Description

Anti-CD19/CD20 CAR-T Cells are produced by high-titer lentiviral transduction of human primary $CD4^+$ and $CD8^+$ T cells using Anti-CD19/CD20 CAR Lentivirus. These ready-to-use CAR (chimeric antigen receptor)-T cells express an anti-CD19/CD20 CAR consisting of the ScFv portions of both anti-CD20 (Clone OMB-157) and anti-CD19 (clone FMC63) linked to a second generation CAR containing IgG4 hinge and CD28 transmembrane domains, 4-1BB and CD3ζ signaling domains (Figure 1).

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

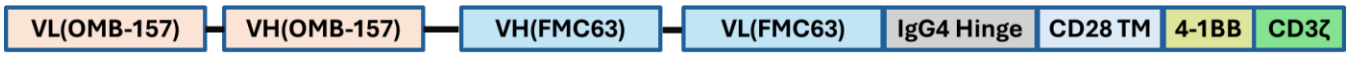

Figure 1. Construct diagram showing components of the Anti-CD19/CD20 CAR expressed in Anti-CD19/CD20 CAR-T Cells.

Background

B-lymphocyte antigen CD19 (Cluster of Differentiation 19), also known as B-Lymphocyte Surface Antigen B4 and CVID3, is a transmembrane protein expressed in follicular dendritic cells and all B lineage cells except plasma cells. CD19 plays two major roles in human B cells. It acts as an adaptor protein to recruit cytoplasmic signaling proteins to the membrane and it works within the CD19/CD21 complex to decrease the threshold for B cell receptor signaling pathways. Due to its presence on all B cells, it is a biomarker for B lymphocyte development and lymphoma diagnosis and can be used as a target for leukemia immunotherapies. CD19-targeted therapies based on T cells that express CD19-specific chimeric antigen receptors (CARs) have been utilized for their antitumor abilities in patients with CD19+ lymphoma and leukemia, such as Non-Hodgkins Lymphoma (NHL), CLL and ALL.

CD20 (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.

Application

- Use as a positive control in the development of anti-CD19/CD20 CAR-T cells.
- Screen modulators of anti-CD19/CD20 CAR-T cytotoxicity.
- Design and optimize co-culture cytotoxicity assays for anti-CD19/CD20 specific CAR-T cell evaluation.

Biosafety

The anti-CD19/CD20 CAR-T cells are produced with 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 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 #100-1061)

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 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
Normal Human Peripheral Blood Mononuclear Cells, Frozen	BPS Bioscience #79059
TCellM™	BPS Bioscience #78753
Human Interleukin-2 Recombinant	BPS Bioscience #90184
Untransduced T Cells (Negative Control for CAR-T cells)	BPS Bioscience #78170
PE-Labeled Anti-FMC63 scFv Monoclonal Antibody	Acrobiosystems # FM3-HPY53-25tests
CD20, FLAG-Tag Recombinant	BPS Bioscience #101572
PE anti-DYKDDDDK Tag Antibody	BioLegend#637310
FluoSite™ Anti-CD3 Antibody, FITC-Labeled	BPS Bioscience #102008
APC anti-human CD107a (LAMP-1) Antibody	BioLegend #328620
Firefly Luciferase K562 Cell Line	BPS Bioscience #78621
Firefly Luciferase CD19 K562 Cell Line	BPS Bioscience #82486
Firefly Luciferase CD20 K562 Cell Line	BPS Bioscience #82487
Firefly Luciferase CD19/CD20 K562 Cell Line	BPS Bioscience #82488
Monensin sodium	MedchemExpress #HY-N0150
Brefeldin A	MedchemExpress #HY-16592
Cell Staining Buffer	BioLegend #420201
Thaw Medium 2	BPS Bioscience #60184
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

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

Cell Culture Protocol

Cell thawing

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 CAR-T Cell Medium.

   Note: 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 CAR-T Cell Medium.

3. Transfer the resuspended cells to a T25 flask.

Cell culture

1. Centrifuge the cells gently at 300 *x g* for 5 minutes.

2. Resuspend in fresh CAR-T Cell Medium.

3. Continue to culture the cells at 37°C with 5% CO_2.

4. Do not allow the cell density to exceed 2.0 x 10^6 cells/ml. Transfer the cells to 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-CD19/CD20 CAR-T cells may stop proliferating after ~one week in culture. It is not recommended to activate/freeze these cells again as these primary cells have a finite lifespan.

Validation Data

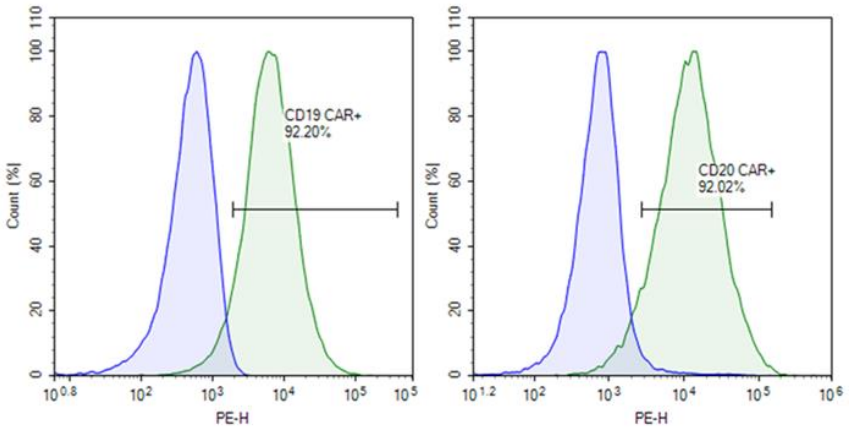

Figure 2. Expression of anti-CD19/CD20 CAR in Anti-CD19/CD20 CAR-T Cells analyzed by flow cytometry.
Anti-CD19/CD20 CAR-T cells (green) and Unstransduced T cells (BPS Bioscience #78170) (blue) were thawed for 24 hours and stained with PE-Labeled Anti-FMC63 scFv Monoclonal Antibody (Acrobiosystems # FM3-HPY53-25tests) **(left)** or CD20, FLAG-Tag Recombinant (BPS Bioscience #101572) followed by and PE anti-DYKDDDDK Tag Antibody (BioLegend #637310) **(right)**. Anti-CD19/CD20 CAR expression was analyzed by flow cytometry. The y axis represents the % of cells, while the x axis indicates PE-intensity.

Functional Validation

A. Cytotoxicity assay of Anti-CD19/CD20 CAR-T Cells using several Firefly Luciferase K562 cell lines as the target cells

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include “Background Control”, “No T Cell Control” and “Test” conditions.
- The following experiment is an example of a co-culture assay where Firefly Luciferase K562 Cell Line (#78621), Firefly Luciferase CD19 K562 Cell Line (#82486), Firefly Luciferase CD20 K562 Cell Line (#82487), and Firefly Luciferase CD19/CD20 K562 Cell Line (#82488) were used to evaluate the cytotoxicity of Anti-CD19/CD20 CAR-T Cells (#82481).
- We recommend the use of Untransduced T Cells (Negative Control for CAR-T cells) (#78170) as control.

Day 0

1. Thaw frozen Anti-CD19/CD20 CAR-T cells and control cells in CAR-T Cell Medium.

2. Incubate at 37°C for 24 hours.

Day 1

1. Seed Firefly Luciferase K562 cells at 500 cells/well in 50 µl of Thaw Medium 2, in a 96-well white, clear bottom tissue culture plate. Leave a few empty wells as “Background Control”.

2. Centrifuge T cells gently and resuspend in fresh CAR-T Cell Medium at the appropriate cell density to reach the desired effector:target (E:T) cell ratio (50 µl/well).

3. Carefully pipet 50 µl of T cells into each "Test" well at the desired effector:target (E:T) cell ratio.

4. Add 50 µl of fresh CAR-T Cell Medium to the "No T Cell Control" wells.

5. Add 100 µl of fresh CAR-T Cell Medium to the "Background Control" wells.

6. Incubate at 37°C for 24 hours.

Day 2

1. Add 100 µl of ONE-Step™ Luciferase assay reagent to each well.

2. Incubate at Room Temperature (RT) 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 K562 cells alone was set as 100%. The % Luminescence was calculated as background-subtracted luminescence of "Test" wells divided by background-subtracted luminescence of the "No T Cell Control" wells (Firefly Luciferase K562 cells only).

$$\%\ Lum = \frac{Lum\ test - background}{Lum\ control - background} \times 100$$

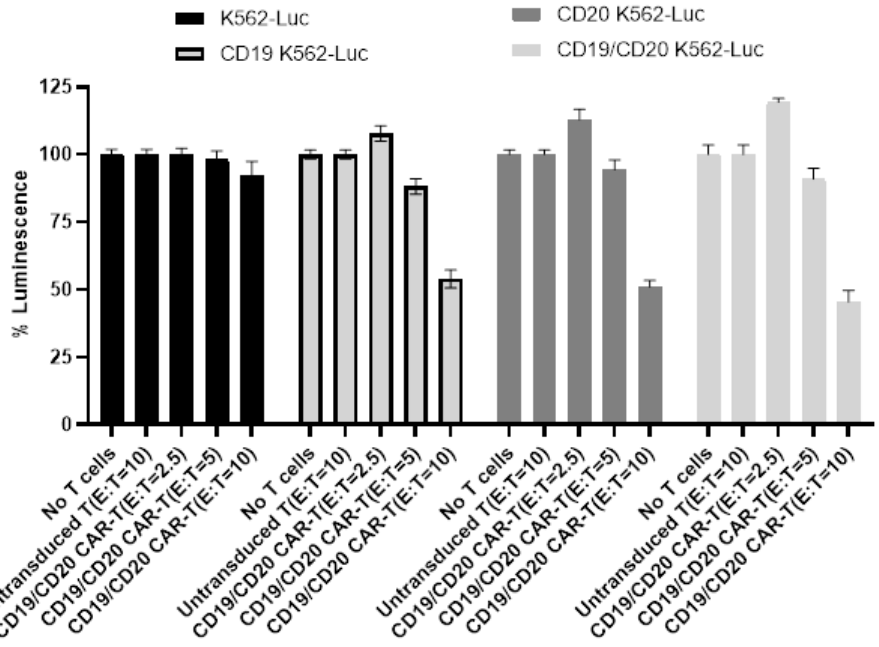

Figure 3. Luciferase-based cytotoxicity assay of Anti-CD19/CD20 CAR-T Cells using several Firefly Luciferase K562 cell lines as the target cells.
Anti-CD19/CD20 CAR-T cells (#82481) (effector, E) were thawed and co-cultured with Firefly Luciferase K562 cells that either not express or express CD20, CD19, or both (#78621, #82486, #82487, and #82488) as the target cells (target T) for 24 hours at the indicated E:T ratios. The lysis of the target cells was determined by measuring luciferase activity with ONE-Step™ Assay System (#60690). The assay was performed in parallel with untransduced T cells as negative control.

B. Degranulation Assay

- The following assay was performed in a 96-well plate with a 100 µl volume. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- We recommend using Untransduced T Cells (#78170) as negative control.
- We recommend using Firefly Luciferase K562 Cell Line (#78621) as negative control.

1. Thaw and culture Anti-CD19/CD20 CAR-T cells and Untransduced T Cells for 24 hours in CAR-T Cell Medium according to the protocol in the "Cell Culture Protocol" Section above.

2. Co-culture 1 x 10^5 Untransduced T or Anti-CD19/CD20 CAR-T cells with 2 x 10^4 Firefly Luciferase K562 target cells (#78621, 82486, 82487, and 82488) in CAR-T Cell Medium, with 5 µl of APC anti-human CD107a (LAMP-1) Antibody.

3. Incubate the cells at 37°C in 5% CO_2 for 6 hours.

   Optional: Add 2 µM of Monensin and 3 µM of Brefeldin A to each well 1 hour after co-culture, to aid with surface CD107a staining, and incubate the cells at 37°C in 5% CO_2 for another 5 hours.

4. Wash cells twice with Cell Staining Buffer and stain with FluoSite™ Anti-CD3 Antibody, FITC-Labeled for 30 minutes at RT.

5. Analyze cells by flow cytometry.

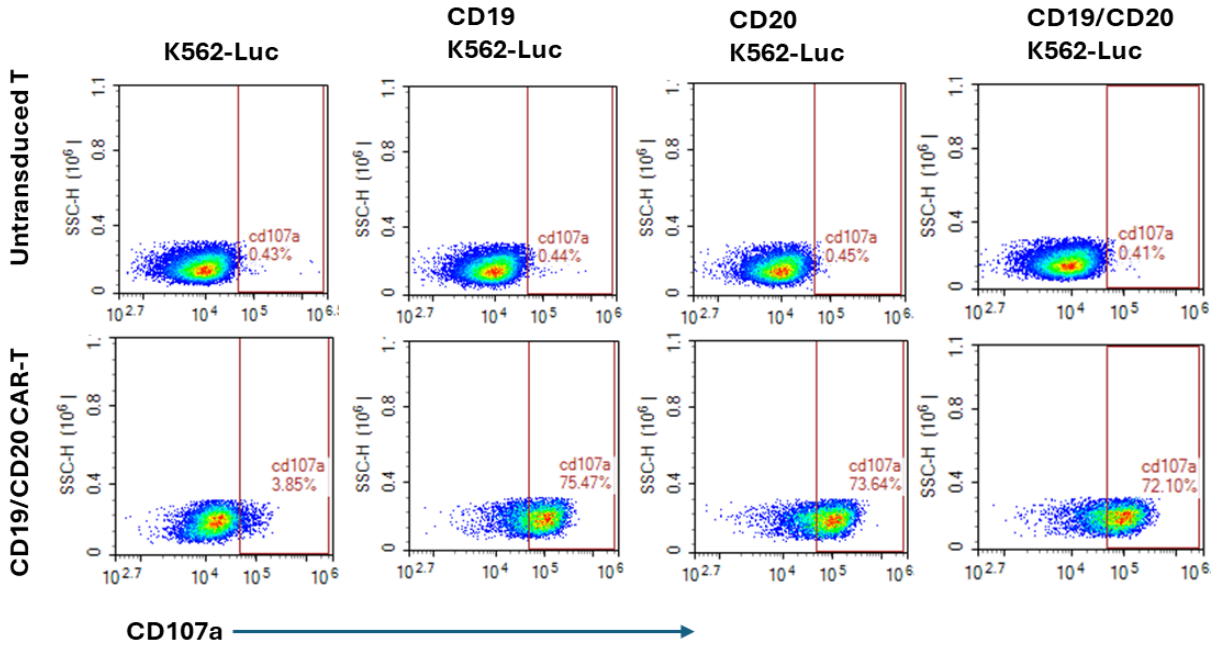

Figure 4: Flow cytometry analysis of CD107a degranulation in Anti-CD19/CD20 CAR-T Cells co-cultured with several Firefly Luciferase K562 cell lines as target.
Anti-CD19/CD20 CAR-T cells and Untransduced T cells were co-cultured with Firefly Luciferase K562 target cells expressing CD19, CD20, or both (#82486, #82487, and #82488) or with negative control Firefly Luciferase K562 cells (#78621) and APC anti-human CD107a (LAMP-1) Antibody (BioLegend #328620) for 6 hours. Cells were then stained with FluoSite™ Anti-CD3 Antibody, FITC-Labeled (BPS Bioscience #102008) and analyzed by flow cytometry. The x axis represents APC intensity, while the y axis shows CD3+ gated Side Scatter.

Data is representative.

References

Depoil D., *et al*., 2008 *Nat Immunol.* 9: 63-72.
van Zelm M.C., *et al*., 2006 *N Engl J Med.* 354: 1901-1912.
Kosmas C., *et al*., 2002 *Leukemia.* 16: 2004-2015.
Martyniszyn A., *et al*., 2017 *Hum Gene Ther.* 28(12): 1147-1157.

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 (BSL-2) precautions should be used.*

Troubleshooting Guide

Visit Cell Line FAQs for more information. For lot-specific information and all other questions, please email visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
Anti-CD19 CAR-T Cells	78171	1 Vial/ 5 Vials
Anti-CD19 CAR-T Cells (eGFP)	78789	1 Vial
Anti-CD20 CAR-T Cells	78611	1 Vial
Anti-BCMA CAR-T Cells	78660	1 Vial/ 5 Vials
Dual Epitope Anti-BCMA CAR-T Cells	78790	1 Vial/ 5 Vials
Anti-CD22 CAR-T Cells	78612	1 Vial

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