Description

The anti-CD20 CAR-T cells are produced by high-titer lentiviral transduction of human primary CD4+CD8+ T cells using the anti-CD20 CAR Lentivirus (CD20 ScFv-CD8-4-1BB-CD3ζ; BPS Bioscience #78606). These ready-to use CAR-T cells express an anti-CD20 CAR consisting of the ScFv (Single chain fragment variable) 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).

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-CD20 CAR expressed in anti-CD20 CAR-T cells.

Background

CD20 (also known as MS4A1) is a glycosylated phosphoprotein expressed on the cell surface of B cells. CD20 is a highly attractive target antigen for immunotherapy because it is highly expressed in more than 90% of patients with B-cell lymphoma. First approved in 1997, Rituximab (Rituxan) is a chimeric monoclonal antibody targeting CD20 and has been classified by the World Health Organization as an "Essential Medicine". Since then, additional monoclonal antibodies against CD20 have been approved or are being tested in clinical trials for the treatment of multiple sclerosis (MS), chronic lymphocytic leukemia (CLL), follicular lymphoma, diffuse large B cell lymphoma (DLBCL), rheumatoid arthritis, non-Hodgkin's lymphoma, systemic lupus erythematosus, and myalgic encephalomyelitis (chronic fatigue syndrome). More recently, anti-CD20-CD19 bispecific CAR-T cells have been developed to address concerns over potential relapse in cancer patients.

Application(s)

- Use as positive control for anti-CD20 CAR-T cells
- Screen modulators of anti-CD20 CAR-T cytotoxicity
- Design and optimize co-culture cytotoxicity assays

Biosafety

The anti-CD20 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-CD20 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 Protein L	Genscript #M00097
PE-Streptavidin	Biolegend #405203
CD20 CHO Recombinant Cell Line (High Expression)	BPS Bioscience #79624-H
IFN-γ (Human) Colorimetric ELISA Detection Kit	BPS Bioscience #79777

Recommended anti-CD20 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. If anti-CD20 CAR-T cells stop proliferation, they can be activated by T cell activator (Stemcell technologies #10970) for 24-48 hours for expansion. Since these are primary cells, the extent of expansion is not predictable. It is not recommended to freeze the cells again once they have been activated and expanded.

Validation

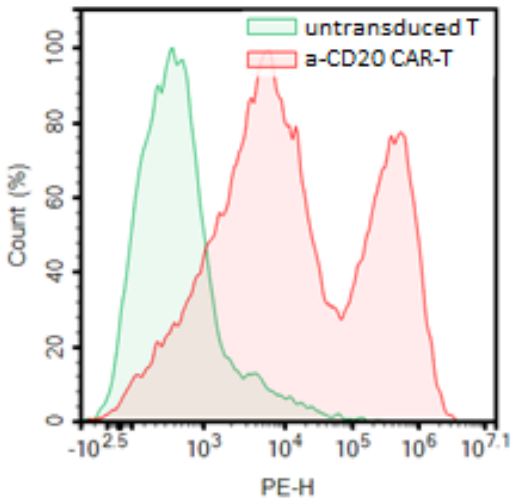

Figure 2: Expression of anti-CD20 CAR in anti-CD20 CAR-T cells.
Anti-CD20 CAR-T cells were thawed and expanded for 4 days. Approximately 50,000 cells were analyzed by flow cytometry using Biotinylated Protein L (Genscript #M00097) and PE-Streptavidin (Biolegend #405203).

Experimental Methods and Results
The following experiments are one example of co-culture assay to evaluate the cytotoxicity of anti-CD20 CAR-T using CD20 CHO Cell Line.

Interferon production assay using CD20 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 "CD20 CHO Cell Line" (BPS Bioscience #79624-H) 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.
   a. Extra wells of CD20 CHO Cell Line or parental CHO Cells were included for the "No T cells" control.
   b. Extra wells of "medium only" were included to determine background reading.

3. Anti-CD20 CAR-T cells were centrifuged gently (300g x 5 min) 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-g analysis.
   IFN-g analysis: IFN-g 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 3.

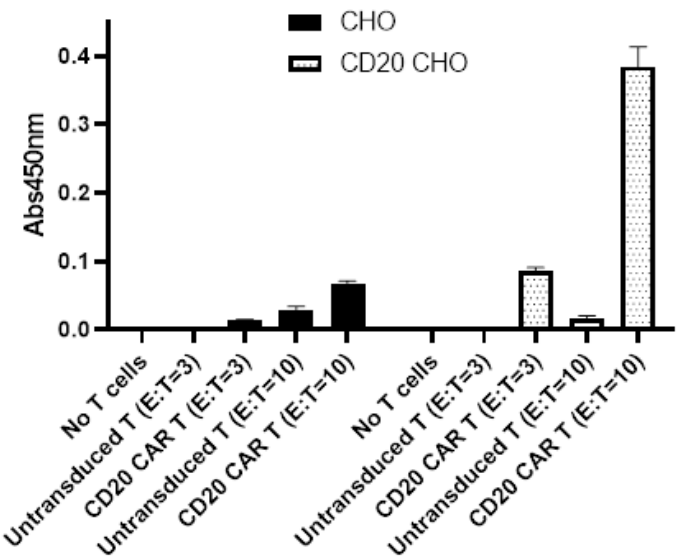

Figure 3: IFN-g expression analysis using CD20 CHO as the target cells.
Anti-CD20 CAR-T cells were thawed and expanded for 4 days, the T cells (effector) were co-cultured with CD20 CHO cells (target) or parental CHO cells for 24 hours at indicated ratio of effector: target. The medium was then collected for IFN-g analysis using IFN-g ELISA Detection Kit (BPS Bioscience #79777).

References

1. Kosmas C, *et al.* Anti-CD20-based therapy of B cell lymphoma: state of the art. *Leukemia.* 2002; **16**: 2004-15
2. Cragg MS, *et al*. The biology of CD20 and its potential as a target for mAb therapy. *Curr Dir Autoimmun.* 2005; **8**: 140-174
3. Martyniszyn A, *et al.* CD20-CD19 bispecific CAR T cells for the treatment of B cell malignancies. *Hum Gene Ther*. 2017; **28(12)**: 1147-1157
4. Lee SY, *et al*. Preclinical optimization of a CD20-specific chimeric antigen receptor vector and culture conditions. *J Immunother*. 2018; **41(1)**: 19-31

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*
CD20 CHO Cell Line	79624-H	2 vials
PBMC, Frozen	79059	30, 100 million cells
Anti-CD19 CAR-T Cells	78171	Various
Anti-Mesothelin CAR-T Cells	78729	Various
Anti-BCMA CAR-T Cells	78660	Various
CD4+ T cells, Negatively Selected (Human)	79752	10 million cells
Untransduced T Cells	78170	Various