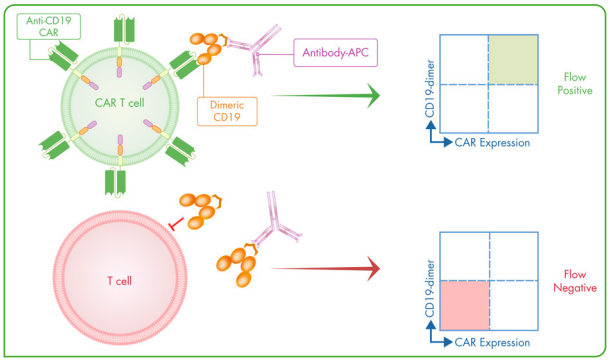
Application Note

Reliable Detection of Anti-CD19 CAR T cells Using Dimeric CD19 by Flow Cytometry

Introduction

Anti-CD19 chimeric antigen receptor (CAR) T-cell therapies have demonstrated remarkable clinical success in the treatment of B-cell malignancies, driving continued development and optimization of new CAR constructs (1). Reliable methods for detecting CAR expression are critical for evaluating and screening candidate constructs during CAR-T development.



Flow cytometry is commonly used for CAR-T detection. The most direct CAR-T detection method uses the target antigen itself to detect CAR expression through direct interaction with the CAR antigen-binding domain (3), although some strategies rely on linker or engineered tag detections (2). Multimeric antigen formats can enhance binding avidity and improve detection sensitivity. To support CAR T-cell detection workflows, we developed a non-Fc dimeric CD19 reagent designed for robust interaction with anti-CD19 CARs.

In this application note, we demonstrate that the non-Fc dimeric CD19 binds multiple FDA-approved anti-CD19 therapeutic antibodies, confirming proper epitope presentation. We further show that the reagent enables efficient detection of anti-CD19 CAR expression in both CD4+ and CD8+ T cells across a range of concentrations suitable for CAR screening applications.

Results

Therapeutic Antibody Recognition of Recombinant Non-Fc CD19 Dimers

Prior to evaluating CAR recognition, the biochemical integrity and antibody binding properties of the recombinant CD19 dimer were assessed.

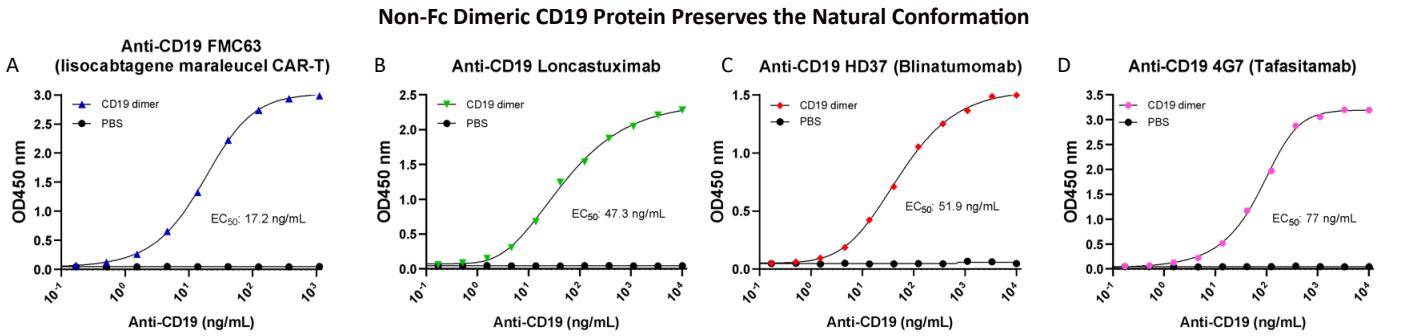


Figure 1. *Non-Fc Dimeric CD19 Protein can be potently and specifically recognized by FDA approved CD19 therapeutic antibody clones measured by ELISA. (**A**) Lisocabtagene maraleucel CAR-T therapeutics (clone FMC63); (**B**) Loncastuximab antibody conjugate drug (ADC); (**C**) Blinatumomab bispecific therapeutic antibody (clone HD37); (D) Tafasitamab therapeutic monoclonal antibody (clone 4G7).*

To verify that the CD19 dimers retain native antigenic conformation, binding was evaluated using enzyme-linked immunosorbent assays (ELISAs) with four clinically relevant anti-CD19 antibodies. These included Lisocabtagene maraleucel CAR-T therapeutics (clone FMC63), Tafasitamab (clone 4G7), Loncastuximab, and Blinatumomab (clone HD37). Each antibody demonstrated specific and potent binding to the recombinant CD19 dimers, indicating that key epitopes recognized by therapeutic antibodies are preserved (Figure 1).

The observed binding across multiple antibody clones spanning distinct therapeutic modalities - including monoclonal antibodies, antibody-drug conjugates, and bispecific antibodies - supports the structural integrity of the recombinant antigen. Collectively, these results demonstrate that Conigen's CD19 dimers are both highly pure and conformationally intact, making them well suited for downstream applications such as CAR T cell detection and characterization assays.

Dimeric CD19 Enables Specific Detection of Anti-CD19 CAR Expression in T Cells.

The non-Fc dimeric CD19 was evaluated for its ability to detect anti-CD19 CAR expression in engineered T cells by flow cytometry. To assess the performance of the CD19 dimer fused with a C-terminal His-tag, CAR-expressing T cells were stained with a titration of dimeric CD19 ranging from 0 to 20 ug/mL and analyzed within both CD4+ and CD8+ T cell populations (Figure 2A & 2B) transfected using CD19-CAR mRNA. Following staining with dimeric CD19, bound protein was detected using an APC-conjugated anti-His-tag antibody recognizing the His-tag on the CD19 dimer, allowing quantification of CD19-specific CAR T-cell binding by flow cytometry. Across this concentration range, dimeric CD19 enabled clear identification of CAR-expressing cells in both CD4 and CD8 T cells, demonstrating consistent binding to the CAR antigen-binding domain. Concentrations of 5 ug/mL and above resulted in greater than 80% detection of CAR-positive cells (Figure 2C).

Detection of Anti-CD19 CAR Expression Using Dimeric CD19 by Flow Cytometry

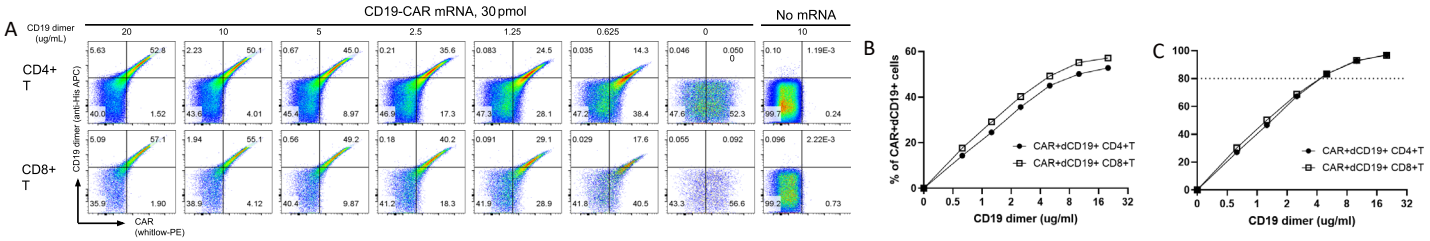


Figure 2. Detection of Anti-CD19 CAR Expression Using Dimeric CD19 by Flow Cytometry. (A) CD4+ and CD8+ T-cells were stained with a titration of dimeric CD19 ranging from 0 to 20 µg/mL and analyzed by flow cytometry to evaluate detection efficiency. Bound CD19 dimer was detected using an APC-conjugated anti-His antibody recognizing the His-tag on the CD19 reagent. T cells transfection with no mRNA were also stained and had no datable signal, confirming staining is dependent on CAR expression. (B). Graph showing the relationship between CD19 dimer concentration (ug/mL) and % of CAR+ and dimeric CD19+ cells. (C) Data from (B) normalized to the maximum signal observed at 20 ug/mL to illustrate relative detection efficiency across CD19 dimer concentrations.

To evaluate assay specificity, a negative control was included in which T cells were processed identically but transfected with no mRNA. When stained with dimeric CD19, these cells showed no detectable signal, as expected. The CD19 dimer does not produce detectable background in the absence of an anti-CD19 CAR. Combined, these results indicate that dimeric CD19 provides specific, sensitive and reliable detection of anti-CD19 CAR expression and can support efficient evaluation of CAR-T cell populations during development and characterization.

Conclusion

Dimeric CD19 was successfully produced and characterized as a functional reagent for detecting anti-CD19 CAR T cells.

1. Analytical characterization confirmed high protein purity, supporting the overall quality and suitability of the reagent for downstream applications.
2. Functional validation further demonstrated that the CD19 dimer binding multiple FDA-approved anti-CD19 therapeutic antibodies, indicating proper folding and preservation of clinically relevant CD19 epitopes.
3. The dimeric CD19 reagent enabled robust detection of anti-CD19 CAR expression on CD19-CAR transfected T cells by flow cytometry. Dimeric CD19 specifically bound CAR-expressing cells and produced no detectable signal in negative control cells lacking CAR expression, confirming assay specificity.
4. A titration experiment demonstrated reliable CAR detection across a wide concentration range, with concentration of 5 ug/mL and above achieving greater than 80% detection of CAR-positive cells in both CD4+ and CD8+ T cells populations.

These results highlight the utility of dimeric CD19 as a sensitive and specific reagent for CAR detection and support its application in CAR construct screening and CAR-T development workflows.

References

1. June CH, et al, *N Engl J Med* 2018, 379:64
2. Hamieh M, et al, *Front Immunol* 2020, 11:1770
3. Mazzoni A, et al, *Cytometry A* 2023, 103:379