Description

The Anti-mesothelin 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 fragment variable) of anti-mesothelin (Clone P4) 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).

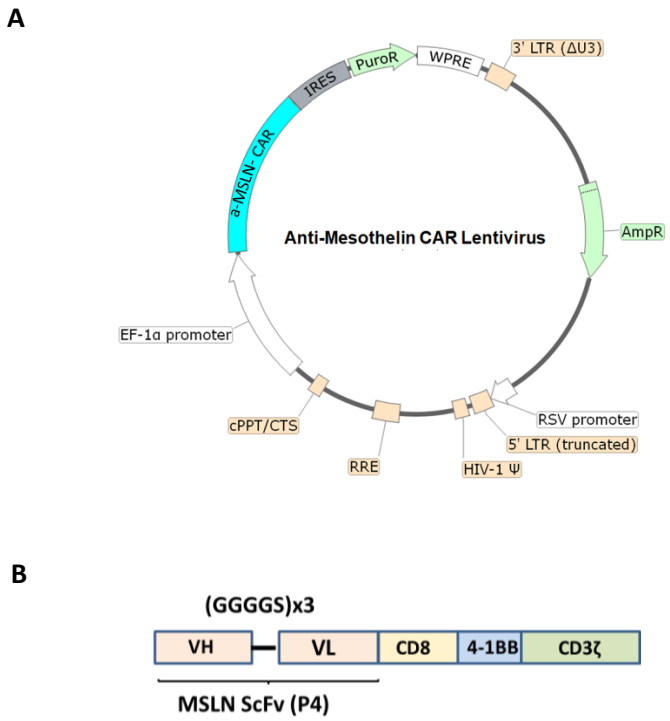

Figure 1. (A) Schematic of the lenti-vector used to generate the anti-mesothelin CAR lentivirus and (B) Construct diagram showing components of the anti-mesothelin CAR.

Background

Mesothelin (MSLN) is a glycophosphatidylinositol (GPI) linked cell-surface protein that is produced as a ~70 kDa precursor protein and cleaved by Furin protease to generate the ~40 kDa mature form. MSLN is frequently overexpressed in mesothelioma, ovarian, pancreatic, and non-small cell lung cancers, while its expression in normal tissues is restricted to the mesothelial lining. MSLN is a tumor-associated antigen and has been an attractive target for targeted immunotherapy, including drug-conjugated antibodies and chimeric antigen receptor T cells (CAR-T Cells).

Application(s)

Positive control for anti-mesothelin 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-mesothelin CAR 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
Biotinylated Human Mesothelin	BPS Bioscience #100291
PE-Streptavidin	Biolegend #405203
Mesothelin - CHO Recombinant Cell Line	BPS Bioscience #78132
IFN-γ (Human) Colorimetric ELISA Detection Kit	BPS Bioscience #79777

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-mesothelin 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 5min) 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-mesothelin 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 10. 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-mesothelin CAR was estimated by flow cytometry using Biotinylated Mesothelin and PE-Labeled Streptavidin, as shown in Figure 3. 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 one example of co-culture assays to evaluate the cytotoxicity of anti-mesothelin CAR-T cells using the Mesothelin-CHO Recombinant Cell Line.

Interferon production Assay using Mesothelin-CHO Recombinant Cell Line as the target cells.

1. **Day 11**: Target cells "Mesothelin- CHO Recombinant Cell Line" (BPS Bioscience #78132) and parental CHO Cells 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 reading.

   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 12**: The medium was transferred to another plate for IFN-g analysis.
   IFN-g analysis: IFNg expression in each well containing the mix of medium/non-attached cells was determined using the Colorimetric Human IFN-g ELISA Detection Kit (BPS Bioscience #79777), following the recommended protocol. Note: If the IFN-g assay is not performed immediately, the collected medium can be stored at -20°C. Results are shown in Figure 4.

Validation Data:

A

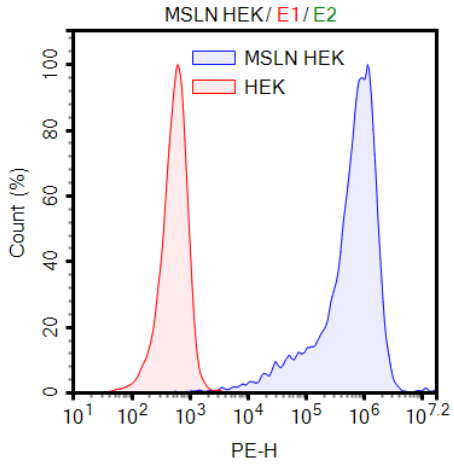

Figure 2. The expression of anti-Mesothelin CAR in HEK293 cells transduced with anti-mesothelin CAR lentivirus.

Approximately 100,000 HEK293 cells were transduced with 1,000,000 TU (at MOI of 10) anti-Mesothelin CAR Lentivirus. 72 hours post-transduction, 100,000 cells were analyzed by flow cytometry using Biotinylated Mesothelin and PE-Streptavidin. Red, HEK293 parental cells; Blue, HEK293 cells transduced with anti-mesothelin CAR Lentivirus.

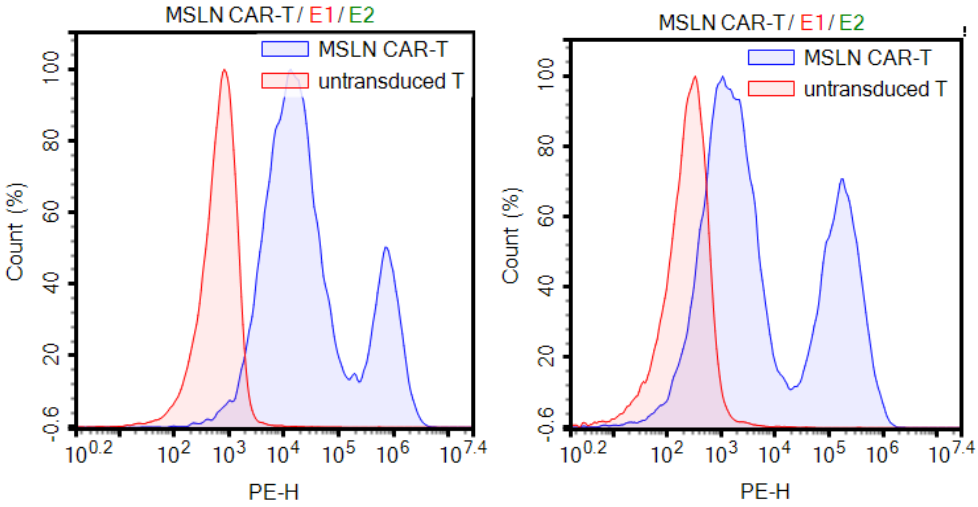

Figure 3. The expression of anti-Mesothelin CAR in T cells transduced with Anti-Mesothelin CAR lentivirus.

Approximately 20,000 CD4+CD8+ T cells were transduced with 400,000 TU (at MOI of 20) anti-Mesothelin 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 Mesothelin and PE-Streptavidin. Red, Untransduced T cells; Blue, T cells transduced with anti-Mesothelin CAR lentivirus.

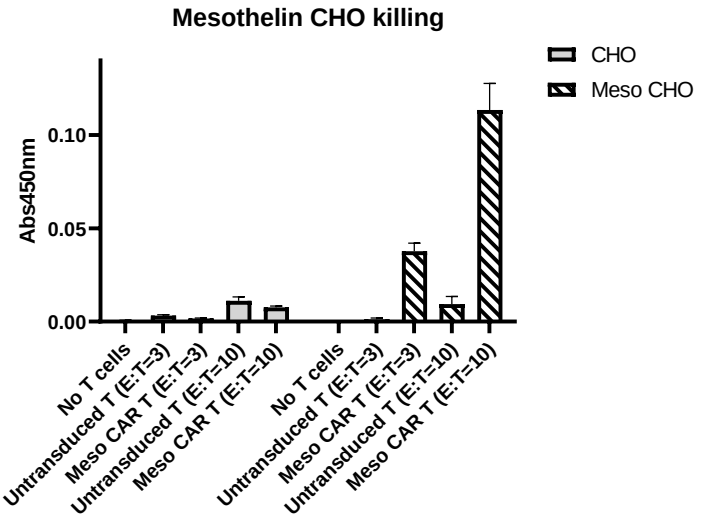

Figure 4. IFNg expression analysis using Mesothelin CHO as the target cells.
Approximately 20,000 CD4+CD8+ T cells were transduced with 400,000 TU (at MOI of 20) Anti-Mesothelin CAR Lentivirus in the presence of 5 µg/ml of polybrene via spinoculation. Ten days post-transduction, the T cells (effector) were co-cultured with CHO cells or Mesothelin CHO cells (target) for 24 hours at indicated ratio of effector: target. The medium was then collected for IFNg analysis using IFN-g ELISA Detection Kit (BPS Bioscience #79777).

References

1. Asgarov, K., *et al.* (2017). A new anti-mesothelin antibody targets selectively the membrane-associated form. *MAbs*, **9(3):** 567–577.
2. Ye, L., *et al.* (2019). Mesothelin-targeted second generation CAR-T cells inhibit growth of mesothelin-expressing tumors in vivo. *Experimental and Therapeutic Medicine,* **17:** 739-747.

License Disclosure
Visit bpsbioscience.com/license for the label license and other key information about this product.

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*
Mesothelin-CHO Recombinant Cell Line	78132	2 vials
Mesothelin, Avi-His-Tag, HiP™ Recombinant	100290	100 µg
Biotinylated Human Mesothelin	100291	25, 50 µg
Human Interleukin-2	90184	10, 50 µg
PBMC, Frozen	79059	30, 100 million cells
IFN-g (Human) Colorimetric ELISA Detection Kit	79777	1, 5 x 96 reactions