TOOLS FOR RESEARCH ON ANTI-CD19

The CD19 molecule (also known as Cluster of Differentiation 19, B-lymphocyte antigen CD19, B-Lymphocyte Surface Antigen B4, or CVID3) belongs to the family of immunoglobulins. It is a glycoprotein of 95 kDa expressed at the surface of B cell lymphocytes through almost all phases of B cell maturation from earliest stages to terminal differentiation, for which it is strictly required [1]. Thus, mutations in the CD19 gene cause severe immunodeficiency syndromes associated with impaired antibody production, such as CVID3 disease (common variable immunodeficiency 3) [2]. CD19 may also be involved in several autoimmune diseases.

CD19 contains two extracellular immunoglobulin-like domains termed C2, a single span hydrophobic transmembrane domain and a large C-terminal cytoplasmic domain containing several conserved tyrosine phosphorylation sites. No specific ligand has been identified; however, it is known that the protein forms a complex with CD21 and CD81. Activation of this complex has been shown to facilitate B cell activation by lowering the signaling threshold by several orders of magnitude [3]. Engagement of the BCR (B Cell antigen Receptor) by a membrane-bound antigen recruits tyrosine kinases SYK and LYN, which in turn phosphorylate the cytoplasmic domain of CD19 to serve as a docking site for SH2-containing signaling proteins (Figure 1).

The majority of B cell malignancies, notably B cell lymphomas, acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL), express normal to high levels of CD19. Since it is a marker of B cells, CD19 has been used to detect leukemia and lymphoma [4]. It is also a nearly ideal target for cancer immunotherapy. Blinatumomab, a CD19/CD3 bi-specific T cell engager (BiTE) has

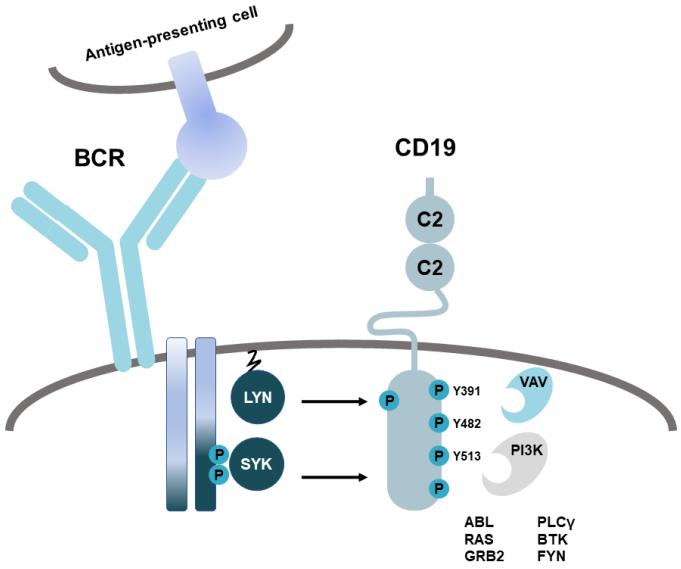


Figure 1: CD19 signaling

remarkable anti-tumor efficacy [5]. It has been approved for relapsed/refractory B-precursor ALL. In addition, CD19 is the target of the first CAR-T cell therapy, approved 30 years after the concept of a chimeric antigen receptor (CAR) was proposed [6].

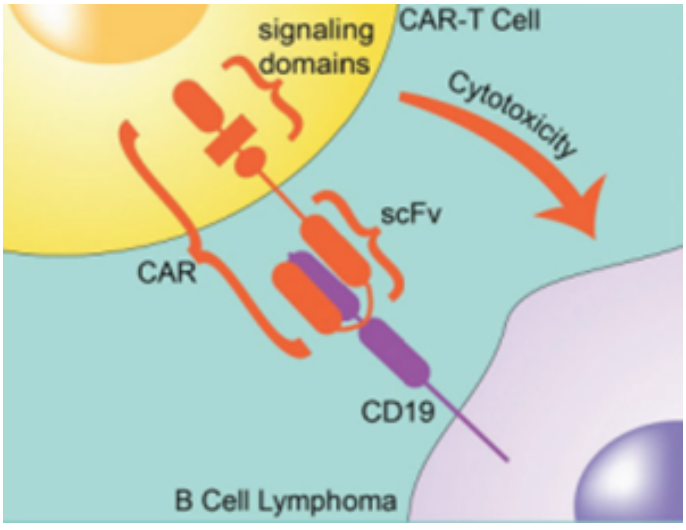


Figure 2: Illustration of the structure of an anti-CD19 CAR (CD19 ScFv-CD8-4-1BB-CD3ζ).

CD19-presenting cells

CD19-presenting cells such as the CD19 CHO (Chinese Hamster Ovary) Recombinant Cell Lines are particularly useful to screen, characterize and validate antibodies against CD19, or to study the binding of CAR constructs. Cells expressing CD19 can also be used to activate CAR-T cells in various experimental settings.

Clonal stable CHO cell lines were generated to constitutively express full length human CD19, as confirmed by flow cytometry (Figure 3). Each cell line was selected for low, medium or high levels of CD19 expression to model tumor cells. Cells with low expression of CD19 may be particularly useful to assess the potential efficacy of a new CAR construct against tumor cells expressing only low levels of CD19.

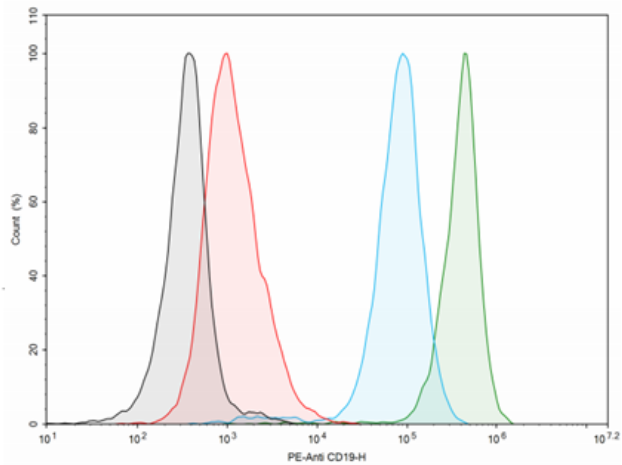


Figure 3: Expression of CD19 in CD19-CHO Recombinant Cell Lines (#79561)**.** The cells were incubated with PE-conjugated anti-human CD19 antibody (Biolegend) and analyzed by flow cytometry to detect surface expression of CD19. Green, blue and red: high, medium and low expression, respectively. Black: parental CHO cells.

CD19 reporter cells

CD19 Luciferase CHO Recombinant Cells constitutively express human CD19, as demonstrated by flow cytometry (Figure 4). The cells also stably express firefly luciferase, so that luciferase activity is directly proportional to the number of cells. This cell line offers a simple way to quantify changes in cell viability, which is particularly useful in co-culture experiments in which the cytotoxicity of T cells is determined against the CD19-presenting cells. Additional reporter cells were designed to co-express other targets of interest together with CD19, including CD20 (#78186), CD38 (#78149) and BCMA (#78030). These cells are used for the characterization of bi-specific antibodies and functional co-culture studies.

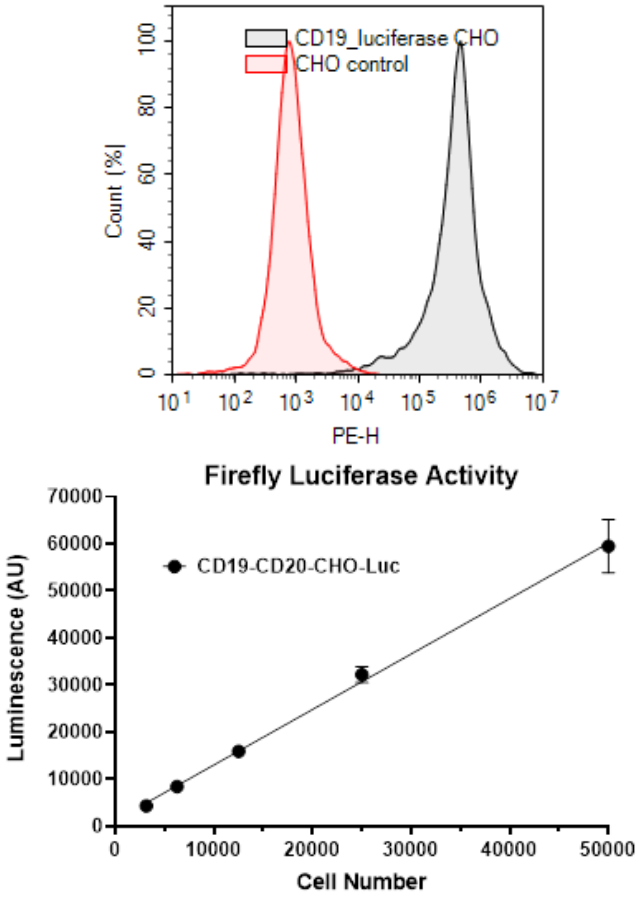
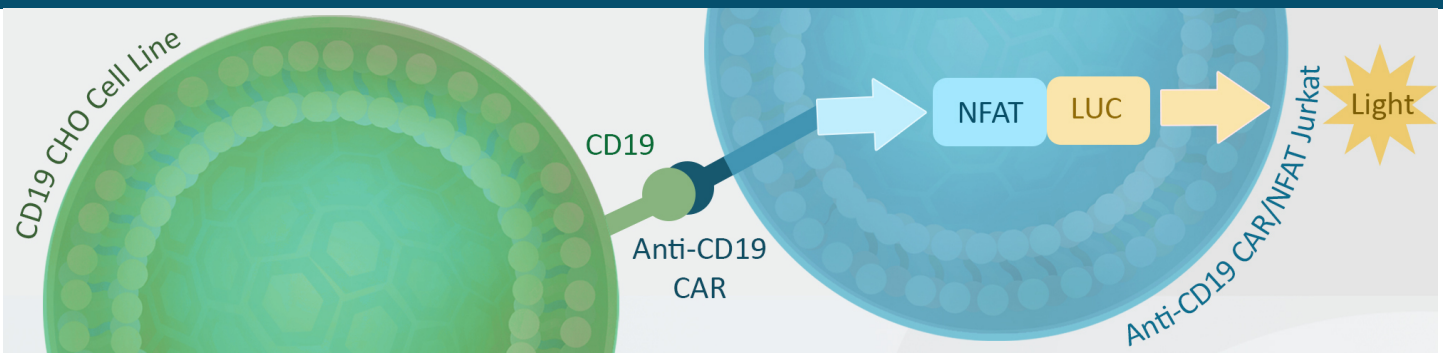
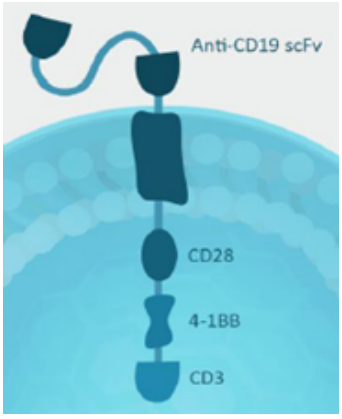


Figure 4: CD19 expression and Luciferase activity in reporter cells.
Left panel: CD19 luciferase cells were incubated with PE-conjugated anti-human CD19 antibody (Biolegend) and analyzed by flow cytometry (left panel). Parental CHO-K1 cells: red. CD19 Luciferase CHO cells #79714: black. **Right panel:** Increasing numbers of CD19/CD20 luciferase cells were seeded in a 96-well plate. Four hours later, luciferase activity was measured using the ONE-Step™ luciferase assay system (#60690).



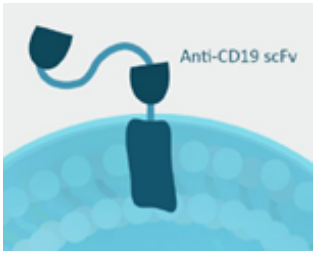
Anti-CD19 CAR Luciferase Reporter Jurkat cells

Jurkat cells are immortalized human T lymphocytes established from a patient with leukemia. A firefly luciferase reporter, expressed under the control of a promoter containing an NFAT response element, monitors the activation of transcription factor NFAT (Nuclear factor of activated T cells). This cell line has been validated for response to NFAT agonists, an anti-CD3 antibody, and the CD3/CD19 bispecific antibody blinatumomab in co-culture with CD19-positive Raji cells. The anti-CD19 CAR/NFAT-luciferase reporter Jurkat cell line (#79853) is a double transfectant cell line that can be used as a surrogate T cell to develop assays for CAR-T cell optimization, and as a positive control in co-culture assays. It is a valuable tools during the generation of CD19-expressing target cells.



This cell line displays constitutive expression of an anti-CD19 CAR in which the anti-CD19 ScFv is linked to the CD28 transmembrane and costimulatory domains, the 4-1BB costimulatory domain and the CD3ζ signaling domain. The CAR is activated when added to cells expressing CD19, ultimately leading to the stimulation of transcription factor NFAT which in turn induces luciferase expression.

The anti-CD19 CAR-negative/NFAT luciferase reporter Jurkat cell line is used as negative control in co-culture experiments with CD19-presenting cells. They express an anti-CD19 ScFv linked to the CD28 transmembrane domain but lacking the T cell activation components.



Although the cells contain the NFAT responsive luciferase reporter, the short CAR protein is incapable of inducing luciferase expression following binding to CD19.

Both cell lines contain the anti-CD19 ScFv, demonstrated by flow cytometry. The functional validation (Figure 5) shows that the anti-CD19 CAR Jurkat cell line was activated when co-cultivated with CD19 CHO cells, indicated by a 10-fold increase in luciferase activity, but not when co-cultivated with parental CHO cells. On the other hand, the luciferase reporter in anti-CD19 CAR-negative Jurkat luciferase cells was not activated by the CD19-presenting or the parental CHO cells.

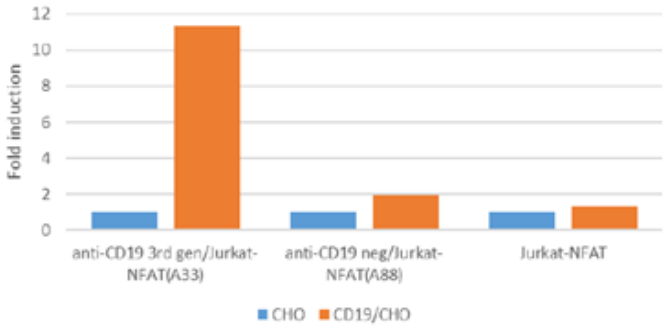


Figure 5: Functional validation of anti-CD19 CAR reporter Jurkat cells. Parental and CD19 CHO cell lines (#79561) were cultivated either with control (#60621), anti-CD19 CAR-negative (#79854), or anti-CD19 CAR (#79853) NFAT luciferase reporter Jurkat cells. Luciferase activity was measured using the ONE-Step™ luciferase assay system (#60690).

Anti-CD19 CAR lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ)

BPS Bioscience has developed off-the-shelf lentiviruses, ready for infection and capable of transducing most types of dividing or non-dividing mammalian cells. None of the HIV genes from the backbone virus are expressed in the transduced cells, therefore these viral particles are replication incompetent and can be used in a Biosafety Level 2 facility, which makes them accessible to many research laboratories.

The anti-CD19 CAR lentivirus is pseudotyped, which means that the HIV envelope protein has been replaced with VSV-G (Vesicular stomatitis virus G), a protein that binds to the ubiquitously expressed LDL receptor (low-density lipoprotein). Upon infection of the target cells, the anti-CD19 CAR protein is transduced into the cells and expressed under the control of constitutive mammalian promoter EF-1α. Stable expression can be achieved following puromycin selection.

The anti-CD19 CAR construct (Figure 6) consists of the ScFv portion of highly specific monoclonal anti-CD19 antibody (clone FMC63) linked to the CD8 hinge, 4-1BB and CD3ζ signaling domains. FMC63 ScFv is the most commonly used ectodomain of anti-CD19 CARs.

Stable expression in transduced cells was demonstrated by flow cytometry. Figure 7 shows

Figure 6: Diagram of the lentiviral CD19 CAR construct (#78600).

Application: Useful as a positive control in experiments that evaluate anti-CD19 CAR constructs, to optimize T cell transduction, and to develop functional assays.

that a proportion of the T cells were not effectively transduced and did not express the CAR construct, whereas the fraction of cells that were transduced displayed robust anti-CD19 ScFv expression.

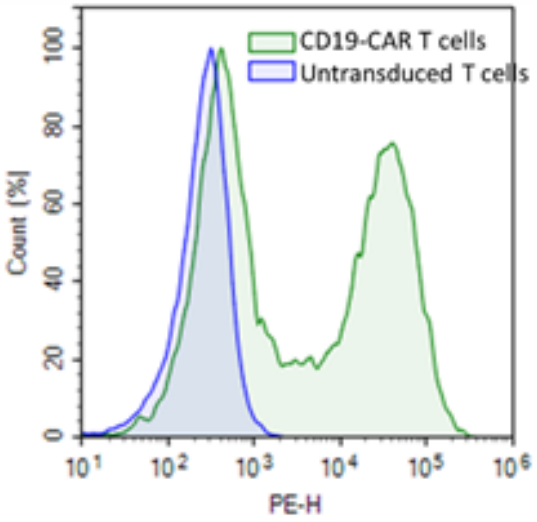


Figure 7: Expression of anti-CD19 CAR in T cells transduced with the lentivirus. Approximately 15,000 CD4+/CD8+ activated T cells were transduced (MOI of 40) in the presence of 5 µg/mL of polybrene via spinoculation. Anti-CD19 CAR expression was analyzed 10 days later by flow cytometry using PE-labeled anti-FMC63 ScFv (Acrobiosystems). Transduced cells: green; non-transduced: blue.

Functional characterization of the stably transduced T cells included measuring interferon release following addition of CD19-expressing cells. IFN-γ is a cytokine secreted by activated T cells that has been recognized as an indicator of T cell activation. The anti-CD19 CAR-T cells released IFN-γ when cultured with CD19-presenting CHO cells but not with CHO cells, while control T cells did not release IFN-γ in any condition (Figure 8, left). In another set of experiments, the transduced T cells were co-cultured with CD19 luciferase CHO cells. As shown in Figure 8 (right), co-culture with anti-CD19 CAR-T cells killed the CD19 luciferase CHO cells, evidenced by a 95% drop in luciferase activity.

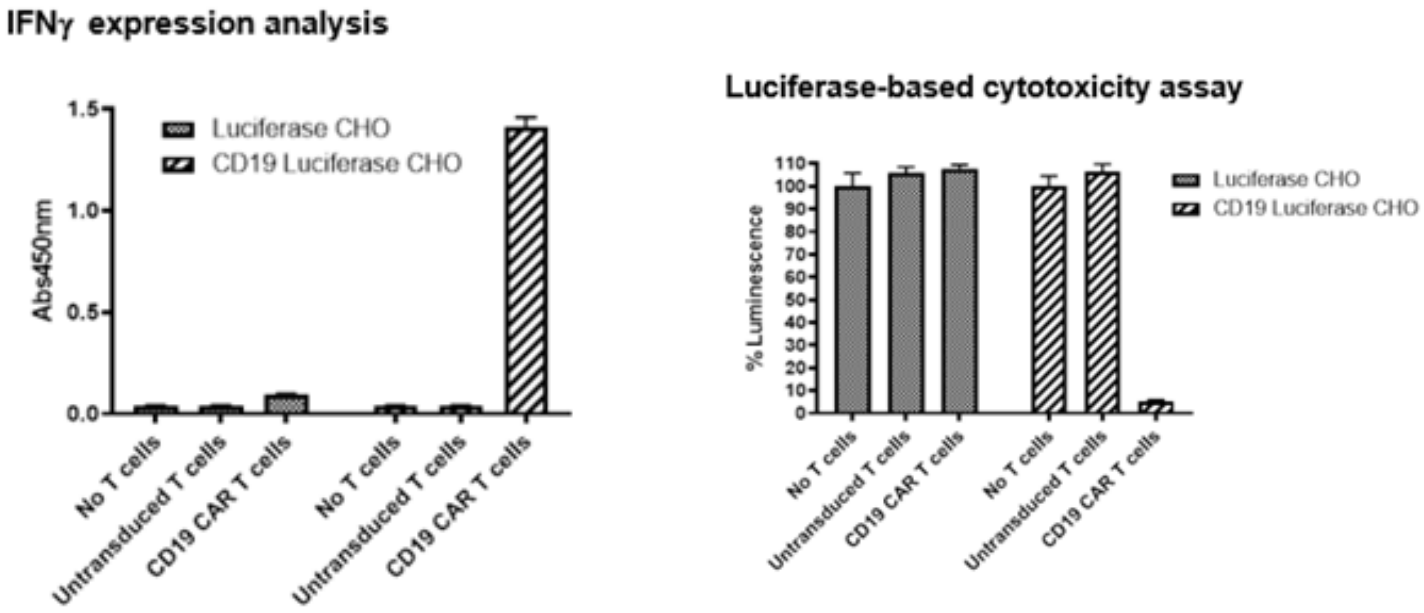


Figure 8: Functional validation of lentivirus-transduced CAR T cells. Approximately 15,000 CD4+/CD8+ activated T cells were transduced with anti-CD19 CAR lentivirus (MOI of 40) in the presence of 5 µg/mL polybrene using spinoculation. Transduced T cells were expanded and 12 days later they were co-cultured with luciferase CHO cells (#79725) or with CD19-expressing luciferase CHO cells (#79714) for 24 hours at a ratio of 1 to 20. Non-transduced T cells were used as negative control. **Left panel:** The culture medium was collected and assayed using the IFN-γ Colorimetric ELISA Detection Kit (#79777). **Right panel:** Luciferase activity was quantified using the ONE-Step™ luciferase assay system (#60690).

Conclusion

In addition to the lentivirus and antigen-presenting cell lines discussed here, our portfolio of CD19-related products [CD19] includes affinity purified recombinant proteins (labeled or not), as well as mono-specific and bi-specific antibodies. Cytokine assay kits and reporter cell systems allow for the functional validation of anti-CD19 CAR-T cells using biologically relevant cellular models.

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