Description

The anti-CD19 CAR-T cells are produced by high-titer lentiviral transduction of human primary CD4+CD8+ T cells using the anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ; SIN Vector, BPS Bioscience, #78601). These ready-to use CAR-T cells express an anti-CD19 CAR consisting the ScFv of CD19 (clone FMC63) linked to a 2nd generation CAR (Chimeric Antigen Receptor) containing CD8 hinge and transmembrane domains, and the 4-1BB and CD3ζ signaling domains (Figure 1).

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

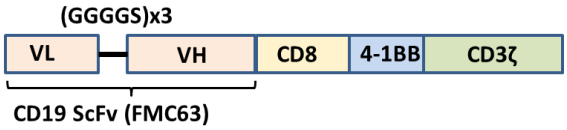

Figure 1: Construct diagram showing components of the anti-CD19 CAR expressed in anti-CD19 CAR-T cells.

Background

CD19 (also known as Cluster of Differentiation 19, 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 syndromes 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, which is 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.

Application

1. Positive control for anti-CD19 CAR-T cells
2. Screening inhibitors or activators of anti-CD19 CAR-T cytotoxicity
3. Design and optimize co-culture cytotoxicity assay

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 2 x 10^6 cells in 1 ml of CryoStor® CS10)

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
Human Interleukin-2	BPS Bioscience #90184
Human CD3/CD28/CD2 T Cell Activator	Stemcell technologies #10970
CD19/Firefly Luciferase CHO Cell Line	BPS Bioscience #79714
Firefly Luciferase CHO Cell Line	BPS Bioscience #79725
Firefly Luciferase Raji Cell Line	BPS Bioscience #78622
Firefly Luciferase K562 Cell Line	BPS Bioscience #78621
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: StemSpan SFEM (Stemcell Technologies #09650) supplemented with 10% heat-inactivated FBS (Life Technologies #10082147), 1% Penicillin/Streptomycin (Hyclone #SV30010.01), plus 10 ng/ml IL-2 (BPS Bioscience #90184)

Cell Thawing and Culture Protocol:

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 T cell growth medium.

   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 T cell growth medium.
3. Transfer the resuspended cells to a T25 flask and add T cell activation reagents to the cells. Activate the cells at 37°C with 5% CO_2 for 24 – 48 hours.
4. Centrifuge the cells gently at 300g for 5 min and resuspend in fresh **CAR-T** cell medium. Continue to culture the cells at 37°C with 5% CO_2. 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. Since these are primary cells, the extent of expansion is not predictable. Perform the cytotoxicity assay as soon as possible to avoid exhaustion. The anti-CD19 CAR-T cells should not be in culture for more than 8-10 days. It is not recommended to freeze the cells again once they have been activated and expanded.

Validation

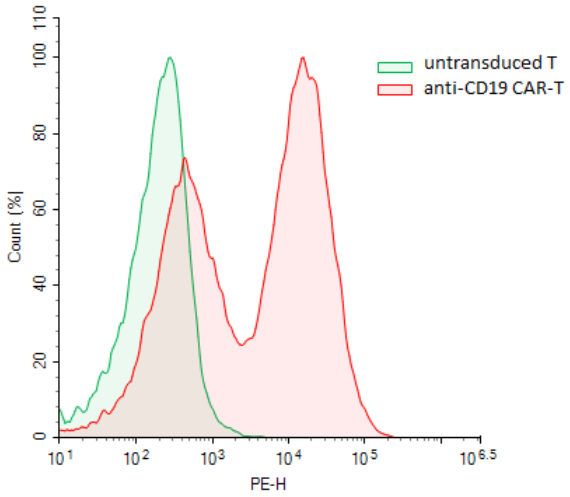

Figure 2: Expression of anti-CD19 CAR in anti-CD19 CAR-T cells. Anti-CD19 CAR-T cells were thawed and activated for 48 hours. Anti-CD19 CAR-T cells were then expanded for another 4 days, and ~50,000 cells were analyzed by flow cytometry using PE-anti-FMC63 ScFv (Acrobiosystems, #FM3-HPY53-25tests).

Experimental Methods and Results

The following experiments are two examples of co-culture assays used to evaluate the cytotoxicity of anti-CD19 CAR-T using **A)** CD19/Firefly Luciferase CHO Cell Line or **B)** Firefly Luciferase Raji Cell Line as the target cells.

A. Cytotoxicity assay using CD19/Firefly Luciferase CHO Cell Line as the target cells

1. T cells were thawed, activated, and expanded according to the protocol in the "**Cell Thawing and Culture Protocol"** Section.

2. Target cells "CD19/Firefly Luciferase CHO Cell Line" (BPS Bioscience #79714) and negative control "Luciferase CHO Cell Line" (BPS Bioscience #79725) were seeded in 50 µl of Thaw Medium 3 (BPS Bioscience #60186) at 500 cells/well in a 96-well white, clear bottom tissue culture plate.

    a. Extra wells of CD19/Firefly Luciferase CHO Cell Line or Firefly Luciferase CHO Cell Line were included for the "no T cell" control.

    b. Extra wells of "medium only" were included to determine background luminescence.

3. Anti-CD19 CAR-T cells were centrifuged gently (300g x 5 min) and resuspended in fresh T cell growth medium. The T cells were carefully pipetted into wells containing the CHO cells, at the desired effector:target (E:T) cell ratio in 50 µl of volume. For "no T cells" wells and "medium only" wells, 50 µl of fresh T cell medium was added. The total volume of each well was 100 µl. The plates were incubated at 37°C with 5% CO_2 for 24 hours.

    Note: No overnight attachment was needed for the CHO cells. T cells were added into the wells right after the CHO cells were seeded.

4. After 24 hours: Each well was pipetted gently up and down 3 to 4 times. The medium containing the non-attached cells was transferred to another plate.

Luciferase assay was performed using the CHO cells remaining on the plate whereas the collected medium/nonattached cells can be subjected to cytokine release analysis. If the cytokine release analysis is not performed immediately, the collected medium can be stored at -20°C.

Luciferase assay: The ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was prepared following the recommended protocol. 50 µl of ONE-Step™ Luciferase assay reagent was added to each well, including empty wells (that had contained medium only) to determine the background luminescence. The plate was incubated at room temperature for ~15 to 30 minutes before measuring luminescence using a luminometer.

Data Analysis: The average background luminescence was subtracted from the luminescence reading of all wells. The luciferase activity of Luciferase CHO cells or CD19/Luciferase CHO cells was set as 100%. The % Luminescence was calculated as luminescence of co-culture well divided by luminescence from the "no T cells" well (Luciferase CHO or CD19/Luciferase CHO cells only). Results are shown in Figure 3.

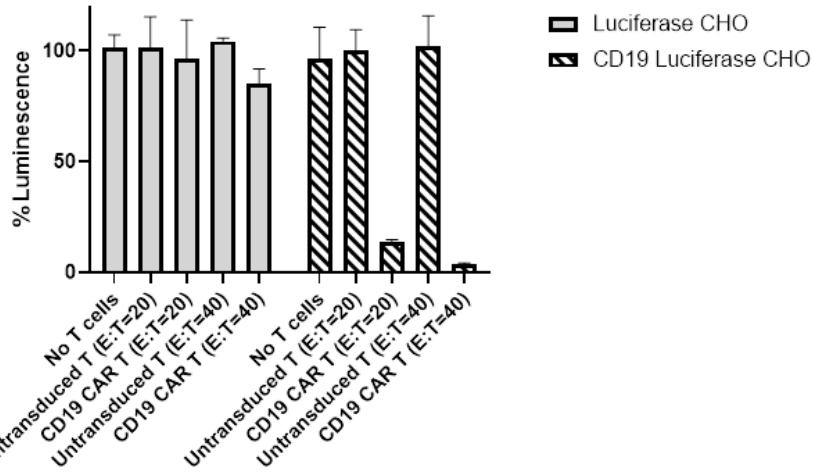

Figure 3: Luciferase-based cytotoxicity assay using CD19-Luciferase CHO cells as the target cells. Anti-CD19 CAR-T cells were thawed, activated for 48 hours, and expanded for another 4 days. The anti-CD19 CAR-T cells (effector) were then co-cultured with CD19/Luciferase CHO cells (target) for 24 hours at the indicated effector:target (E:T) ratio. The lysis of target cells was determined by measuring Luciferase activity using the OneStep™ Luciferase reagent (BPS Bioscience #60690). The anti-CD19 CAR-T cells showed specific toxicity towards CD19/Luciferase CHO cells. The assay was performed in parallel with untransduced T cells (BPS Bioscience #78170) and Luciferase CHO cells (BPS Bioscience #79725) as negative controls.

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

1. T cells were thawed, activated, and expanded according to the protocol in the "**Cell Thawing and Culture Protocol"** Section.

2. Target cells "Firefly Luciferase Raji Cell Line" (BPS Bioscience #78622) that endogenously express CD19 or negative control cells "Firefly Luciferase K562 Cell Line" (BPS Bioscience #78621), which do not express CD19, were seeded in 50 µl of Thaw Medium 2 (BPS Bioscience #60184) at 5,000 cells/well in a 96-well white, clear bottom tissue culture plate.

a. Extra wells of Firefly Luciferase Raji cells or Firefly Luciferase K562 cells were included for the "no T cell" control wells

b. Extra wells of "medium only" were included to determine background luminescence.

3. Anti-CD19 CAR-T cells were centrifuged gently (300g x 5min) and resuspended in fresh T cell growth medium. T cells were carefully pipetted into wells containing the CHO cells, at the desired effector:target (E:T) cell ratio in 50 µl of volume. For "no T cells" wells and "medium only" wells, 50 µl of fresh T cell medium was added. The total volume of each well was 100 µl. The plates were incubated at 37°C with 5% CO_2 for 24 hours.

4. After 24 hours, the ONE-Step™ Luciferase reagent (BPS Bioscience, #60690) was prepared following the recommended protocol. 100 µl of ONE-Step™ Luciferase assay reagent was added to each well and incubated at room temperature for ~15 to 30 minutes before measuring 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 or Firefly Luciferase K562 Cell Line was set as 100%. The % Luminescence was calculated as luminescence of co-culture well divided by luminescence from the "no T cells" well (Luciferase Raji or Luciferase K562 cells only). Results are shown in Figure 4.

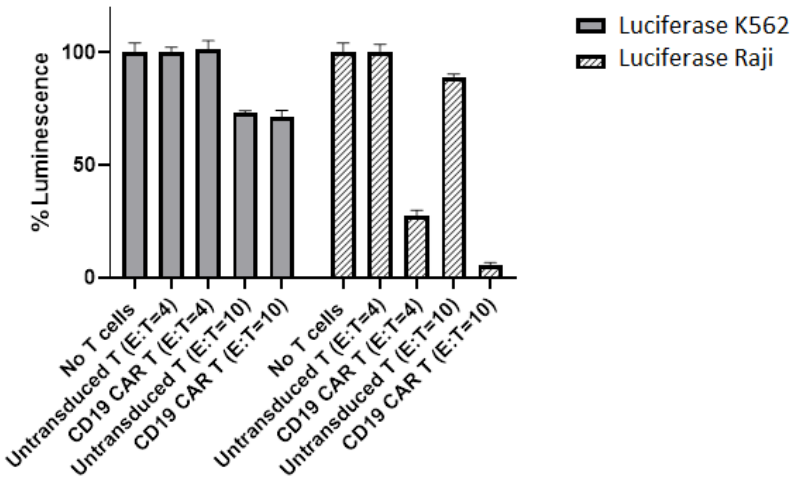

Figure 4: Luciferase-based cytotoxicity assay using Firefly Luciferase-Raji Recombinant Cell Line as the target cells. Anti-CD19 CAR-T cells and control untransduced T cells (BPS Bioscience #78170) were thawed, activated for 48 hours, and expanded for another 4 days. The anti-CD19 CAR-T cells (effector) were then co-cultured with Firefly Luciferase Raji Cells for 24 hours at the indicated effector:target ratio. The lysis of target cells was determined by measuring Luciferase activity. The assay was performed in parallel with untransduced T cells and Firefly Luciferase-K562 Cells as negative controls.

References

1. Depoil D, *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.
2. van Zelm MC, *et al*. An antibody-deficiency syndrome due to mutations in the CD19 gene. *N Engl J Med.* 2006; **354:** 1901-1912.
3. Goebeler ME, Bargou R. Blinatumomab: a CD19/CD3 bispecific T cell engager (BiTE) with unique anti-tumor efficacy. *Leuk Lymphoma* 2016; **57**: 1021-1032.
4. Braendstrup P, *et al.* The long road to the first FDA-approved gene therapy: chimeric antigen receptor T cells targeting CD19. *Cytotherapy* 2020; **22**: 57-69.

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Troubleshooting Guide

For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Untransduced T Cells	78170	1 vial
Firefly Luciferase Raji Cell Line	78622	2 vials
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)	78603	50 µl
Anti-CD20 CAR Lentivirus (Clone Leu-16 ScFv-CD8-4-1BB-CD3ζ)	78606	50 µl
Anti-CD22 CAR Lentivirus (Clone m971 ScFv-CD8-4-1BB-CD3ζ)	78608	50 µl
Anti-CD19/CD22 Bispecific CAR Lentivirus (Clones FMC63/m971 ScFv-CD8-4-1BB-CD3ζ)	78609	50 µl