Detection of Rare and Low-expressing BCMA CAR-T cells with BD® 1-step CAR Detection Reagent

Poster# 2166

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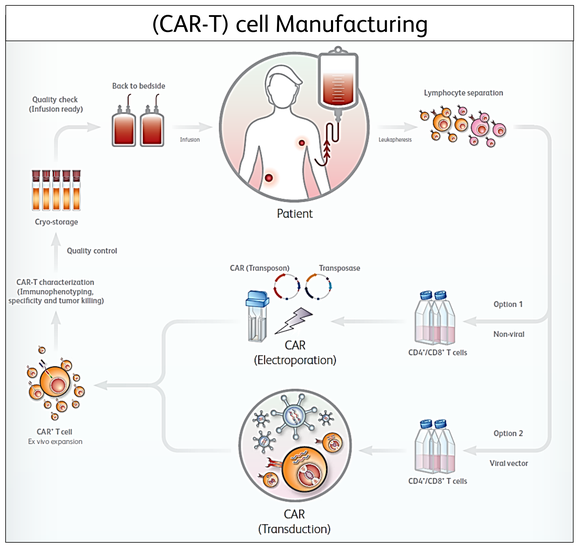
Abstract

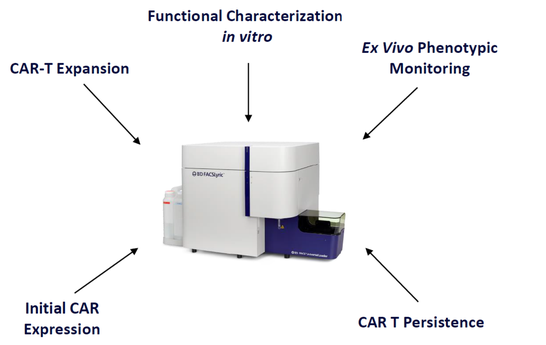
Flow cytometric identification and analysis of CAR-expressing cells is applied in many stages of early drug discovery and translational research. However, surface CAR protein expression can vary widely, making accurate identification and flow cytometric analysis of CAR+ cells difficult. BD® has developed 1-step reagents for resolving CAR-expressing cells using flow cytometry that are compatible with multiple fluorochrome options to accommodate varying panel needs and instrument constraints. Here, we show that our CAR Detection Reagents specifically stain CAR-expressing CHO, Jurkat CAR-T, and primary human CAR-T cells. In addition to *in vitro* cell culture settings, our CAR Detection Reagents performed well on lysed whole blood and in simple multi-color panels and showed compatibility with intracellular staining workflows. CAR-T cells added to donor PBMC were detected by this reagent at <0.1% proportions, showing potential value in research studies on CAR-T persistence. Importantly, background staining was minimal on CAR-negative blood cell populations, and CAR^{lo} cells could be resolved from negative cells. Overall, our data indicate that these new reagents address the challenges of CAR+ cell detection and will empower CAR cell research.

Objective



Goal:

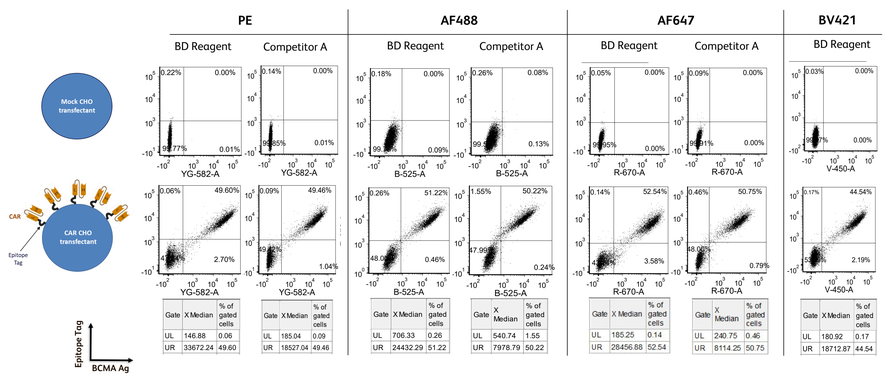
Create a High-performance CAR Staining Reagent to Meet CAR T Workflow Needs



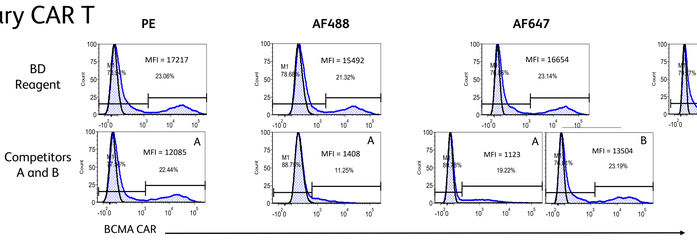
Results

Staining Performance

A) CHO-CAR Transfectants



B) Primary CAR T



C) VHH BCMA CAR

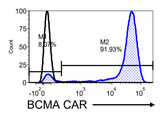
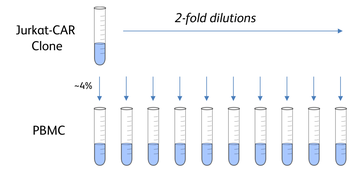


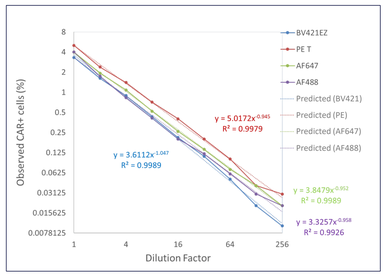
Figure 1. Staining Performance. **(A)** CHO cells were transfected with mock- or BCMA-(scFv)CAR and stained with BD (0.4 µg for BV421, others at 0.1 µg/test) or competitor BCMA CAR Detection Reagents (at recommended concentrations) with epitope tag antibody co-stain for 30 min at RT. **(B)** T cells purified by negative depletion from healthy donors were activated with anti-CD3/28 beads + IL-2 for 2d before infection with BCMA-CAR lentivirus, followed by expansion for 7-10 days and cryopreservation. Thawed cells were stained with the indicated CAR T detection reagents (BD at 0.25 µg/test, competitors at recommended concentrations.) **(C)** Jurkat T cells were infected with BCMA (VHH) CAR before staining with BD BCMA CAR AF647 Detection Reagent.

Rare Cell Detection

A) Model



C) Summary



B) Representative Data (PE)

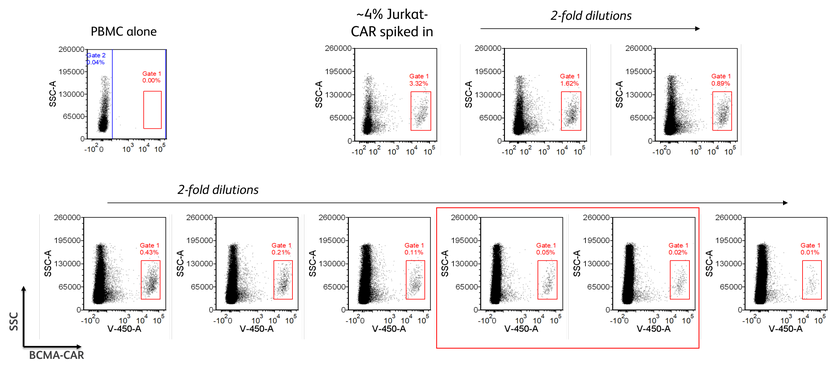
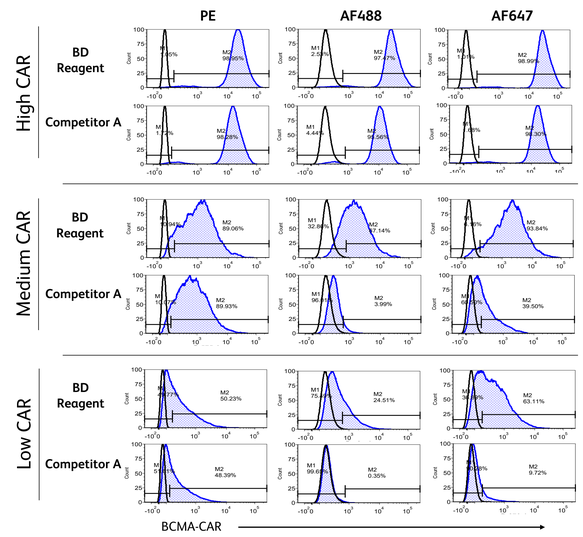


Figure 2 Rare Cell Detection. **(A)** *Model.* Jurkat-CAR clone isolated from BCMA (scFv)CAR lentivirus-infected cells was spiked into PBMC from a healthy donor in the Associate Sample Collection Program (ASCP) at approximately 4% and 9 additional descending 2-fold dilutions. **(B)** *Representative Conjugate Data (PE).* Jurkat-CAR-spiked PBMC were stained with PE-conjugated BD BCMA CAR Detection Reagent at 0.1 µg/test 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 differing CAR levels

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



B) Normalized staining index vs. competitor

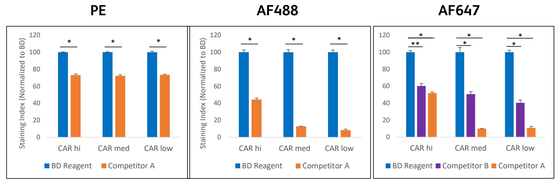
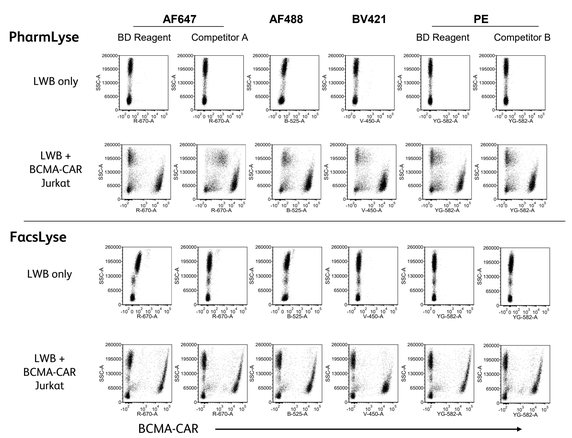


Figure 3. Detection of low CAR expression with BCMA CAR Detection Reagent. **(A)** Jurkat cells were infected with BCMA-(scFv)CAR lentivirus and single-cell cloned to obtain CAR hi, CAR med, and CAR low clones. These were stained with BD vs. indicated competitor reagents at recommended concentrations for 30 min at RT (BD reagents at 0.25 µg for PE and AF488; 0.125 µg/test for AF647 conjugate). **(B)** Data from (A) was used to compare ability to resolve high, medium, and low-CAR expression between BD and competitor reagents. Data is displayed as % of BD staining index. Error bars are S.E.M. of 3 replicate staining wells. **p*-value < 0.00015; ***p*-value < 0.0015

Buffer Compatibility

A) Lysis Buffers



B) Fixation Buffers

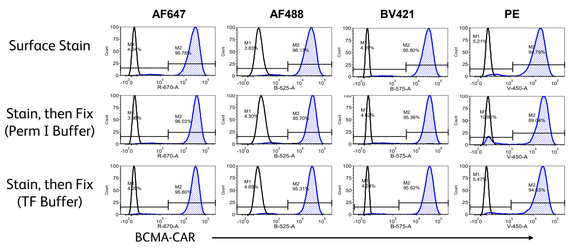


Figure 4. Buffer Compatibility. **(A)** Lysed whole blood (LWB) from healthy donors was stained with BD or competitor BCMA CAR Detection Reagents (BD at 0.1 µg/test, competitors at recommended concentrations), following by lysis with PharmLyse or FacsLyse buffers. **(B)** BCMA CAR hi clone from Fig. 3 was stained with BD BCMA CAR Detection Reagents at 0.125 for AF647, 0.25µg/test for other conjugates in the indicated BD buffers following standard protocols.

BCMA CAR Detection Reagent

- Advantages over linker Abs
  - Highly specific
  - Useful for dual & tandem CARs
- 4 Direct conjugates for flexibility
- Very competitive performance
- Excellent rare cell detection
- Sensitivity to detect low CAR expression
- Long shelf-life (up to 1.5 yrs at 4°C)
- Broad Compatibility
  - Lysis buffers
  - Permeabilization buffer
  - Transcription factor buffer