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- Trockeneiszuschlag
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Description

Anti-CD19 CAR-T Cells (eGFP) were produced by high-titer lentiviral transduction of human primary $CD4^+$ and $CD8^+$ T cells with SIN Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ eGFP) (#78775). These ready-to-use CAR (Chimeric Antigen Receptor)-T cells express an anti-CD19 CAR consisting of the ScFv (Single chain fragment variable) of anti-CD19 (clone FMC63) linked to a 2nd generation CAR containing CD8 hinge and transmembrane domains, and the 4-1BB and CD3ζ signaling domains (Figure 1). The presence of eGFP (enhanced green fluorescent protein) allows for easy fluorescent detection of CAR-expressing cells.

These CAR-T cells have been validated by flow cytometry (to determine the CAR expression) and co-culture cytotoxicity assays.

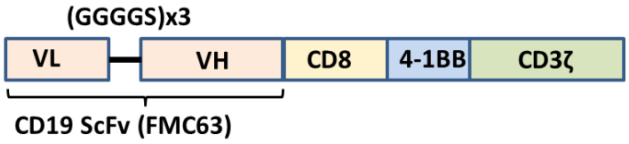

Figure 1. Construct diagram showing components of the anti-CD19 CAR expressed in Anti-CD19 CAR-T Cells (eGFP).

Background

CD19 (Cluster of Differentiation 19), also known as B-lymphocyte surface antigen B4, or CVID3, is a glycoprotein expressed at the surface of B lymphocytes through most phases of B cell maturation. It is strictly required for B cell terminal differentiation. Mutations in the CD19 gene cause severe immune-deficiency disorders associated with impaired antibody production such as CVID3 (common variable immuno-deficiency 3). The majority of B cell malignancies express normal to high levels of CD19, making it a nearly ideal target for cancer immunotherapy. Blinatumomab, a CD19/CD3 bi-specific T cell engager (BiTE) has been approved for relapsed/refractory B precursor ALL (Acute lymphoblastic leukemia). In addition, CD19 was the target of the first approved CAR-T cell therapy. Studies of CD19 function and expression profiles will continue to broaden our knowledge and support broader applications in cancer therapy.

Application

- Use as positive control in anti-CD19 CAR-T cell development.
- Screen modulators of anti-CD19 CAR-T driven cytotoxicity.
- Design and optimize co-culture cytotoxicity assays for anti-CD19 specific CAR-T cell evaluation.

Biosafety

The anti-CD19 CAR-T cells are produced with the third generation SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal and are not present in the lentivirus particle.

Materials Provided

Components	Format
One vial of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of CryoStor® CS10 (Stemcell Technologies #100-1061)

Mycoplasma Testing

The cells have been screened to confirm the absence of Mycoplasma species.

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Materials Required but Not Supplied

These materials are not supplied with the CAR-T cells but are necessary for cell culture and for the cellular assays described below. BPS Bioscience's reagents are validated and optimized for use with these cells and are highly recommended for best results.

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Human Interleukin-2 Recombinant	BPS Bioscience #90184
Human CD3/CD28/CD2 T Cell Activator	STEMCELL Technologies #10970
PE-Labeled Monoclonal Anti-FMC63 Antibody, Mouse IgG1	Acrobiosystems # FM3-HPY53-25tests
Firefly Luciferase Raji Cell Line	BPS Bioscience #78622
Firefly Luciferase CD19 Knockout Raji Cell Line	BPS Bioscience #82167
Untransduced T Cells (Negative Control for CAR-T cells)	BPS Bioscience #78170
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Recommended Anti-CD19 CAR-T Cell Medium: TCellM™ (#78753) supplemented with 10 ng/ml Interleukin-2 (#90184).

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Anti-CD19 CAR-T Cell Medium.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Anti-CD19 CAR-T Cell Medium.

3. Transfer the resuspended cells to a T25 flask.

4. If desired, activate the cells using Human CD3/CD28/CD2 T Cell Activator, following the manufacturer's recommendations, at 37°C with 5% CO_2 for 24-48 hours.

Cell Culture

1. Centrifuge the cells gently at 300 *x g* for 5 minutes.

2. Resuspend in fresh Anti-CD19 CAR-T Cell Medium.

3. Continue to culture the cells at 37°C with 5% CO_2.

4. Do not allow the cell density to exceed 2.0 x 10^6 cells/ml. Transfer the cells in larger culture vessels and add fresh medium when the density reaches 2.0 x 10^6 cells/ml.

It is recommended to activate the anti-CD19 CAR-T cells for expansion after thawing, following the activation reagents manufacturer's intructions. Since these are primary cells, the extent of expansion is not predictable. Perform the cytotoxicity assay as soon as possible to avoid T Cell exhaustion. Anti-CD19 CAR-T Cells (eGFP) should not be in culture for more than 8-10 days. It is not recommended to freeze the cells again.

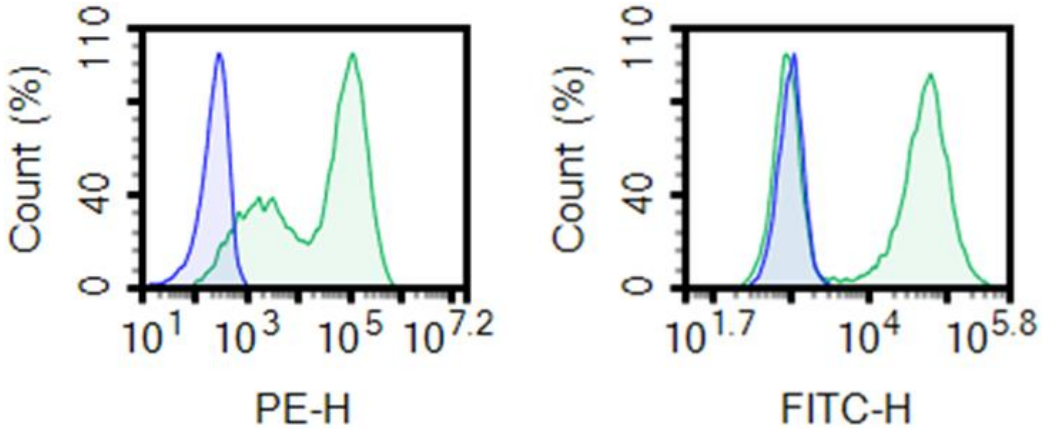

Figure 2. Assessment of expression of anti-CD19 CAR and eGFP in Anti-CD19 CAR-T Cells (eGFP) by flow cytometry.
Anti-CD19 CAR-T cells were activated for 24 hours and cultured for 4 days. ~50,000 cells of Anti-CD19 CAR-T cells (green) and Untransduced T cells (#78170) (blue) were stained with PE-Labeled Monoclonal Anti-FMC63 Antibody, Mouse IgG1 (Acrobiosystems #FM3-HPY53-25tests). Anti-CD19 CAR (left) and eGFP (right) expression was analyzed by flow cytometry. The y axis represents the % of cells, while the x axis indicates PE-intensity and GFP-intensity, respectively.

Functional Validation

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- The following experiment is an example of a co-culture assay used to evaluate the cytotoxicity of anti-CD19 CAR-T Cells (eGFP) against Firefly Luciferase Raji Cell Line as the target cells.
- All conditions should be performed in triplicate.
- The assay should include "Luminescence Background", "No T Cell Control" (contain Luciferase Raji cells but no T cells) and "Test Condition" wells.
- We recommend using Untransduced T Cells as negative control.
- We recommend using Firefly Luciferase CD19 Knockout Raji Cell Line as a control for Firefly Luciferase Raji Cell Line.

Cytotoxicity assay using Firefly Luciferase Raji Cell Line as the target cells.

Day 1:

1. Thaw T cells, activate, and expand according to the protocol described in the "**Cell Culture Protocol"** section.

2. Seed Firefly Luciferase Raji cells (express endogenous CD19) at 5,000 cells/well in 50 µl of Thaw Medium 2 in a 96-well white, clear bottom tissue culture plate. Leave a few empty wells as "Luminescence Background" wells.

3. Centrifuge T cells at 300 *x g* for 5 minutes and resuspended the cell pellet in fresh Anti-CD19 CAR-T Cell Medium.

4. Determine the desired Effector to Target ratio (E:T) and prepare appropriate cell suspensions (50 µl/well).

5. Carefully pipet 50 µl of T cell suspension into the appropriate "Test Condition" wells, containing the Firefly Luciferase Raji cell line.

6. Add 50 µl of Anti-CD19 CAR-T Cell Medium to the "No T Cell Control" wells.

7. Add 100 µl of Anti-CD19 CAR-T Cell Medium to the "Background Luminescence" wells.

8. Incubate the plates at 37°C with 5% CO_2 for 24 hours.

Day 2:

1. Add 100 µl of ONE-Step™ Luciferase assay reagent to each well.

2. Incubate at room temperature for ~15 to 30 minutes.

3. Measure luminescence using a luminometer.

Data Analysis: the average background luminescence was subtracted from the luminescence reading of all wells. The luciferase activity of Firefly Luciferase Raji Cell Line was set as 100%. The % Luminescence was calculated as: (luminescence of co-culture well)/ (luminescence from the "No T Cell Control" well).

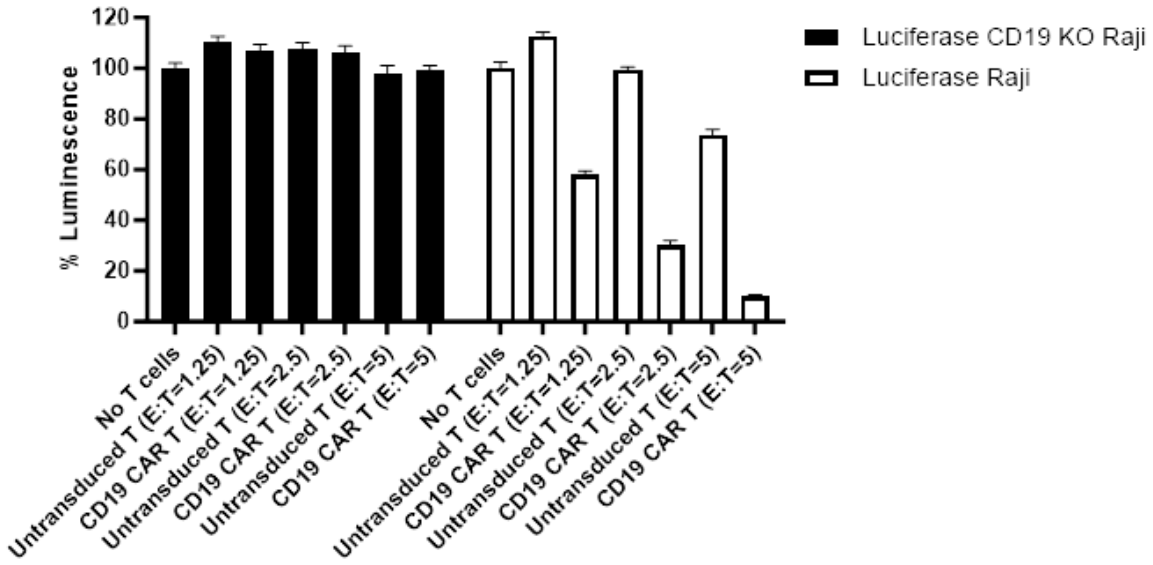

Figure 3. Luciferase-based cytotoxicity assay using Anti-CD19 CAR-T Cells (eGFP) co-cultured with Firefly Luciferase Raji Cell Line as the target cells.
Anti-CD19 CAR-T cells and control Untransduced T cells (#78170) were thawed, activated for 24 hours, and expanded for 4 days. The T cells (effector cells) were then co-cultured with Firefly Luciferase Raji cells (target cells) for 24 hours at the indicated effector:target (E:T) ratio. The lysis of target cells was determined by measuring luciferase activity with ONE-Step™ Luciferase Assay System. Untransduced T cells (#78170) and Firefly Luciferase CD19 Knockout Raji cells (#82167) were run in parallel as negative controls.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Depoil D., *et al.*, 2008 *Nat Immunol.* 9: 63-72.
Van Zelm M.C., *et al.*, 2006 *N Engl J Med.* 354: 1901-1912.
Goebeler M.E. and Bargou R., 2016 *Leuk Lymphoma* 57: 1021-1032.
Braendstrup P., *et al.*, 2020 *Cytotherapy* 22: 57-69.

Warnings

- Donors have been screened and determined negative for:
- Hepatitis B (anti-HBc EIA, HBsAg EIA)
- Hepatitis C (anti-HCV EIA)
- Human Immunodeficiency Virus (HIV-1/HIV-2 plus O)
- Human T-Lymphotropic Virus (HTLV-I/II)
- HIV-1/HCV/HBV
- West Nile Virus
- Trypanasoma cruzi

Note: Testing cannot guarantee that any sample is completely virus-free. These cells should be treated as potentially infectious and appropriate Biological Safety Level 2 (BSL-2) precautions should be used.

Troubleshooting Guide
Visit Cell Line FAQs for more information.
For further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Anti-CD19 CAR-T Cells	78171	1 vial
Firefly Luciferase K562 Cell Line	78621	2 vials
Firefly Luciferase-CHO Recombinant Cell Line	79725	2 vials
CD19/Firefly Luciferase-CHO Recombinant Cell Line	79714	2 vials
Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ)	78600	50 µl
Anti-BCMA CAR Lentivirus (Clone C11D5.3 ScFv-CD8-CD28-CD3ζ)	78655	50 µl

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