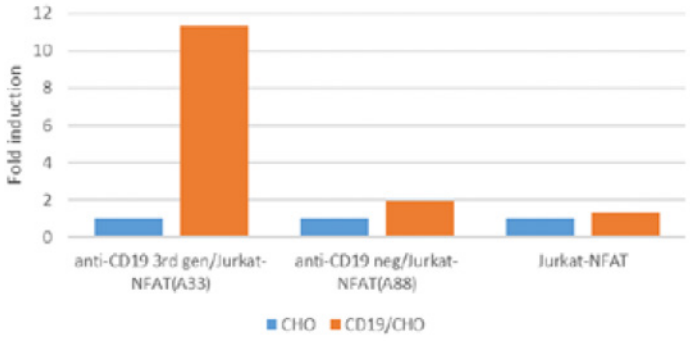


Figure 5: Functional validation of anti-CD19 CAR reporter Jurkat cells. Parental and CD19 CHO cell lines (#79561) were cultivated either with control (#60621), anti-CD19 CAR-negative (#79854), or anti-CD19 CAR (#79853) NFAT luciferase reporter Jurkat cells. Luciferase activity was measured using the ONE-Step™ luciferase assay system (#60690).

reporter in anti-CD19 CAR-negative Jurkat luciferase cells was not activated by the CD19-presenting or the parental CHO cells.

Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ)

BPS Bioscience has developed off-the-shelf lentiviruses, ready for infection and capable of transducing most types of dividing or non-dividing mammalian cells. None of the HIV genes from the backbone virus are expressed in the transduced cells, therefore these viral particles are replication incompetent and can be used in a Biosafety Level 2 facility, which makes them accessible to many research laboratories.

The anti-CD19 CAR lentivirus is pseudotyped, which means that the HIV envelope protein has been replaced with VSV-G (Vesicular stomatitis virus G), a protein that binds to the ubiquitously expressed LDL receptor (low-density lipoprotein). Upon infection of the target cells, the anti-CD19 CAR protein is transduced into the cells and expressed under the control of constitutive mammalian promoter EF-1α. Stable expression can be achieved following puromycin selection.

The anti-CD19 CAR construct (Figure 6) consists of the **ScFv** portion of highly specific monoclonal anti-CD19 antibody (clone **FMC63**) linked to the CD8 hinge, 4-1BB and CD3ζ signaling domains. FMC63 ScFv is the most commonly used ectodomain of anti-CD19 CARs.

Stable expression in transduced cells was demonstrated by flow cytometry. Figure 7 shows that a proportion of the T cells were not effectively transduced and did not express the CAR construct, whereas the fraction of cells that were transduced displayed robust anti-CD19 ScFv expression.

Functional characterization of the stably transduced T cells included measuring interferon release

*Figure 6: Diagram of the lentiviral CD19 CAR construct (#78600). **Application:** Useful as a positive control in experiments that evaluate anti-CD19 CAR constructs, to optimize T cell transduction, and to develop functional assays.*

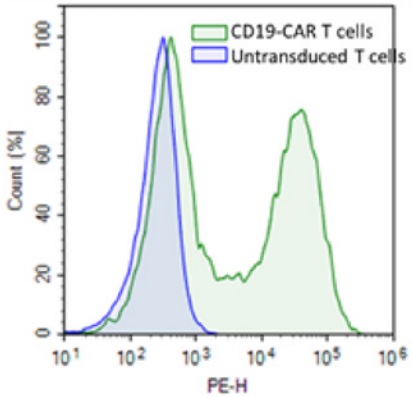


Figure 7: Expression of anti-CD19 CAR in T cells transduced with the lentivirus. Approximately 15,000 CD4+/CD8+ activated T cells were transduced (MOI of 40) in the presence of 5 µg/mL of polybrene via spinoculation. Anti-CD19 CAR expression was analyzed 10 days later by flow cytometry using PE-labeled anti-FMC63 ScFv (Acrobiosystems). Transduced cells: green; non-transduced: blue.

following addition of CD19-expressing cells. IFN-γ is a cytokine secreted by activated T cells that has been recognized as an indicator of T cell activation. The anti-CD19 CAR-T cells released IFN-γ when cultured with CD19-presenting CHO cells but not with CHO cells, while control T cells did not release IFN-γ in any condition (Figure 8, left). In another set of experiments, the transduced T cells were co-cultured with CD19 luciferase CHO cells. As shown in Figure 8 (right), co-culture with anti-CD19 CAR-T cells killed the CD19 luciferase CHO cells, evidenced by a 95% drop in luciferase activity.

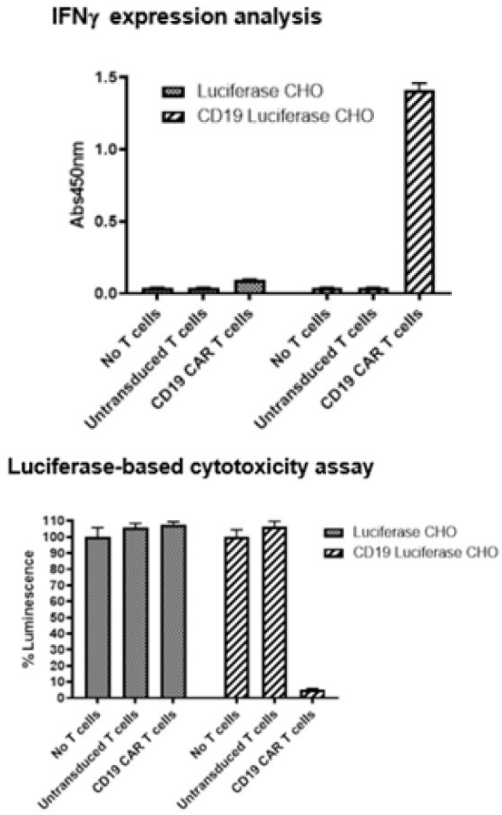


Figure 8: Functional validation of lentivirus-transduced CAR T cells. Approximately 15,000 CD4+/CD8+ activated T cells were transduced with anti-CD19 CAR lentivirus (MOI of 40) in the presence of 5 µg/mL polybrene using spinoculation. Transduced T cells were expanded and 12 days later they were co-cultured with luciferase CHO cells (#79725) or with CD19-expressing luciferase CHO cells (#79714) for 24 hours at a ratio of 1 to 20. Non-transduced T cells were used as negative control. Left panel: The culture medium was collected and assayed using the IFN-γ Colorimetric ELISA Detection Kit (#79777). Right panel: Luciferase activity was quantified using the ONE-Step™ luciferase assay system (#60690).

Conclusion

In addition to the lentivirus and antigen-presenting cell lines discussed here, our portfolio of CD19-related products [CD19] includes affinity purified recombinant proteins (labeled or unlabeled), as well as mono-specific and bi-specific antibodies. Cytokine assay kits and reporter cell systems allow for the functional validation of anti-CD19 CAR-T cells using biologically relevant cellular models.

Bibliography

1. Depoil, D., Fleire, S., Treanor, B.*et al*. CD19 is essential for B cell activation by promoting B cell receptor-antigen microcluster formation in response to membrane-bound ligand. *Nat Immunol.* (2008) 9: 63-72. PMID: 18059271. https://pubmed.ncbi.nlm.nih.gov/18059271/
2. van Zelm MC, Reisli I, van der Burg M.*et al*. An antibody-deficiency syndrome due to mutations in the CD19 gene. *N Engl JMed.* (2006) 354: 1901-1912. PMID: 16672701. https://pubmed.ncbi.nlm.nih.gov/16672701/
3. Carter RH, Fearon DT. CD19: lowering the threshold for antigen receptor stimulation of B lymphocytes. *Science* (1992) 256: 105-107. PMID: 1373518. https://pubmed.ncbi.nlm.nih.gov/1373518/
4. Scheuermann RH, Racila E. CD19 antigen in leukemia and lymphoma diagnosis and immunotherapy. *Leuk Lymphoma* (1995) 18: 385-397. PMID: 8528044. https://pubmed.ncbi.nlm.nih.gov/8528044/
5. Goebeler ME, Bargou R. Blinatumomab: a CD19/CD3 bispecific T cell engager (BiTE) with unique anti-tumor efficacy. *Leuk Lymphoma* (2016) 57: 1021-1032. PMID: 27050240. https://pubmed.ncbi.nlm.nih.gov/27050240/
6. Braendstrup P, Levine BL, Ruella M. The long road to the first FDA-approved gene therapy: chimeric antigen receptor T cells targeting CD19. *Cytotherapy* (2020) 22: 57-69. PMID: 32014447. https://pubmed.ncbi.nlm.nih.gov/32014447/

The Rise of CAR-NK Cell Therapy

Rise tied to its potential to be the most effective, safest, and reproducible treatment.

Adoptive cellular immunotherapy is a rising star, having racked up impressive wins against many hematological cancers in the past decade in clinical trials. Among its greatest successes are chimeric antigen receptor (CAR)-T cells. Currently more than 500 CAR-T trials are being conducted world-wide, resulting in five FDA-approved therapeutics against two different therapeutic targets on B lymphocytes.

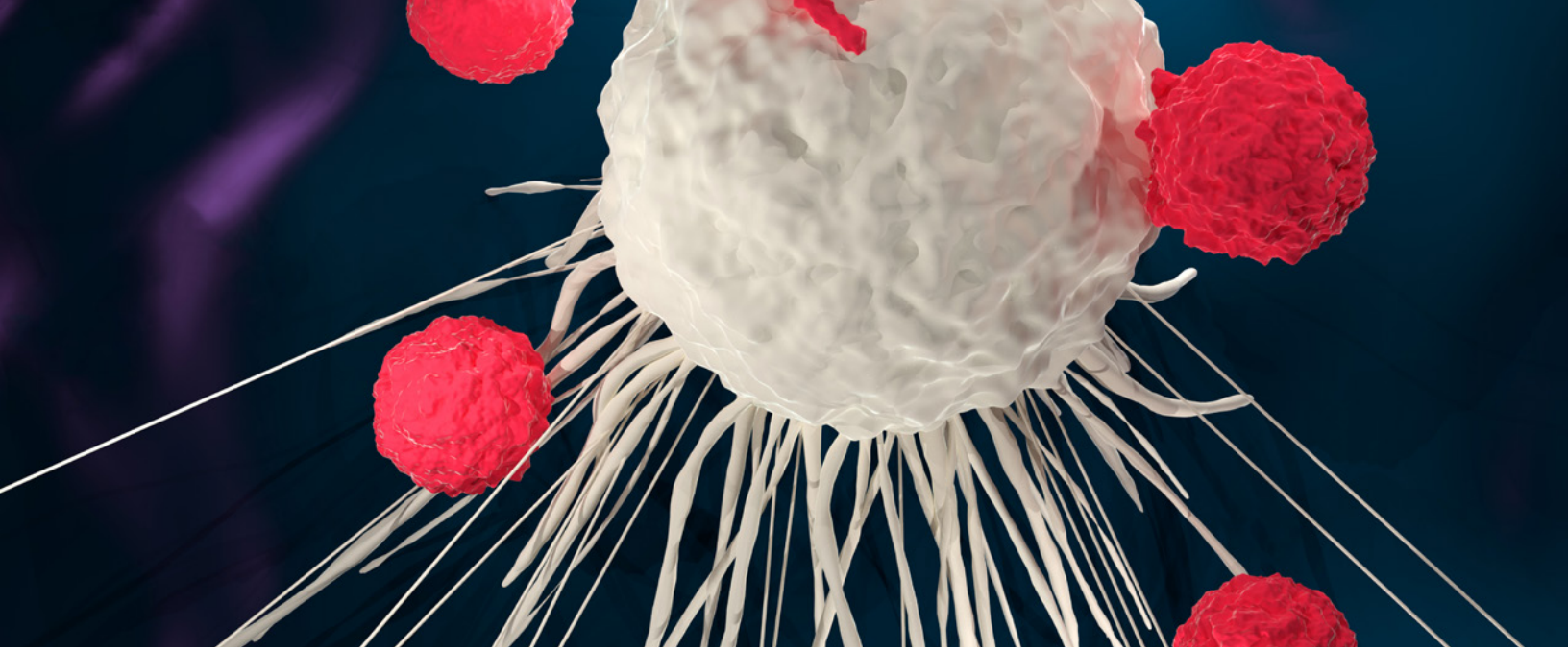
These manufactured drug products typically consist of an extracellular domain derived from an antigen-specific antibody linked to an intracellular effector domain, which, when the CAR encounters its cognate antigen, activates the T cell's cytotoxic programming. CARs have become more sophisticated, with the third generation containing three distinct activation signals. Fourth generation CARs may boast safety (suicide) switches or other modifications to improve persistence, enhance tumor activity, prevent antigen escape, or even control CAR expression.

While most CAR research has focused on T cells, CARs have also been introduced into other immune effector cells, such as γδ T cells, macrophages, natural killer (NK) cells, and natural killer T (NKT) cells. Several of these CAR constructs have demonstrated anticancer effects, but CAR-NK cells—with enhanced safety profiles, multiple cytotoxic mechanisms, and the possibility of an off-the-shelf product—have gained the most traction, with at least 19 trials currently being conducted worldwide and many more preclinical studies in progress.

Meet NK cells

NK cells have certain properties that make them well-suited as anticancer effector cells. NK cells are in many ways akin to CD8+ "killer" T cells, killing their targets with similar cytotoxic mechanisms. But unlike T cells, NK cells do not rely on somatically rearranged antigen-specific receptors. Rather, these innate immune effector cells—identified by their ability to non-specifically kill tumor cells—become activated to kill by integrating signals from a bevy of both activating and inhibitory, germline-encoded, receptors.

CD8+ T cells rely on their T cell receptors (TCRs) to recognize antigen presented in the context of human leukocyte antigen (HLA) expressed on the surface of a target cell. Tumors and virally infected or otherwise stressed cells will often downregulate the HLA, making them less visible to the T cell. Converse-



ly, the absence of HLA, is also a sign of "non-self," can help to trigger an NK cell's cytotoxic response.

CD19, a biomarker for B lymphocytes, is often utilized as a target for leukemia immunotherapies. NK cells express CD16, an Fcgamma receptor, that can bind to the Fc region of antibodies. Antibodies to CD19 bound to the target B cell can bind to the CD16, triggering the NK cell to lyse the target cell in a process termed antibody-dependent cell-mediated cytotoxicity (ADCC).

Activated NK cells also secrete inflammatory cytokines and chemokines, enlisting the aid of other innate and adaptive immune cells.

Off the shelf

Features of unmodified NK cells are shared by CAR-NK cells, but with the added benefit of an antigen-specific receptor that can trigger activation—or other responses engineered into the CAR such as secreting molecules designed to reprogram the tumor microenvironment. CAR-NK therapy offers several potential advantages over CAR-T therapy. Hosts may be seen as foreign to the CAR-T cells' endogenous TCRs, making graft versus host disease (GvHD) a potential risk with a CAR-T infusion. Using autologous cells derived from the host can avoid this issue, but this means that every CAR-T therapy would be unique, and therefore time-consuming, expensive, and only available to patients with sufficient numbers of healthy T cells. Even autologous CAR-T cells have the potential to induce cytokine release syndrome (CRS) and severe neurotoxicity. Activated NKs (and CAR-NKs) predominantly secrete a different spectrum of cytokines not associated with CRS and severe neurotoxicity.

Potential advantages

CAR-NK therapy offers several potential advantages over CAR-T therapy. Many features of unmodified NK cells are shared by CAR-NK cells, but with the added benefit of an antigen-specific receptor that can trigger activation. Other responses can also be engineered into the CAR, such as secreting molecules designed to reprogram the tumor microenvironment.

The presence of HLA on the surface of the host cells makes NK cells less likely to become triggered at all, reducing the threats of GvHD and severe neurotoxicity. In fact, these toxicities have yet to be seen in either unmodified or engineered NK therapies.

Another advantage of NK cells "standing down" in the presence of cell-surface HLA is that manufacturing allogeneic CAR-NK therapies becomes more feasible. Such products could be produced in bulk and supplied off-the-shelf, which would improve consistency, decrease costs, and make them available to patients much sooner.

Where from?

Only 5–30% of the leukocytes in a healthy human are NK cells. Large numbers of NK cells are required due to the need for extensive *ex vivo* handling prior to CAR-NK cell infusion. However, expanding NK cells *in vitro* can be challenging due to the short life span and limited proliferative capacity of native NK. Since cancer patient's (autologous) NK cells are often compromised—and are silenced by the presence of self-MHC—allogeneic sources from healthy donors are typically used. These may be from peripheral or umbilical cord blood, derived from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) or stem cell lines, or from NK-92 cells (currently the only NK cell line approved for clinical trials).

The main advantage of peripheral blood is that the NKs cells are already mature. Transduction efficiency is usually low, but this can be circumvented using proper culture conditions. The risk of host versus graft disease (HvGD) requires careful depletion of T cells from peripheral blood. Cord blood-derived NK cells have a high proliferative capacity. Their haplotype can be determined at collection, allowing a cell bank can be generated and matches can be selected. The disadvantage of both peripheral blood- and cord blood-derived cells as sources of CAR-NKs is that heterogeneity among donors can result in an inconsistent product.

Stem cell-derived NKs, on the other hand, can be homologous and result in a uniform product. Unfortunately stem cell-derived NKs take 3–5 weeks to produce, may require a continuous infusion of cytokines to continue to proliferate *in vivo*, and have the potential for malignant transformation.

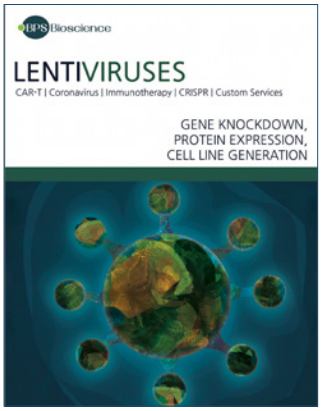
This is perhaps the main drawback of the NK-92 cell lines as well. While it can be quick to generate a large number of cells that are relatively easy to genetically manipulate, cells derived for this cell line must be irradiated prior to infusion, preventing their proliferation and limiting their lifespan *in vivo*.

The phenomenal successes of CAR-T therapies—especially when coupled with other modalities such as immune checkpoint inhibitors—have enhanced interest in passive immunotherapies in general, and engineered immune effector cell therapy in particular. CAR-NK-based therapies will be at the forefront as researchers continue to seek out and refine the most effective, safest, and most reproducible treatments for previously intractable cancers.

Resources

CRISPR-Cas9: Screening, Knock-out, Knock-in, Cell-Line Development

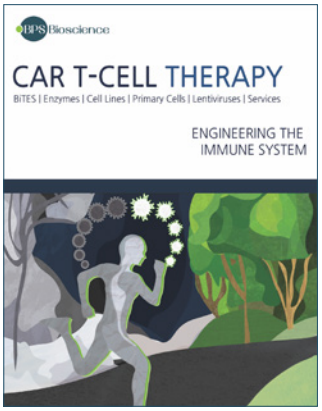
Innovative Products & Services to Advance Gene Editing Research



Lentiviruses: CAR-T, Coronavirus, Immunotherapy, CRISPR, Custom Services

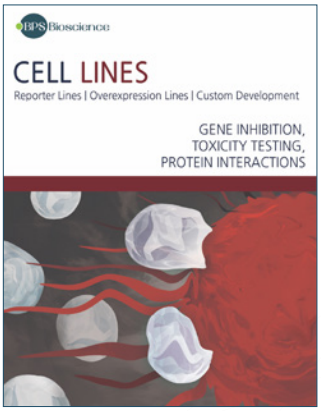
Gene Knockdown, Protein Expression, Cell Line Generation

Improving Immunotherapy Through Immune Checkpoint Assays, CAR-T Target Display, and Effector Function Optimization



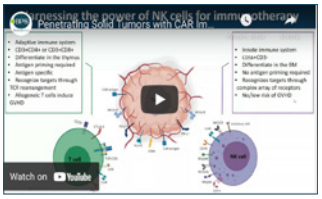
CAR T Cell Therapy Booklet

Engineering the Immune System



Cell Lines Booklet

Gene Inhibition, Toxicity Testing, Protein Interactions



Webinar: Lentiviruses: Versatile Tools for Cell Biology