Anti-CD20 CAR Lentivirus (Clone Leu-16 ScFv-CD8-4-1BB-CD3ζ)

#78606

Description

The anti-CD20 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-CD20 (clone Leu-16) 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).

Application

Ideal as a positive control for anti-CD20 CAR evaluation in T cells; useful for transduction optimization.

Formulation

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

Titer

50 µl of anti-CD20 CAR at a titer $\geqslant 10^8$ 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 Protein L	Genscript #M00097
PE-Streptavidin	Biolegend #405203
IFN-γ (Human) Colorimetric ELISA Detection Kit	BPS Bioscience #79777
CD20/Firefly Luciferase CHO Cell Line	BPS Bioscience #78620
Firefly Luciferase CHO Cell Line	BPS Bioscience #79725
Firefly Luciferase Raji Cell Line	BPS Bioscience #78622
Firefly Luciferase K562 Cell Line	BPS Bioscience #78621
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690

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

Experimental Methods and Results:

The following protocol was used to transduce CD4+CD8+ primary T cells with the anti-CD20 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 reagents were 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-CD20 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.
      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-CD20 CAR was estimated by flow cytometry using Biotinylated Protein L 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. In the experience of scientists at BPS Bioscience, the T cells had expanded >1000 fold by 11 days post-transduction, using the recommended T cell medium.

The following experiments are two examples of co-culture assays to evaluate the cytotoxicity of anti-CD20 CAR-T using CD20/Firefly Luciferase CHO Cell Line or Firefly Luciferase Raji Cell Line as the target cells.

Cytotoxicity assay using CD20/Firefly Luciferase CHO Cell Line as the target cells

1. Day 5: Target cells "CD20/Firefly Luciferase CHO Cell Line" (BPS Bioscience, #78620) 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.
   1) Extra wells of Firefly Luciferase CHO cells were included for the "no T cell" control
   2) Extra wells of medium only were included to determine background luminescence.

   T cells were centrifuged gently (300*g* x 5 min) 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 was subjected to **IFNγ expression analysis.**

   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 CD20/Luciferase CHO cells was set as 100%. The % Luminescence was calculated as luminescence of co-culture well/ luminescence from the "no T cells" well (Luciferase CHO or CD20/Luciferase CHO cells only).
   Results are shown in Figure 4.

   IFNγ analysis: IFNγ expression in each well containing the mix of medium/non-attached cells was determined using the Colorimetric Human IFN-γ ELISA Detection Kit (BPS Bioscience, #79777), following the recommended protocol. Results are shown in Figure 3.

   Note: If the IFNγ assay is not performed immediately, the collected medium can be stored at -20°C.

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

1. Day 5: Target cells "Firefly Luciferase Raji Cell Line" (BPS Bioscience, #78622) or negative control cells "Firefly Luciferase K562 Cell Line" (BPS Bioscience, #78621), which do not express CD20, 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 Raji cells were included for the "no T cell" control wells

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

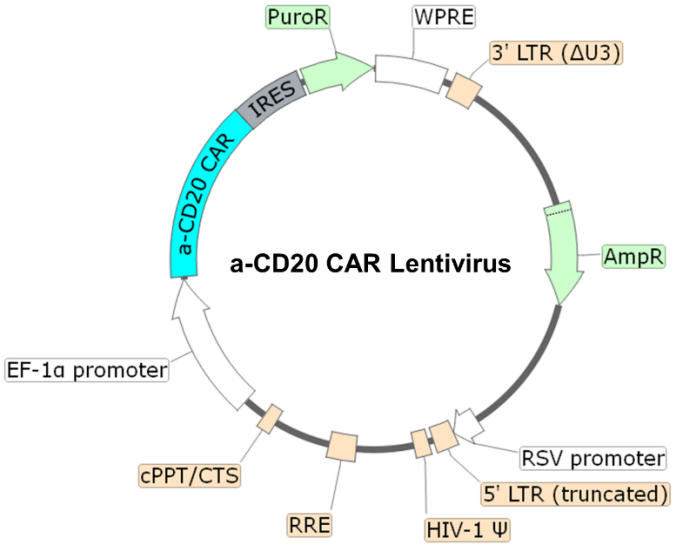
T cells were centrifuged gently (300*g* x 5min) and resuspended in fresh T cell growth medium. T cells were carefully pipetted into each well at the desired effector:target (E:T) cell ratio in 50 µl of volume. For "no T cells" wells and "medium only" wells, 50 µl of fresh T cell medium was added. The 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.

   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/ luminescence from the "no T cells" well (Luciferase Raji or Luciferase K562 cells only). Percent specific lysis was calculated as: 1-% Luminescence. Firefly Luciferase K562 cells (BPS Bioscience, #78621), which do not express endogenous CD20, were used as a negative control.

Figures and Validation Data:

A



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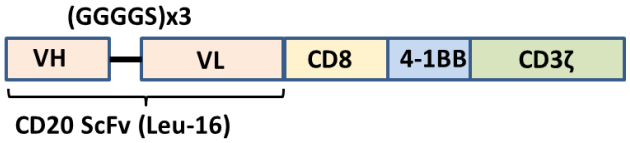

Figure 1. *(A) Schematic of the lenti-vector used to generate the anti-CD20 CAR lentivirus and (B) Construct diagram showing components of the anti-CD20 CAR*

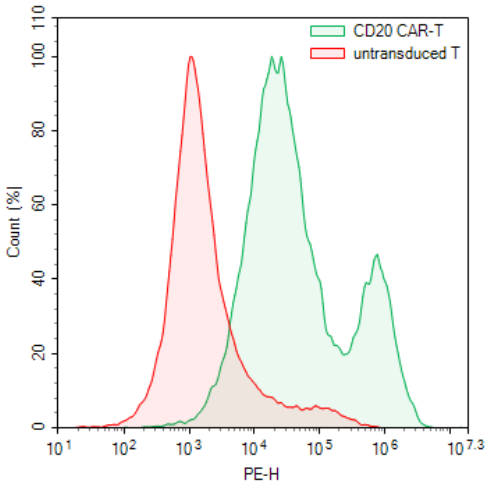

Figure 2. *The expression of anti-CD20 CAR in T cells transduced with anti-CD20 CAR lentivirus*
Approximately 100,000 CD4+CD8+ T cells were transduced with 4,000,000 TU (at MOI of 40) anti-CD20 CAR Lentivirus in the presence of 5 µg/mL of polybrene via spinoculation. Three days post-transduction, 100,000 cells were analyzed by flow cytometry using Biotinylated Protein-L and PE-Streptavidin. Red, Untransduced T cells; Green, T cells transduced with anti-CD20 CAR lentivirus.

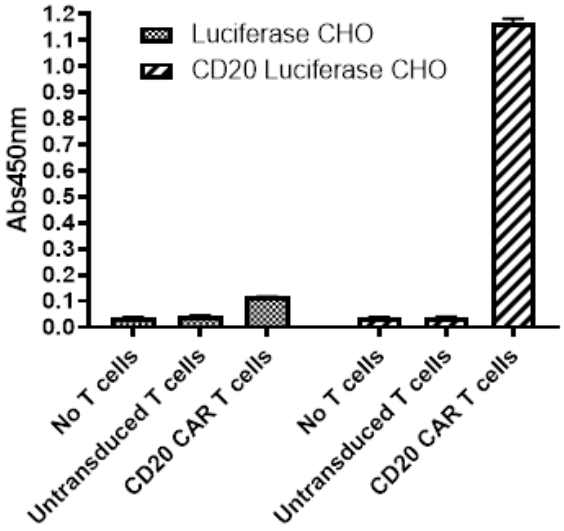

Figure 3. *IFNγ expression analysis using CD20/Firefly Luciferase CHO as the target cells*
Approximately 100,000 CD4+CD8+ T cells were transduced with 4,000,000 TU (at MOI of 40) anti-CD20 CAR Lentivirus in the presence of 5 µg/mL of polybrene via spinoculation. Three days post-transduction, the T cells (effector) were co-cultured with Firefly Luciferase CHO cell or CD20/Firefly Luciferase CHO cells (target) for 24 hours at a ratio of effector: target=30. The medium was then collected for IFNγ analysis using IFN-γ ELISA Detection Kit (BPS Bioscience, #79777).

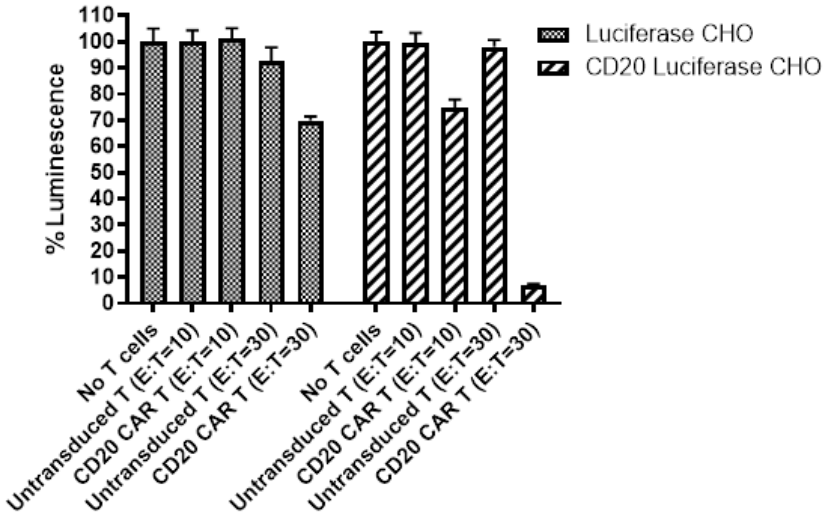

Figure 4. *Luciferase-based cytotoxicity assay using CD20-Luciferase CHO as the target cells*
Approximately 100,000 CD4+CD8+ T cells were transduced with 4,000,000 TU (at MOI of 40) anti-CD20 CAR Lentivirus in the presence of 5 µg/mL of polybrene via spinoculation. Three days post-transduction, the T cells (effector) were co-cultured with Luciferase CHO cell or CD20/Luciferase CHO cells (target) for 24 hours at an effector:target ratio of either 10 or 30. The lysis of target cells was determined by measuring Luciferase activity. The anti-CD20 CAR lentivirus transduced T cells showed specific toxicity towards CD20/Luciferase CHO cells. The assay was performed in parallel with untransduced T cells as a negative control.

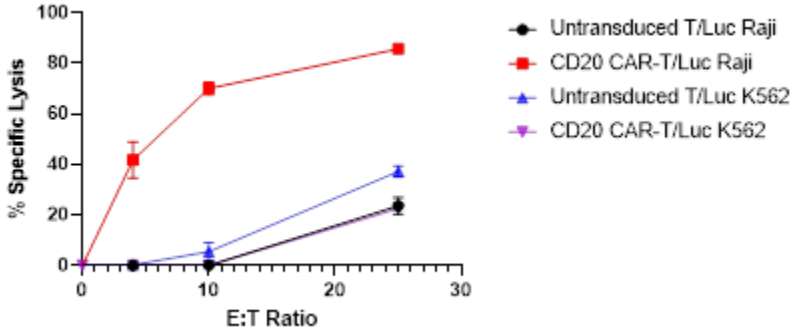

Figure 5*. Luciferase-based cytotoxicity assay using Firefly Luciferase-Raji Recombinant Cell Line as the target cells*
Approximately 100,000 CD4+CD8+ T cells were transduced with 4,000,000 TU (at MOI of 40) anti-CD20 CAR Lentivirus in the presence of 5 µg/mL of polybrene via spinoculation. Three days post-transduction, the T cells (effector) were co-cultured with Firefly Luciferase Raji Cells for 24 hours at 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.

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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*
CD20/Firefly Luciferase CHO Cell Line	78620	2 vials
Firefly Luciferase CHO Cell Line	79725	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
IFN-γ (Human) Colorimetric ELISA Detection Kit	79777	96 reactions
Human Interleukin-2	90184-A	10 µg
Normal Human Peripheral Blood Mononuclear Cells	79059	30 x 10^6 cells
Firefly Luciferase Raji Cell Line	78622	2 vials
Firefly Luciferase K562 Cell Line	78621	2 vials
Firefly Luciferase CHO Cell Line	79725	2 vials
Anti-CD19 CAR Lentivirus	78600	50 µL
Anti-BCMA CAR Lentivirus	78603	50 µL
Anti-CD22 CAR Lentivirus	78608	50 µL