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Produktinformation

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

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See the following pages for more information!

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-mesothelin CAR-T cells are produced by high-titer lentiviral transduction of human primary CD4+CD8+ T cells using the anti-Mesothelin CAR Lentivirus (Mesothelin ScFv-CD8-4-1BB-CD3ζ; BPS Bioscience #78703). These ready-to use CAR-T cells express an anti-Mesothelin CAR consisting of 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).

These CAR-T cells have been validated using flow cytometry (to determine the CAR expression) and co-culture cytotoxicity assays.

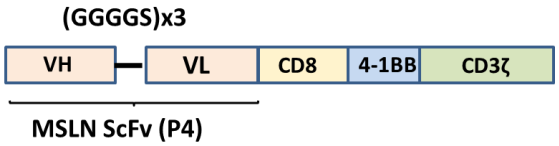

Figure 1: Construct diagram showing components of the anti-Mesothelin CAR expressed in anti-Mesothelin CAR-T cells.

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 approaches, including drug-conjugated antibodies and chimeric antigen receptor T cells (CAR-T Cells).

Application(s)

- Use as positive control for anti-Mesothelin CAR-T cells
- Screen modulators of anti-Mesothelin CAR-T cytotoxicity
- Design and optimize co-culture cytotoxicity assays

Biosafety

The Anti-Mesothelin CAR-T cells are produced with the third generation SIN (self-inactivation) lenti vector 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 (Stemcell Technologies)

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 Anti-Mesothelin 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
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 anti-Mesothelin CAR-T Cell Medium: TCellM™ (BPS Bioscience #78753) supplemented with 10 ng/ml Interleukin-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-Mesothelin 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

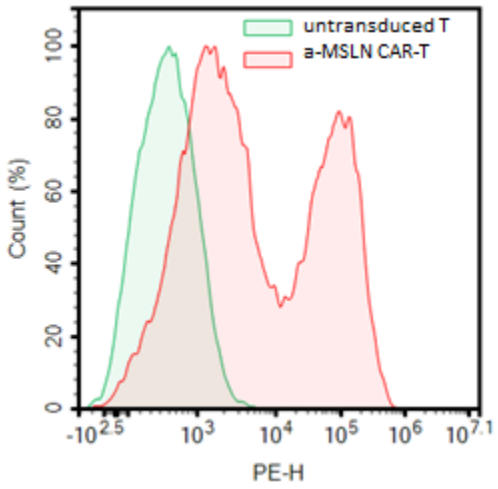

Figure 2: Expression of anti-Mesothelin CAR in Anti-Mesothelin CAR-T cells.
Anti-Mesothelin CAR-T cells were thawed and expanded for 4 days. Approximately 50,000 cells were analyzed by flow cytometry using Biotinylated Mesothelin (BPS Bioscience #100291) and PE-Streptavidin.

Experimental Methods and Results
The following experiments are one example of co-culture assay to evaluate the cytotoxicity of anti- Mesothelin CAR-T using the Mesothelin-CHO Recombinant Cell Line.

Interferon Production assay using Mesothelin 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 "Mesothelin CHO Cell Line" (BPS Bioscience #78132) and parental "CHO Cell Line" 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 Mesothelin CHO Cell Line or CHO Cell Line were included for the "no T cells" control.

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

3. Anti-Mesothelin CAR-T cells were centrifuged gently (300g x 5 minutes) 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: The medium was transferred to another plate for IFN-γ analysis.

IFN-γ analysis: IFN-γ 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. Note: If the IFN-γ assay is not performed immediately, the collected medium can be stored at -20°C. Results are shown in Figure 3.

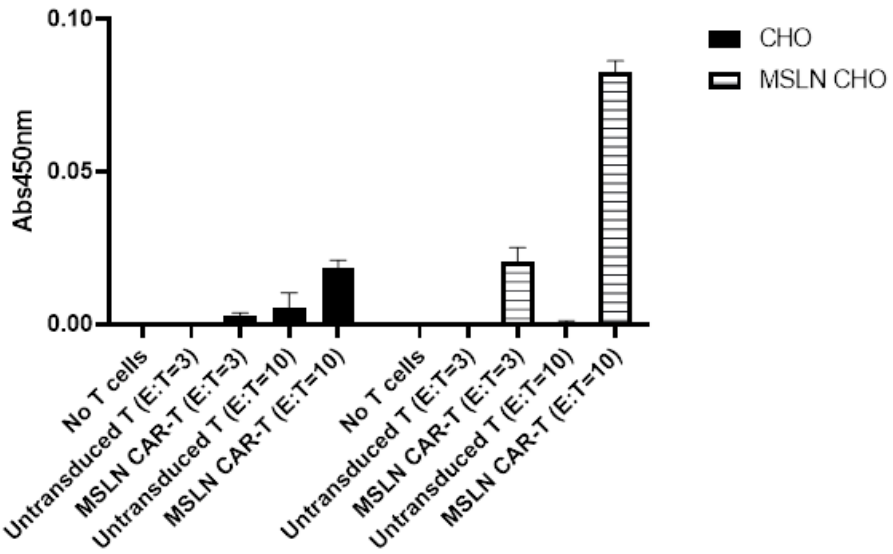

Figure 3: IFN-g expression analysis using Mesothelin CHO as the target cells.
Anti-Mesothelin CAR-T cells were thawed and expanded for 4 days. The T cells (effector) were co-cultured with CHO cell or Mesothelin CHO cells (target) for 24 hours at the indicated ratio of effector:target. The medium was then collected for IFN-g 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.

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

Visit Cell Line FAQs for more information.
For 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
Anti-CD19 CAR-T Cells	78171	Various
Anti-CD20 CAR-T Cells	78611	Various
Anti-BCMA CAR-T Cells	78660	Various