

CAR-T cell monitoring using specific multimers: a fast and specific method allowing uniform evaluation.

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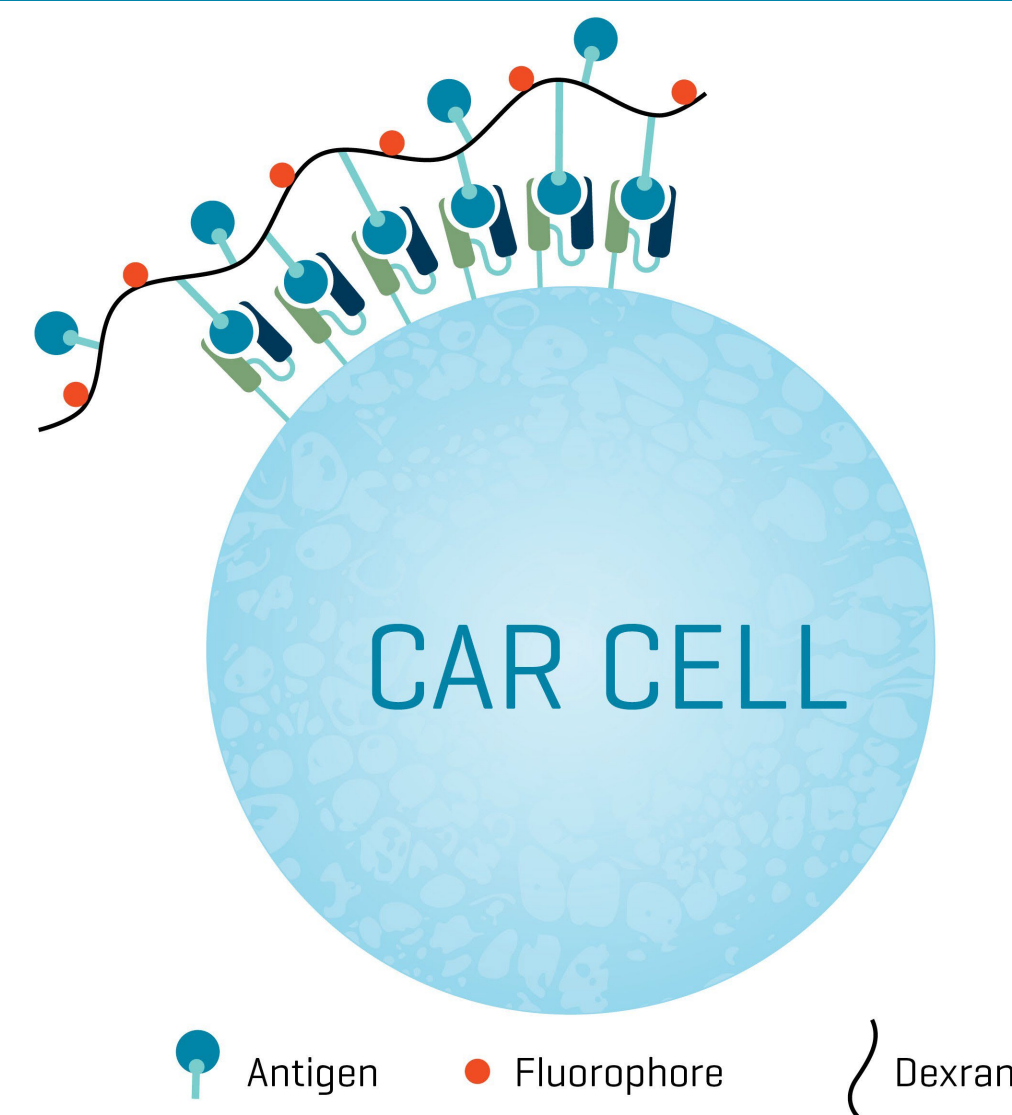
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Introduction

Chimeric antigen receptor (CAR)-T cell therapy is a revolutionary new pillar in cancer treatment. In research CAR-T cells are often detected through tags added to the CAR construct. However, these methods lack sensitivity and do not address CAR recognition of its target. Detection methods using the targeted ligand (ie: antigen + fluorescent antibody) exist but they are indirect and laborious.

In this study we developed:

- a flexible platform for developing CAR Dextramer[®] reagents
- CD19, BCMA and Mesothelin CAR Dextramer[®] reagents which can specifically detect CAR-T cells.



CAR reagent development platform

CAR Dextramer[®] reagents of different specificities (CD19, BCMA, Mesothelin...) were developed using a CAR Dextramer[®] reagent development platform (Fig. 1). During development, antigen specifications (Fig. 2) as well as target protein identity (Fig. 4 and 5) and ability to detect CAR T-cells (Fig 6) are tested and evaluated using different assays.

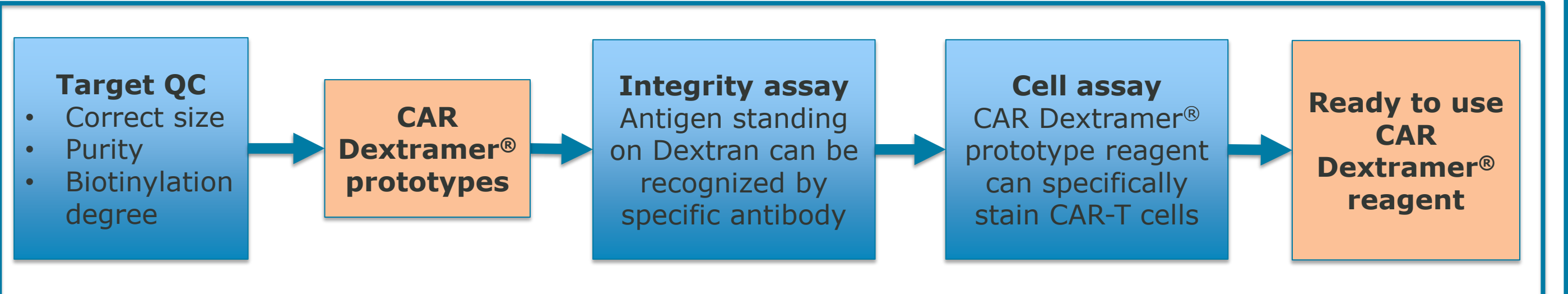
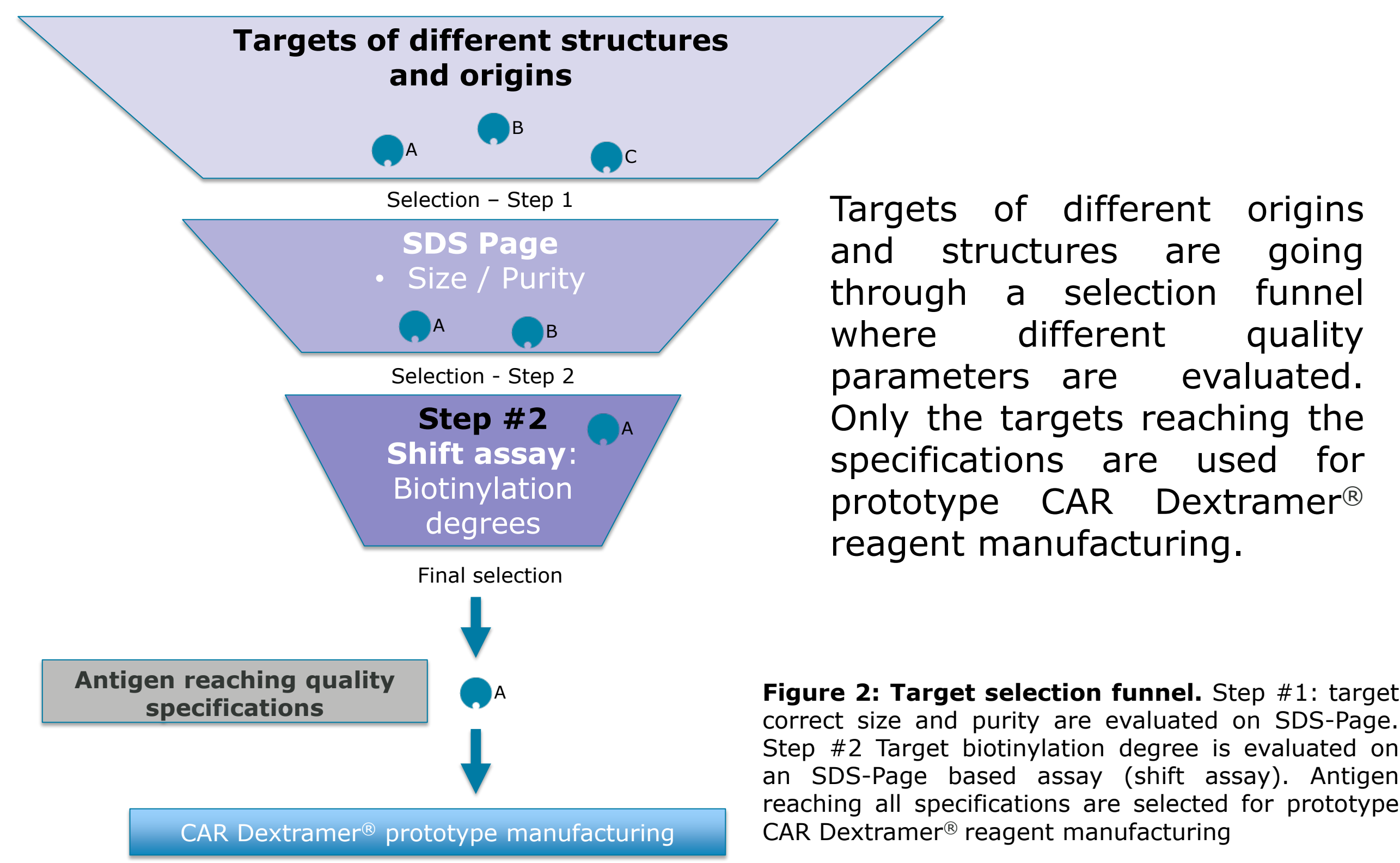


Figure 1: CAR Dextramer[®] reagent development platform.

Target quality evaluation and selection



Targets of different origins and structures are going through a selection funnel where different quality parameters are evaluated. Only the targets reaching the specifications are used for prototype CAR Dextramer[®] reagent manufacturing.

Figure 2: Target selection funnel. Step #1: target correct size and purity are evaluated on SDS-Page. Step #2 Target biotinylation degree is evaluated on an SDS-Page based assay (shift assay). Antigen reaching all specifications are selected for prototype CAR Dextramer[®] reagent manufacturing

Target integrity evaluation and CAR Dextramer[®] prototype screening

Targets fulfilling the quality requirements were used to manufacture CAR Dextramer prototypes. The prototypes had different specificities (CD19, BCMA or Mesothelin) and stoichiometries. They were run through an artificial cell assay to evaluate (i) target integrity: ability to be recognized by a specific antibody and (ii) influence of stoichiometry on signal intensity. All prototypes could be specifically recognized by anti-target mAb-coated beads assessing target accessibility and correct refolding. The prototypes, however, did not give the same signal-to-noise ratio showing the importance of dextran:target stoichiometry.

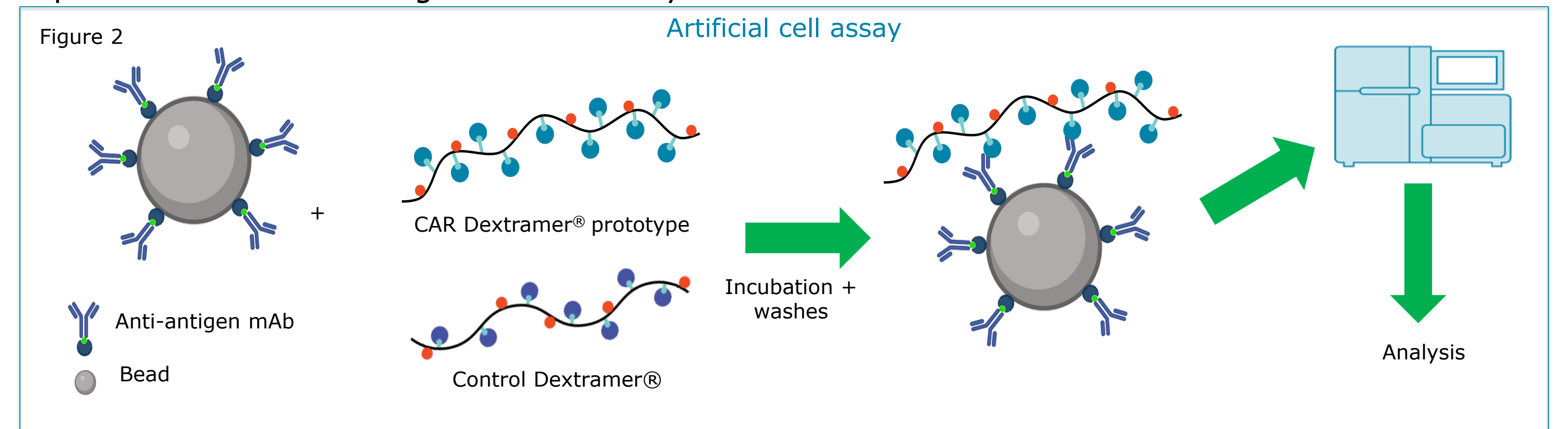
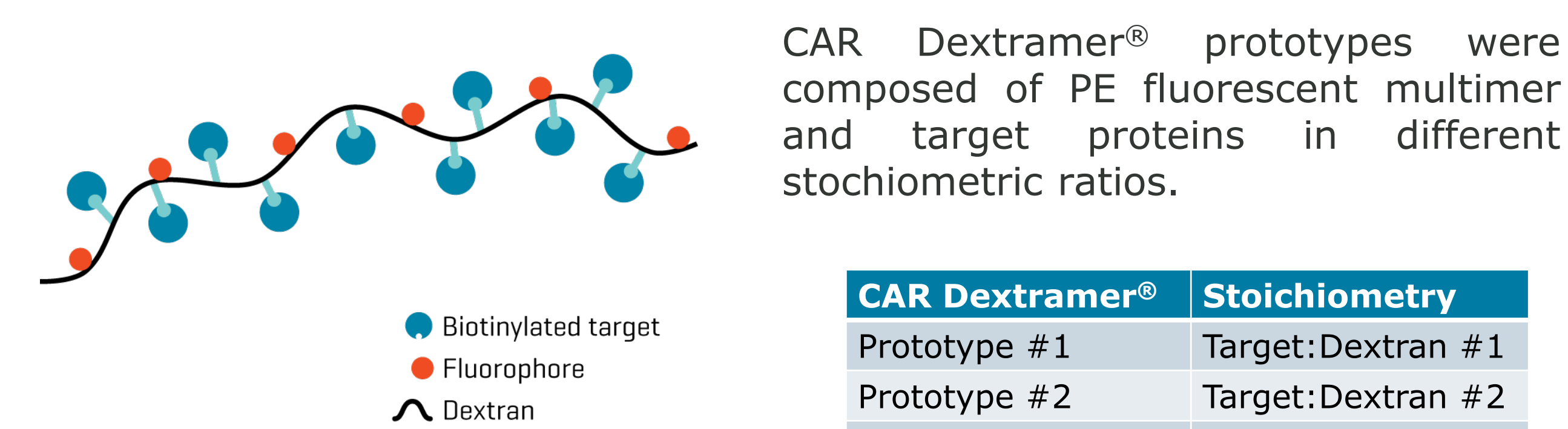


Figure 4: Staining artificial cells with CAR Dextramer[®] prototypes. CAR Dextramer[®] prototype reagents + controls were incubated together with anti target antibody coated beads, washed and acquired on FACS to assess target correct refolding and functionality.

CAR Dextramer[®] prototype structure



CAR Dextramer[®] prototypes were composed of PE fluorescent multimer and target proteins in different stoichiometric ratios.

CAR Dextramer [®]	Stoichiometry
Prototype #1	Target:Dextran #1
Prototype #2	Target:Dextran #2
Prototype #3	Target:Dextran #3

Table 1: CAR Dextramer[®] prototypes.

Figure 3: CAR Dextramer[®]. PE Fluorescent backbone coated with target proteins.

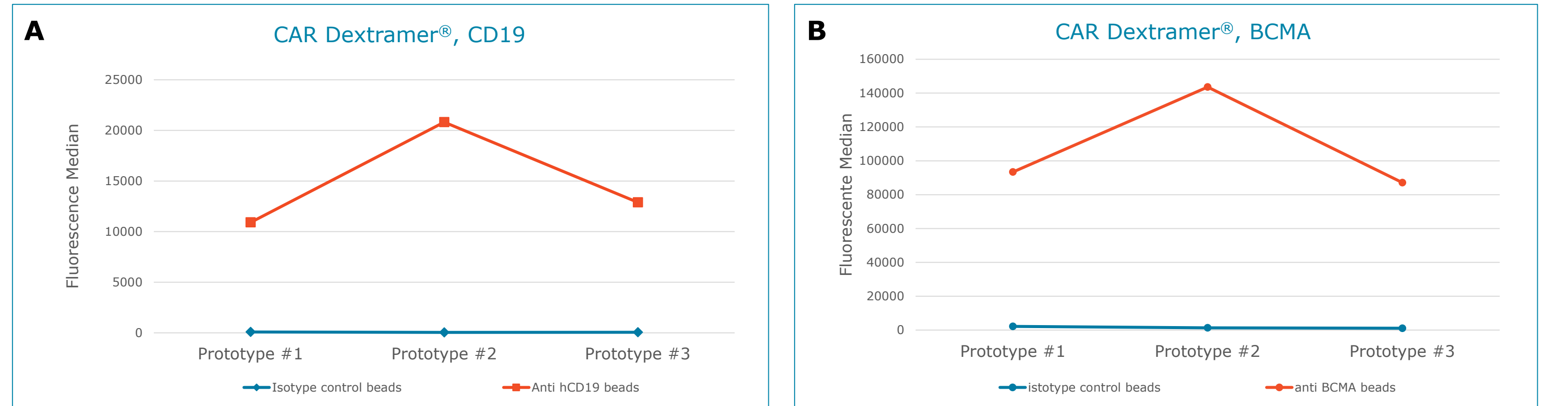


Figure 5a and 5b: CAR Dextramer[®] prototypes are functional and bind specifically to artificial cells. Three prototype reagents and one control were run into an artificial cell assay. Fluorescence values emitted by beads bound to the reagents were measured by flow cytometry and MFI values were plotted into charts to evaluate each prototype ability to bind the target. All 6 prototypes could specifically recognize the anti CD19 or BCMA beads and not the isotype control Ab coated beads. CAR Dextramer[®] prototype performances are different according to target:Dextran ratio. Both parameters have an influence on reagent performance in this artificial setting.

CAR Dextramer[®] - Specific detection of transduced T Cells

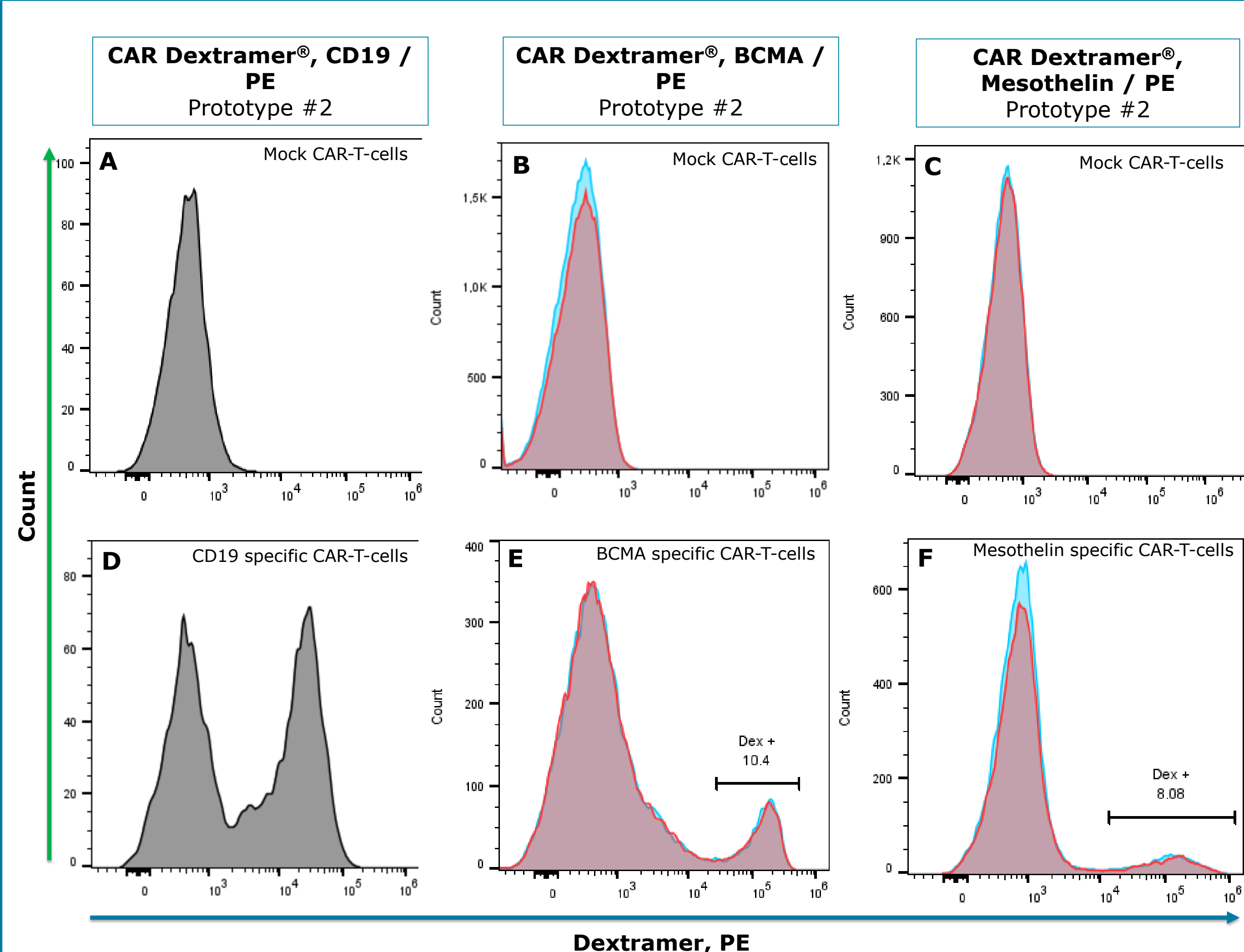


Figure 6: CAR Dextramer[®] reagents PE fluorescent can specifically detect CAR T-cells. Screened CAR Dextramer prototypes were used to stain (i) Control non-transduced primary T-cells (A, B and C) (ii) Primary CAR T cells specific for CD19 (D) , BCMA (E) or Mesothelin (F). None of the reagents showed background on control cells. All prototypes could specifically detect CAR T-cells.

CAR Dextramer[®] - Detect CAR-T cells in PBMC and whole blood

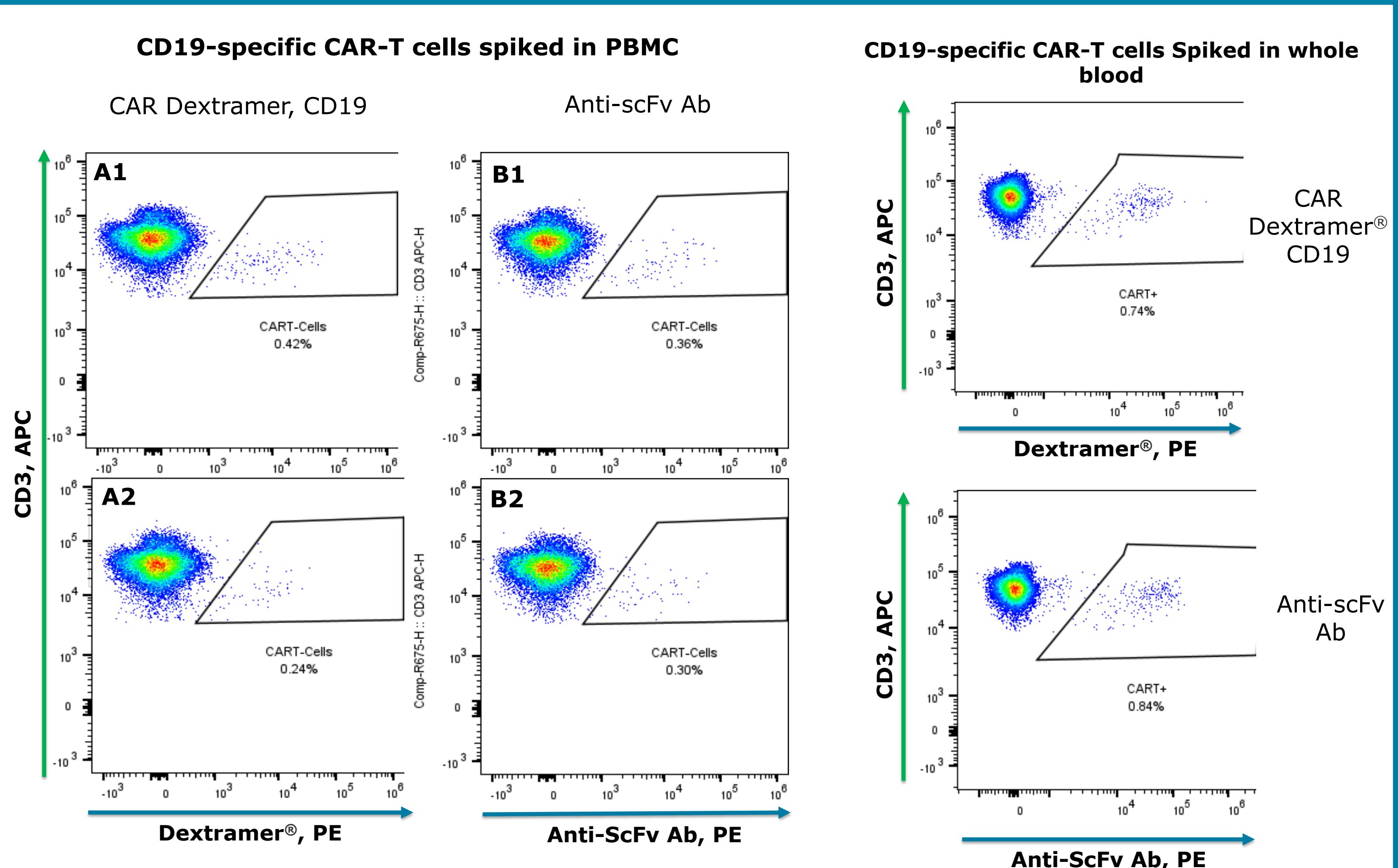


Figure 7: CAR Dextramer[®], CD19 can detect CAR-T cells in PBMC. CAR Dextramer[®], CD19 was used to stain spiked in cells in PBMC (A1-2, B1-2) Spiked in CD19 specific CAR T-cells were detected in comparable numbers to the control anti-scFv antibody.

Figure 8: CAR Dextramer[®], CD19 can detect CAR-T cells in whole blood. CAR Dextramer[®], CD19 was used to stain cells in whole blood (C1, D1). Spiked-in CD19-specific CAR-T cells were detected in comparable numbers to the control anti-scFv antibody control.

Conclusions and perspectives

- We have built a flexible platform focused on quality, allowing fast development of CAR Dextramer[®] reagents including (i) functionality evaluation (ii) selection of most optimal reagent.
- CAR Dextramer[®] allows quick, sensitive and specific detection of CAR-T cells based on receptor recognition of target and can detect CAR-T cells with high- and low-avidity for their target.
- CAR Dextramer[®] can be used to quantify and QC CAR-T Cell products and to measure persistency of CAR-T Cells in patient blood samples.

