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Produktinformation

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

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

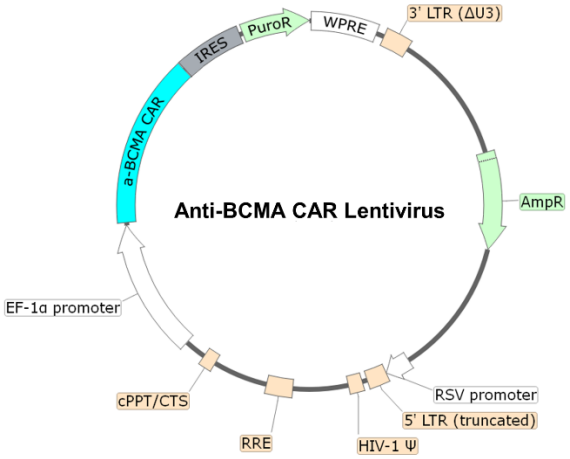
www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The anti-BCMA CAR lentiviruses are replication incompetent, HIV-based, VSV-G-pseudotyped lentiviral particles that are ready to transduce most mammalian cells, including primary and non-dividing cells. These viruses transduce cells with the ScFv (single-chain variable fragment) that recognizes two BCMA epitopes (clones VHH1 and VHH2), linked to a CD8 hinge and transmembrane domains, and the 4-1BB and CD3ζ signaling domains. The lentiviruses also include a puromycin selection marker (Figure 1).

A



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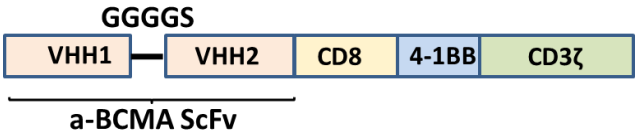

Figure 1. (A) Schematic of the lenti-vector used to generate the anti-BCMA CAR lentivirus. The vector is a SIN vector, and it contains a puromycin selection marker. (B) Construct diagram showing components of the anti-BCMA CAR.

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) and is involved in B cell proliferation and maturation. BCMA is preferentially expressed in mature B lymphocytes and the soluble form of BCMA can be found at higher levels in the serum of Multiple Myeloma (MM) patients. BCMA is a highly attractive target antigen for immunotherapy. BCMA, similarly to CD19, is restricted in expression to mature B cells allowing the progenitor's population to be spared during treatment and to replenish the patient's B cell population. BMCA targeting therapies include bispecific antibodies, antibody-drug conjugates and chimeric antigen receptor (CAR) T cells. To date, the FDA has approved two BCMA CAR-T therapies for the treatment of MM, that resulted in promising outcomes for patients. Further studies will allow a better understanding of the role of BCMA in cancer and fine tune cancer therapy tools.

Application(s)

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

Formulation

The lentiviruses were produced from HEK293T cells, concentrated and resuspended in DMEM. Virus particles can be packaged in custom formulations by special request, for an additional fee.

Size and Titer

50 µl of anti-BCMA CAR Lentivirus at a titer ≥3x10^8 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
BCMA, Fc-Fusion, Avi-Tag, PE-Labeled Recombinant	BPS Bioscience #100733
Firefly Luciferase K562 Recombinant Cell Line	BPS Bioscience #78621
Firefly Luciferase-RPMI 8226 Recombinant Cell Line	BPS Bioscience #79834
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690

Media Formulations

T Cell Medium: TCellM™ (BPS Bioscience #78753) supplemented with 10 ng/ml Interleukin-2 (BPS Bioscience #90184).

Media required for the Proposed Assay

Thaw Medium 2 (BPS Bioscience #60184):

RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

Assay Protocol

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

Day 0:

1. Isolate $CD4^+$ T cells and $CD8^+$ T cells from previously frozen human PBMC by negative selection, according to manufacturer's instructions. Mix the isolated $CD4^+$ T cells and $CD8^+$ T cells at a 1:1 ratio.

2. Culture cells in TCellM™ at 1 x 10^6 cells/ml density, at 37°C with 5% CO_2 overnight.

Day 1:

1. Activate T cells with the appropriate reagents and incubate cells at 37°C with 5% CO_2 for 24 - 48 hours.

Day 2:

1. Centrifuge the cells at 300 *x g* for 5 minutes and resuspend in fresh T cell medium at 0.1 - 1 x 10^6 cells/ml.

2. Add polybrene (5 µg/ml) to the cells.

3. Thaw anti-BCMA CAR lentivirus 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.

4. Perform spinoculation, as follows:

   a) Dispense 100 µl of T cells (~10,000-100,000) into 1.5 ml Eppendorf tubes.

   b) Create a titration of the viruses MOI starting from a MOI of 20.

   c) Incubate in the hood at Room Temperature (RT) for 10 minutes.

   d) Spin down the cells/virus at 800 *x g* for 2 hours at 32°C.

   e) If using 10,000 cells: add 900 µl of fresh T cell medium into each well of a 24-well plate, followed by the cells/virus from the spinoculation step.

      If using 100,000 cells: add 3 ml of fresh T cell medium into each well of a 6-well plate, followed by the cells/virus from the spinoculation step.

5. It is not necessary to remove the virus after spinoculation. Incubate at 37°C with 5% CO_2 for ~48-72 hours.

Day 5-9:

1. Analyze the expression of the anti-BCMA CAR by flow cytometry using PE-Labeled BCMA (BPS Bioscience #100733), as shown in Figure 2. The remaining transduced T cells can be expanded further using TCellM™.

Note: Once the transduced cells have proliferated sufficiently to reach the desired cell number required for your experiments it is recommended to use the cells as soon as possible, in order to minimize cellular exhaustion. In the experience of scientists at BPS Bioscience, when using TCellM™, T cells can expand >1000 fold by 11 days post-transduction.

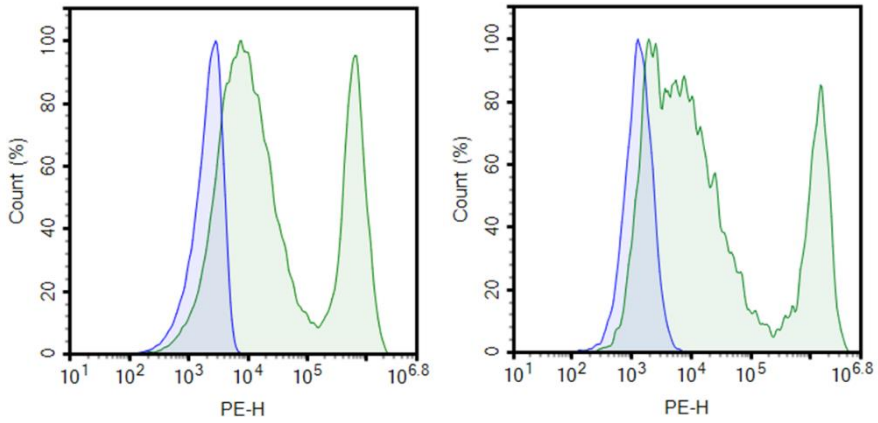

Figure 2. Expression of anti-BCMA CAR in T cells transduced with anti-BCMA CAR lentivirus. Approximately 100,000 $CD4^+$ and $CD8^+$ T cells were transduced with 2,000,000 TU (MOI of 20) anti-BCMA CAR Lentivirus in the presence of 5 µg/ml of polybrene, by spinoculation. Anti-BCMA CAR expression was analyzed by flow cytometry using PE-Labeled BCMA (BPS Bioscience #100733) three days (left) and seven days (right) post-transduction. Blue = untransduced T cells; Green = T cells transduced with anti-BCMA CAR lentivirus.

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

The following experiment is one example of a co-culture assay to evaluate the cytotoxicity of anti-BCMA CAR-T cells using Firefly Luciferase-RPMI 8226 Recombinant Cell Line (BPS Bioscience #79834) as the target cells. Firefly Luciferase K562 Cell Line (BPS Bioscience #78621) should be used as negative control.

The assay should include "No T cell Control", "Background Luminescence Control" and "Test" conditions.

Day 9-13:

1. Seed the target cells (Firefly Luciferase RPMI 8226 Recombinant Cell Line) and negative control cells (Firefly Luciferase K562 Cell Line, which do not express BCMA) in 50 µl of Thaw Medium 2 at 5000 cells/well in a 96-well white, clear bottom tissue culture plate. These are the "Test" wells. Include extra wells of Firefly Luciferase RPMI 8226 cells or Firefly Luciferase K562 cells as "No T cell Control", and wells containing only media as "Background Luminescence Control".

2. Centrifuge transduced T cells at 300 *x g* for 5 minutes.

3. Resuspend T cells in fresh TCellM™.

4. Carefully pipet 50 µl of T cells, at the appropriate density to reach the desired effector:target (E:T) cell ratio, into each "Test" well.

5. For "No T cell Control" and "Background Luminescence Control" wells add 50 µl of fresh TCellM™.

6. Incubate at 37°C for 24 hours.

Next day:

1. Add 100 µl of ONE-Step™ Luciferase assay reagent to each well, including the control wells.
2. Incubate at RT for ~15 to 30 minutes.
3. Measure luminescence using a luminometer.

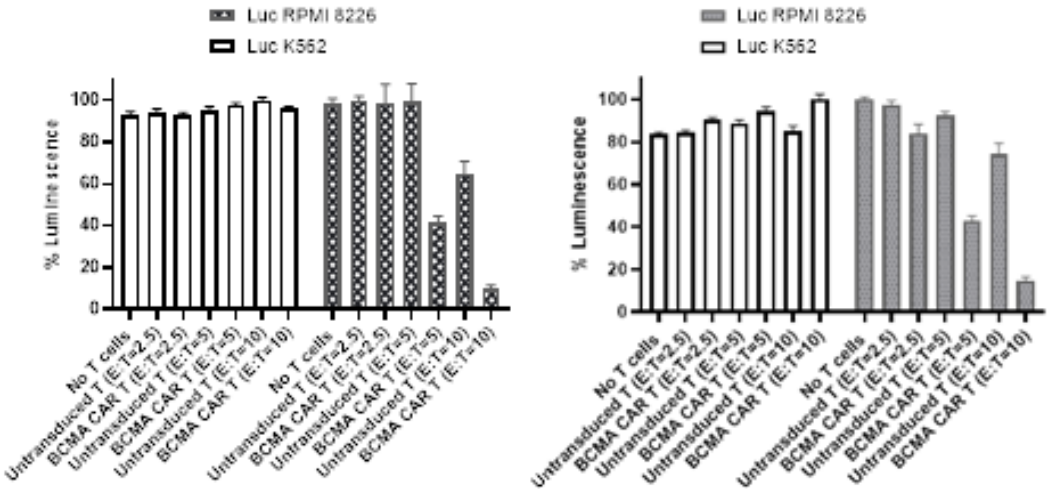

Figure 3. Cytotoxicity profile of T Cells transduced with anti-BCMA CAR lentivirus against Firefly Luciferase RPMI 8226 target cells.
Approximately 100,000 $CD4^+$ and $CD8^+$ T cells were transduced with 2,000,000 TU (MOI of 20) anti-BCMA CAR Lentivirus in the presence of 5 µg/ml of polybrene, by spinoculation. Seven days (left) and eleven days (right) post-transduction, the T cells (effector) were co-cultured with Firefly Luciferase RPMI 8226 Cells and Firefly Luciferase K562 (target) for 24 hours at an effector:target ratio of 2.5, 5, and 10. The lysis of target cells was determined by measuring luciferase activity with ONE-Step™ Luciferase. The anti-BCMA CAR lentivirus-transduced T cells showed specific cytoxicity towards Firefly Luciferase-RPMI 8226 cells even at an E:T ratio of 5 (lower % luminescence). Untransduced T cells were run in parallel as a negative control. The luciferase activity of Luciferase K562 cells or Luciferase-RPMI 8226 cells alone was set as 100%.

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	78170	1 vial
$CD4^+$ T cells, Negatively Selected (Human)	79752	10 million cells
$CD8^+$ T cells, Negatively Selected (Human)	79753	10 million cells
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