Description

The anti-BCMA CAR-T cells are produced by high-titer lentiviral transduction of human primary CD4+CD8+ T cells using the anti-BCMA CAR Lentivirus (BPS Bioscience #78655). These ready-to-use CAR-T cells express an anti-BCMA CAR consisting of the ScFv of BCMA (clone C11D5.3) 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.

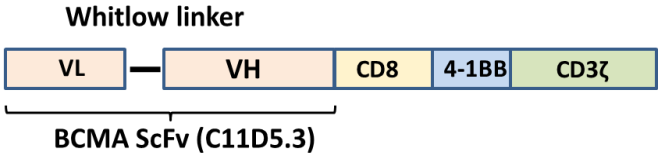

Figure 1: Construct diagram showing components of the anti-BCMA CAR expressed in anti-BCMA CAR-T cells.

Background

B-cell maturation antigen (BCMA), also known as CD269 or tumor necrosis factor receptor superfamily member 17 (TNFRSF17), is a cell surface receptor of the TNF receptor superfamily that recognizes B-cell activating factor (BAFF). BCMA is preferentially expressed in mature B lymphocytes and on Multiple Myeloma (MM) cells. BCMA is a highly attractive target antigen for immunotherapy because of its restricted expression in nonmalignant tissue but almost universal expression on MM cells. So far FDA has approved two BCMA CAR-T therapies for the treatment of multiple myeloma.

Application(s)

- Positive control for anti-BCMA CAR-T cells
- Screen inhibitors or activators of anti-BCMA CAR-T cytotoxicity
- Design and optimize co-culture cytotoxicity assay

Biosafety

The anti-BCMA 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
BCMA/Firefly Luciferase CHO Cell Line	BPS Bioscience #79724
Firefly Luciferase CHO Cell Line	BPS Bioscience #79725
Firefly Luciferase RPMI8226 Cell Line	BPS Bioscience #79834
Untransduced T Cells (Negative Control for CAR-T cells)	BPS Bioscience #78170
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Recommended anti-BCMA 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. 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.

Perform the cytotoxicity assay as soon as possible to avoid exhaustion. Anti-BCMA CAR-T cells may stop proliferation after ~one week in culture. Cells can be activated again for expansion. It is not recommended to freeze the cells again once they have been activated and expanded.

Validation

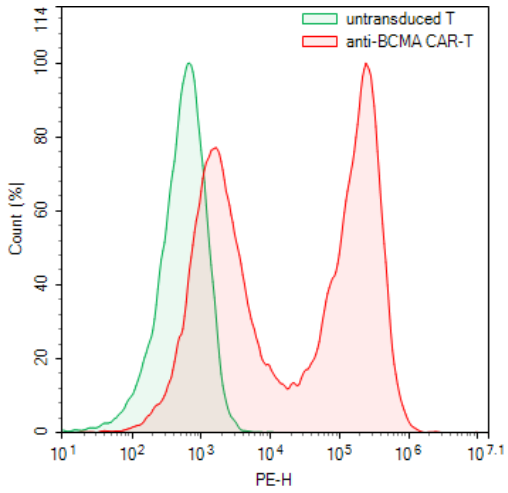

Figure 2: Expression of anti-BCMA CAR in anti-BCMA CAR-T cells.
Anti-BCMA CAR-T cells were thawed and expanded for 4 days and ~50,000 cells were analyzed by flow cytometry using Biotinylated BCMA (BPS Bioscience #79467) and PE-Streptavidin.

Experimental Methods and Results
The following experiments are two examples of co-culture assays used to evaluate the cytotoxicity of anti-BCMA CAR-T using **A)** BCMA/Firefly Luciferase CHO Cell Line or **B)** Firefly Luciferase RPMI 8226 Cell Line as the target cells.

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

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

2. Target cells "BCMA/Firefly Luciferase CHO Cell Line" (BPS Bioscience #79724) 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 BCMA/Firefly Luciferase CHO Cell Line or Firefly Luciferase CHO Cell Line were included for the "no T cells" control.

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

3. Anti-BCMA CAR-T cells were centrifuged gently (300 g 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 BCMA/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 BCMA/Luciferase CHO cells only). Results are shown in Figure 3.

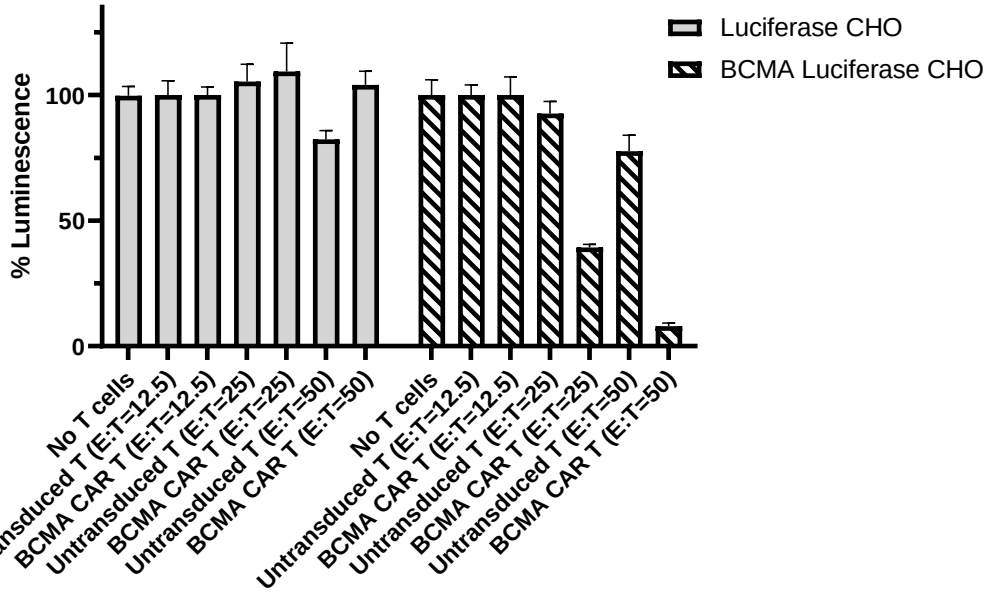

Figure 3: Luciferase-based cytotoxicity assay using BCMA-Luciferase CHO cells as the target cells. Anti-BCMA CAR-T cells were thawed and expanded for 4 days. The anti-BCMA CAR-T cells (effector) were then co-cultured with BCMA/Luciferase CHO cells (target) for 24 hours at the indicated effector:target (E:T) ratio. The lysis of target cells was determined by measuring Luciferase activity using the ONE-Step™ Luciferase reagent (BPS Bioscience #60690). The anti-BCMA CAR-T cells showed specific toxicity towards BCMA/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 RPMI 8226 Cell Line as the target cells

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

2. Target cells "Firefly Luciferase RPMI8226 Cell Line" (BPS Bioscience #79834) that endogenously express BCMA 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 RPMI 8226 cells were included for the "no T cells" control wells

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

3. Anti-BCMA CAR-T cells were centrifuged gently (300 g x 5 min.) and resuspended in fresh T cell growth medium. T cells were carefully pipetted into wells containing the target 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 RPMI 8226 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 RPMI 8226 cells only). Results are shown in Figure 4.

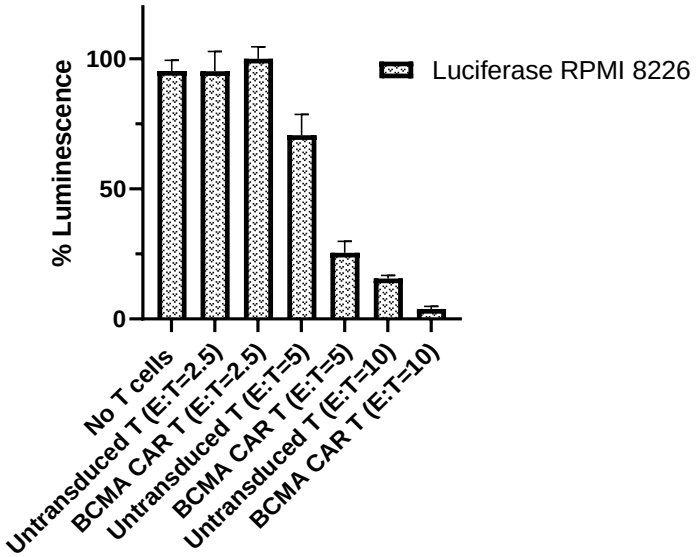

Figure 4: Luciferase-based cytotoxicity assay using Firefly Luciferase-RPMI8226 Recombinant Cell Line as the target cells.
Anti-BCMA CAR-T cells and control untransduced T cells (BPS Bioscience #78170) were thawed and expanded for 4 days. The anti-BCMA CAR-T cells (effector) were then co-cultured with Firefly Luciferase RPMI 8226 Cells for 24 hours at the indicated effector:target ratio. The lysis of target cells was determined by measuring Luciferase activity.

References

1. Ghosh A, *et al.* CAR T cell therapy for multiple myeloma. *Leuk. Lymphoma.* 2017; 6: 1-12
2. Sanchez E, *et al*. The clinical significance of B-cell maturation antigen as a therapeutic target and biomarker. Expert Rev Mol Diagn. 2018; 7: 1-11.
3. Sohail A., *et al*. Emerging immune targets for the treatment of multiple myeloma. *Immunotherapy.* 2018; 10(4): 265-282.
4. Sidaway P., *et al.* Anti-BCMA CAR T cells show promise in MM. *Nat Rev Clin Oncol.* 2016; 13(9): 530.

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 precautions should be used.

Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
Untransduced T cells (Negative Control for CAR-T Cells)	78170	1 vial
Firefly Luciferase RPMI8226 Cell Line	79834	2 vials
Firefly Luciferase CHO Cell Line	79725	2 vials
BCMA/Firefly Luciferase CHO Cell Line	79724	2 vials
Anti-BCMA CAR Lentivirus	78655	50 µL
Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ; SIN Vector)	78601	50 µL
Anti-CD19 CAR-T Cells	78171	1 vial/5 vials