Simplifying CD19 CAR Detection: A Bright Single-Step Solution for Flow Cytometry

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Abstract

Introduction/Rationale
Accurate flow cytometric detection of CD19 CAR-expressing cells is critical across CAR-T cell discovery and translational research workflows. However, variability in surface CD19 CAR protein expression presents challenges in reliably identifying CAR+ cells. Additionally, detection using recombinant CD19 protein is hindered by its instability in soluble form.

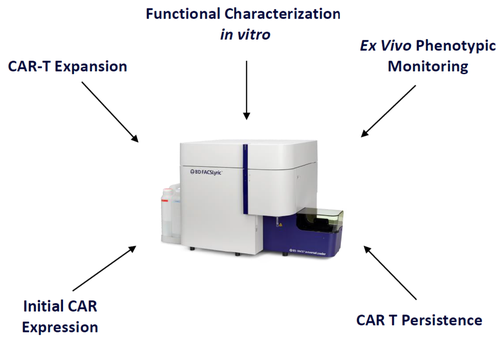
Methods
To address these limitations, BD has developed a novel, bright, and stable one-step CD19 CAR Detection Reagent, available in multiple fluorochrome formats to support diverse panel designs and instrument configurations.

Results
We demonstrate that this reagent specifically stains CD19 CAR-expressing CHO cells, Jurkat CAR-T cells, and primary human CD19 CAR-T cells. Furthermore, it performs effectively in lysed whole blood, multi-color panels, and workflows involving intracellular staining. Notably, CAR-T cells spiked into donor PBMCs were detected at frequencies as low as < 0.04% of live cells, highlighting potential utility in studies of CAR-T cell persistence. Minimal background staining was observed in CAR-negative and mismatched CAR populations, and low-expressing CAR cells were distinguishable from negative cells.

Conclusion
Overall, these findings highlight the BD® CD19 CAR Detection Reagent as a robust solution for overcoming key challenges in CD19 CAR+ cell detection, enabling progressive CAR-T cell research.

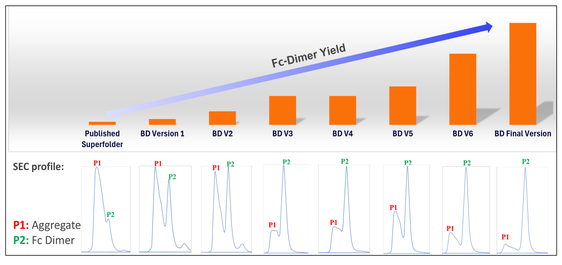
Objective

High-performance CD19 CAR Detection Reagent to Meet CAR T Workflow Needs



Development of a Stable CD19 Reagent

A) Iterative improvement



B) Final Design

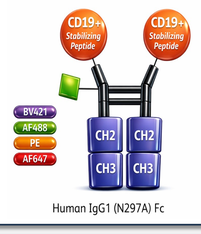
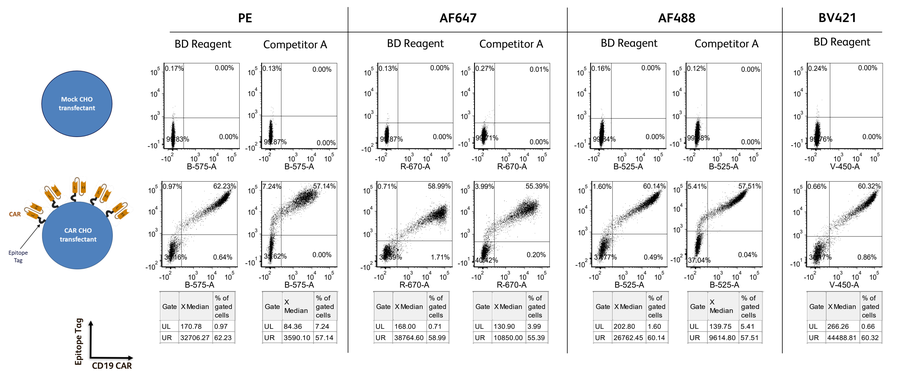


Figure 1. Development of a stable soluble CD19 CAR Detection Reagent. **(A)** Successive design iterations improved dimer yield >50-fold and dramatically reduced aggregation. **(B)** Final design of BD CD19 CAR Detection Reagent is a proprietary fusion protein containing $CD19_{21\text{-}278}$, a stabilizing peptide, and huIgG1(N297A) Fc. These fusion protein dye conjugates have a shelf life >18 months at 4°C.

Results

Staining Performance

A) CHO-CAR Transfectants



B) Primary CAR T

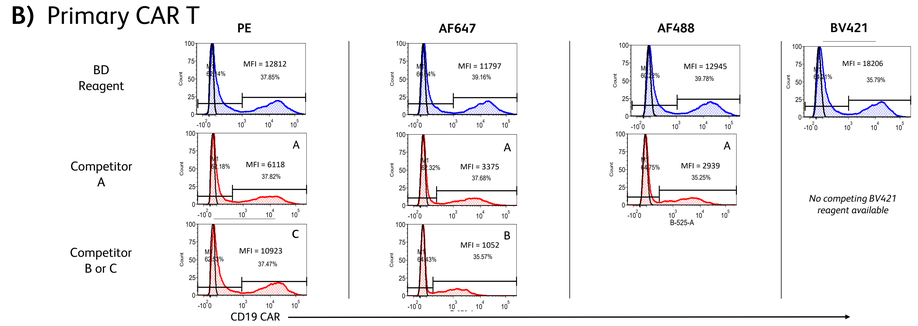
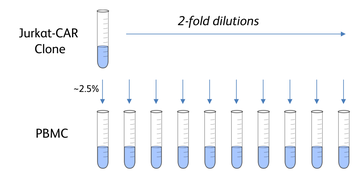


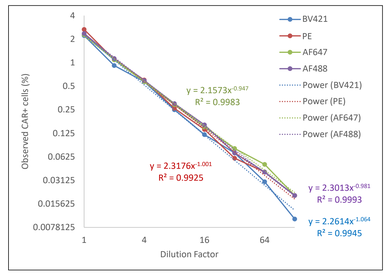
Figure 1. Staining Performance. **(A)** CHO cells were transfected with mock- or CD19-CAR and stained with BD or competitor CD19 CAR Detection Reagents (at recommended concentrations) with epitope tag antibody co-stain for 30 min at RT. **(B)** T cells purified by negative depletion from a healthy donor in the Associate Sample Collection Program (ASCP) were activated with anti-CD3/28 beads + IL-2 for 2d before infection with CD19-CAR lentivirus, followed by expansion for 7-10 days with IL-2 and cryopreservation. Thawed cells were stained with the indicated CAR T detection reagents at recommended concentrations.

Rare Cell Detection

A) Model



C) Summary



B) Representative Data (PE)

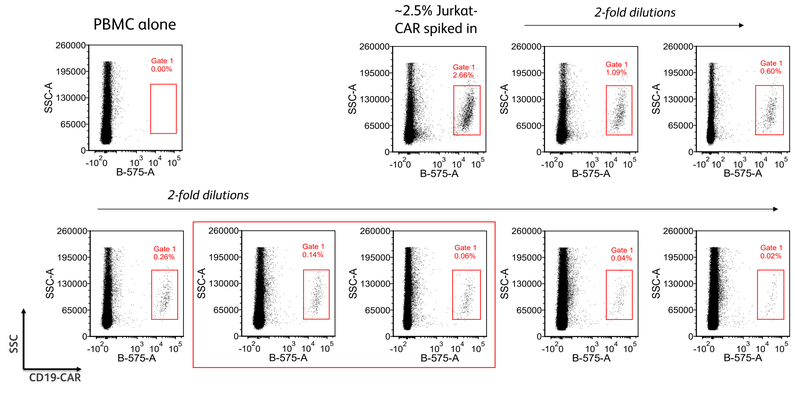
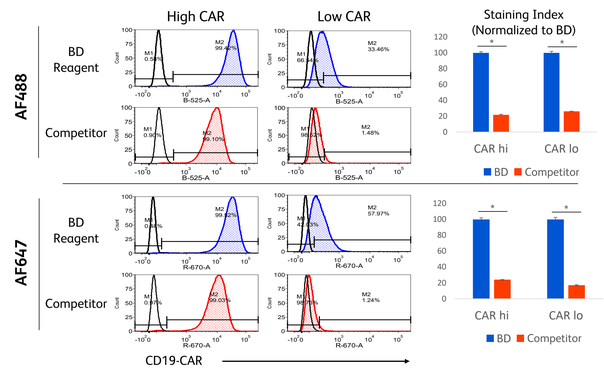


Figure 2. Rare Cell Detection. **(A)** *Model.* Jurkat-CAR clone isolated from CD19 CAR lentivirus-infected cells was spiked into PBMC from a healthy donor in the Associate Sample Collection Program (ASCP) at approximately 2.5% or one of 9 descending 2-fold Jurkat clone dilutions. **(B)** *Representative Data (PE).* Jurkat-CAR-spiked PBMC were stained with PE-conjugated BD CD19 CAR Detection Reagent at test size for 30 min at RT, following by flow cytometric analysis. Red box highlights detection of CAR+ cells at ≤0.1% of PBMC. **(C)** *Summary.* Observed percentages of CAR+ cells were plotted vs. predicted % based on 2-fold division of initial % to estimate ability to accurately detect CAR+ rare cells at ≤0.1%.

Sensitivity on low-level or low-affinity CARs

A) Staining on CAR^{hi} and CAR^{lo} Jurkat clones



B) Staining on high and low affinity CAR: detection options

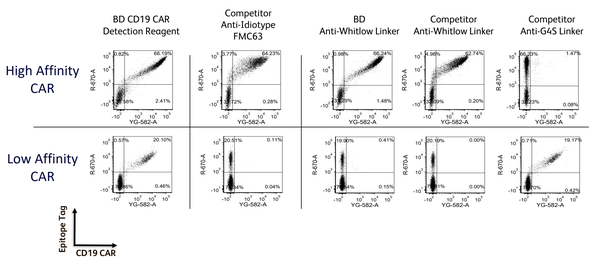
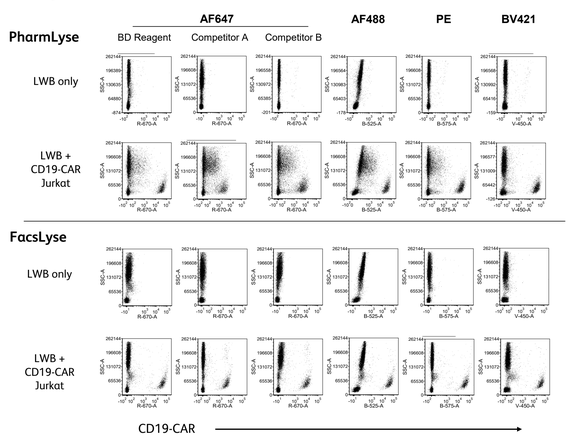


Figure 3. Detection of low CAR expression with CD19 CAR Detection Reagents. **(A)** Jurkat cells were infected with CD19 CAR lentivirus and single-cell cloned to obtain CAR hi and CAR low clones. These were stained with BD vs. indicated competitor reagents at recommended concentrations for 30 min at RT. Histograms are representative data; bar graphs summarize data as % of BD staining index. Error bars are S.E.M. of triplicate staining wells. *p-value < 0.00001 **(B)** CHO cells were transfected with high affinity (FMC63 scFv containing Whitlow linker) or low affinity (CAT13.1E10 scFv containing G4S linker) CD19-CAR and stained with indicated BD or competitor PE-conjugated reagents (at recommended concentrations) for 30 min at RT.

Buffer Compatibility

A) Lysis Buffers



B) Fixation Buffers

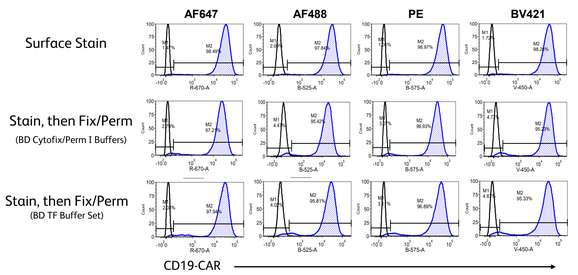


Figure 4. Buffer Compatibility. **(A)** Lysed whole blood (LWB) from a healthy donor was spiked with CD19 CAR hi Jurkat clone from Fig. 3. and stained with BD or competitor CD19 CAR Detection Reagents at recommended concentrations, followed by lysis with BD Pharm Lyse™ or BD FACS™ Lysing Solution. **(B)** CD19 CAR hi clone from Fig. 3 was stained using BD CD19 CAR Detection Reagents and BD Cytofix™ Fixation Buffer and BD Perm/Wash™ Buffer or the BD Pharmingen™ Transcription Factor Buffer Set using the recommended protocol.

CD19 CAR Reagents Summary

- Advantages over linker Abs
  - Antigen binds CAR consistently
  - Specificity allows dual/tandem CAR study
- BV421, AF488, PE, & AF647 conjugates
- Bright one-step reagents
- Low background
- Capable of detecting rare CAR+ T cells in PMBC
- Sensitivity to detect low level/affinity CAR
- Broad Compatibility
  - Lysis buffers
  - Permeabilization buffer
  - Pre-fixed cells
- Long shelf-life (at least 2 yrs at 4°C)*
- Compatible with lyophilization*

* Data not shown in poster; tested during development