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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The anti-BCMA CAR lentiviruses are replication incompetent, HIV-based, VSV-G-pseudotyped lentiviral particles that are ready to infect almost all types of mammalian cells, including primary and non-dividing cells. These viruses transduce the ScFv (single-chain variable fragment) of anti-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). The lentiviruses also transduce a puromycin selection gene.

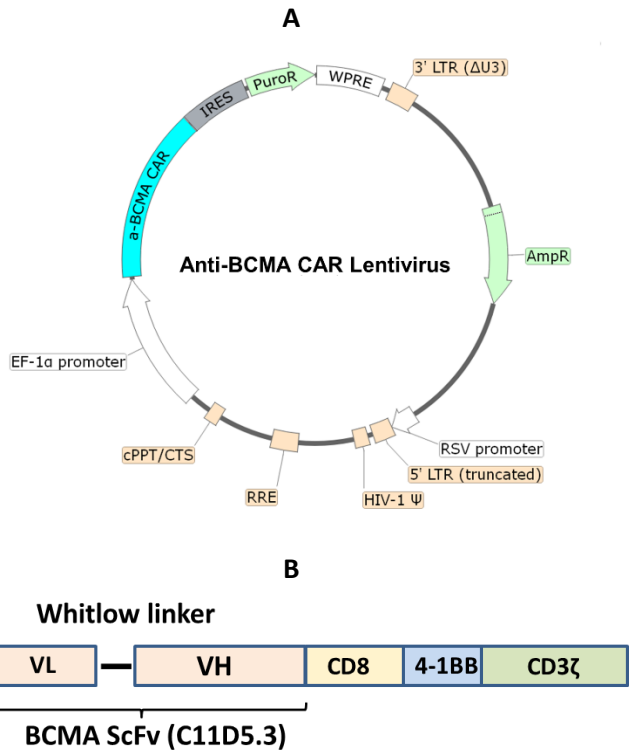

Figure 1. (A) Schematic of the lenti-vector used to generate the anti-BCMA CAR lentivirus with puromycin selection, and (B) Construct diagram showing components of the anti-BCMA CAR

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. To date, the FDA has approved two BCMA CAR-T therapies for the treatment of multiple myeloma.

Application(s)

- Positive control for anti-BCMA CAR evaluation in T cells
- Transduction optimization studies

Formulation

The lentiviruses were produced from HEK293T cells, concentrated and resuspended in DMEM.

Titer

50 µl of anti-BCMA CAR at a titer ≥ 10^8 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

Storage

Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

The lentiviruses are produced with a 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. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Materials Required but Not Supplied

These materials are not supplied with this lentivirus but are necessary to follow the designed protocol. BPS Bioscience media, reagents, and luciferase assay systems are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
PBMC, Frozen	BPS Bioscience #79059
Human Interleukin-2	BPS Bioscience #90184
EasySep™ Human CD4+ T Cell Isolation Kit	Stemcell Technologies #17952
EasySep™ Human CD8+ T Cell Isolation Kit	Stemcell Technologies #17953
Human CD3/CD28/CD2 T Cell Activator	Stemcell Technologies #10970
Biotinylated Human BCMA	Acrobiosystems #BC7-H82F0-25ug
PE-Streptavidin	Biolegend #405203
BCMA / Firefly Luciferase - CHO Recombinant Cell Line	BPS Bioscience #79724
Firefly Luciferase - CHO Recombinant Cell Line	BPS Bioscience #79725
Firefly Luciferase-RPMI 8226 Recombinant Cell Line	BPS Bioscience #79834
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690

Recommended CD4+CD8+ 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)

Experimental Methods and Results:

The following protocol was used to transduce CD4+CD8+ primary T cells with the anti-BCMA CAR Lentivirus. The transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements.

1. Day 0: CD4+ T cells and CD8+ T cells were isolated from previously frozen human PBMC by negative selection, according to manufacturer's instruction. The isolated CD4+ T cells and CD8+ T cells were mixed at a 1:1 ratio and culture the cells using the recommended T cell medium at 1 x 10^6 cells/ml density. The cells were incubated at 37°C with 5% CO_2 overnight.

2. Day 1: T cell activation reagent was added to the cells and incubated at 37°C with 5% CO_2 for 24 - 48 hours.

3. Day 2: The T cells were centrifuged (300 *g* x 5 min) and resuspended in fresh T cell medium at 0.1 - 1 x 10^6 cells/ml; Polybrene (5 µg/ml) was added to the cells.

   The anti-BCMA CAR lentivirus was thawed on ice. Note: Lentiviruses are very sensitive to freeze/thaw cycles. Following the first thaw, prepare small aliquots of virus to limit cycles of freeze/thaw.

Spinoculation:

1) 100 µl of T cells (~10,000-100,000) were distributed into each 1.5 ml Eppendorf tube.

2) The MOI was titrated, starting from 20. The lentivirus was incubated in the hood at room temperature for 10 minutes; the cells/virus were spun gently at 800 x g for 2 hours at 32°C.

3) Using 10,000 cells: 900 µl of fresh T cell medium was added into each well of a 24-well plate. The cells/virus from the spinoculation step were added to the 24-well plate.

4) Using 100,000 cells: 3 ml of fresh T cell medium was added into each well of a 6-well plate. The cells/virus from the spinoculation step were added to the 6-well plate.
   It was not necessary to remove the virus. The cells were incubated at 37°C with 5% CO_2 for ~48-72 hours.

4. Day 5: The expression of the anti-BCMA CAR was estimated by flow cytometry using Biotinylated BCMA and PE-Labeled Streptavidin, as shown in *Figure 2*. The transduced T cells were expanded using the recommended T cell medium.

   Note: Once the transduced cells have proliferated sufficiently to reach the desired cell number required for your experiments, use the cells as soon as possible to minimize cellular exhaustion. It has been observed that the T cells expanded >1000 fold by 11 days post-transduction, using the recommended T cell medium.

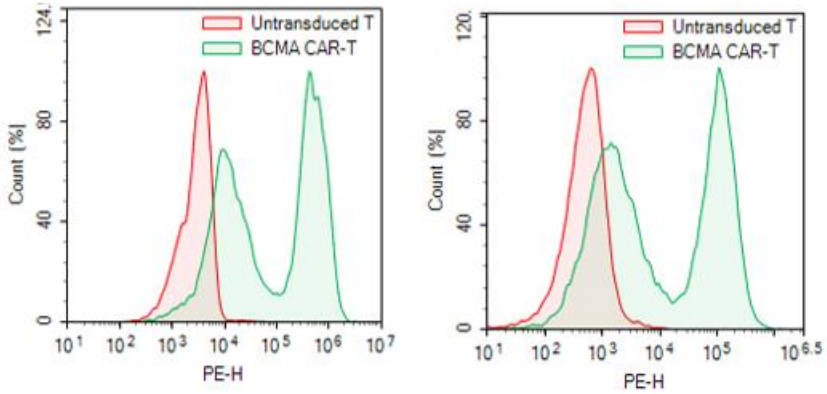

Figure 2. Expression of anti-BCMA CAR in T cells transduced with anti-BCMA CAR lentivirus. Approximately 100,000 CD4+CD8+ T cells were transduced with 4,000,000 TU (at MOI of 40) anti-BCMA CAR Lentivirus in the presence of 5 µg/ml of polybrene via spinoculation. Three days (left) and ten days (right) post-transduction, 100,000 cells were analyzed by flow cytometry using Biotinylated BCMA and PE-Streptavidin. Untransduced T cells are shown in red. T cells transduced with anti-BCMA CAR lentivirus are shown in green.

The following experiments are two examples of co-culture assays to evaluate the cytotoxicity of anti-BCMA CAR-T cells using BCMA / Firefly Luciferase - CHO Recombinant Cell Line or Firefly Luciferase-RPMI8226 Recombinant Cell Line as the target cells.

Cytotoxicity assay using BCMA / Firefly Luciferase - CHO Recombinant Cell Line as the target cells

1. Day 5: Target cells "BCMA / Firefly Luciferase - CHO Recombinant Cell Line" (BPS Bioscience #79724) and negative control "Luciferase CHO Recombinant 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.

   1) Extra wells of CHO cells were included for the "No T cells" control.

   2) Extra wells of medium only were included to determine background luminescence.

   T cells were centrifuged gently and resuspended in fresh T cell growth medium. T cells were carefully pipetted into each well 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 for 24 hours.

   Note: No overnight attachment was needed for the CHO cells. T cells were added into the wells 1-2 hours after the CHO cells were seeded.

2. Day 6: 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 CAR-T/CHO cells remaining on the plate whereas the collected medium/nonattached cells can be subjected to cytokine expression analysis. If the cytokine expression assay 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 the luminescence from the "no T cells" well (Luciferase CHO or BCMA/Luciferase CHO cells only). Results are shown in *Figure 3*.

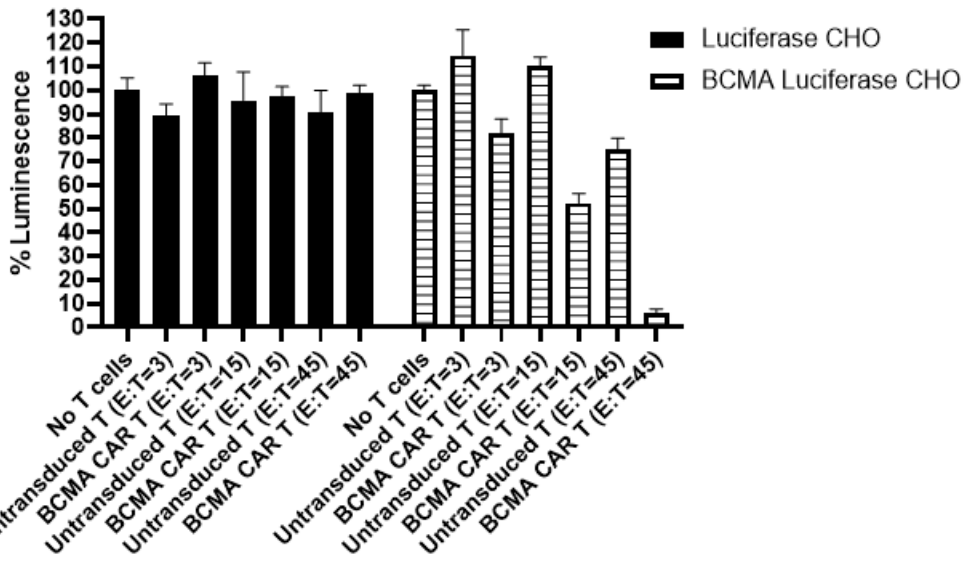

Figure 3. Luciferase-based cytotoxicity assay using BCMA-Luciferase CHO as the target cells. Approximately 100,000 CD4+CD8+ T cells were transduced with 4,000,000 TU (at MOI of 40) anti-BCMA CAR Lentivirus in the presence of 5 µg/ml of polybrene via spinoculation. Ten days post-transduction, the T cells (effector) were co-cultured with Luciferase CHO cell or BCMA/Luciferase CHO cells (target) for 24 hours at an effector:target ratio of 3, 15, and 45. The lysis of target cells was determined by measuring Luciferase activity. The T cells transduced with the anti-BCMA CAR lentivirus showed specific toxicity towards BCMA/Luciferase CHO cells. The assay was performed in parallel with untransduced T cells as a negative control

Cytotoxicity assay using Firefly Luciferase RPMI 8226 Recombinant Cell Line as the target cells

1. Day 5: Target cells "Firefly Luciferase RPMI 8226 Recombinant Cell Line" (BPS Bioscience #79834) were seeded in 50 µl of Thaw Medium 2 (BPS Bioscience #60184) at 5000 cells/well in a 96-well white, clear bottom tissue culture plate.

   1) Extra wells of Firefly Luciferase RPMI 8226 cells were included for the "No T cells" control wells

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

   T cells were centrifuged gently and resuspended in fresh T cell growth medium. T cells were carefully pipetted into each well 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 for 24 hours.

2. Luciferase assay: 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. Results are shown in *Figure 4*.

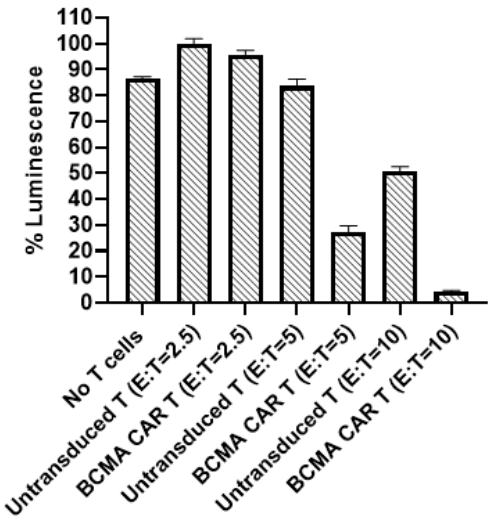

Figure 4. Luciferase-based cytotoxicity assay using Firefly Luciferase-RPMI8226 Recombinant Cell Line as the target cells.
Approximately 100,000 CD4+CD8+ T cells were transduced with 4,000,000 TU (at MOI of 40) anti-BCMA CAR Lentivirus in the presence of 5 µg/ml of polybrene via spinoculation. Five days post-transduction, the T cells (effector) were co-cultured with Firefly Luciferase-RPMI8226 Cells for 24 hours at an effector:target ratio of 2.5, 5, and 10. The lysis of RPMI8226 target cells was determined by measuring Luciferase activity. The assay was performed in parallel with untransduced T cells as a negative control.

Validation Data

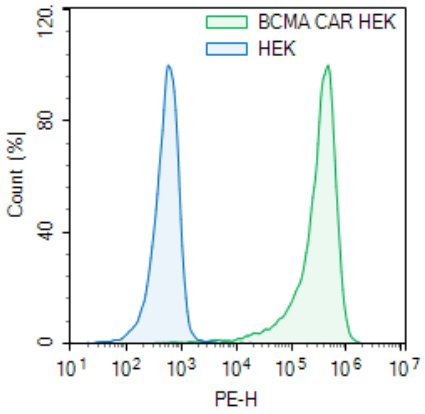

Figure 5. Expression of anti-BCMA CAR in HEK293 cells transduced with anti-BCMA CAR lentivirus.
Approximately 100,000 HEK293 cells were transduced with 1,000,000 TU (at MOI of 10) anti-BCMA CAR Lentivirus. 72 hours post-transduction, 100,000 cells were analyzed by flow cytometry using Biotinylated BCMA and PE-Streptavidin. HEK293 parental cells are shown in blue. HEK293 cells transduced with anti-BCMA CAR Lentivirus are shown in green.

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Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. 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-T Cells	78660	2 vials
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