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

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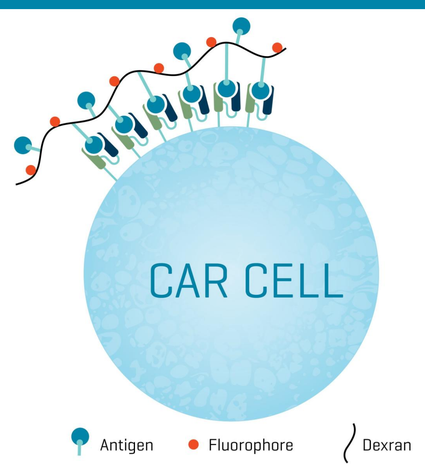
Introduction

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 MSLN (Mesothelin) CAR Dextramer® reagents which can specifically detect CAR-T cells in whole blood.



CAR reagent development platform

CAR Dextramer® reagents of different specificities (CD19, BCMA, MSLN…) were developed using a CAR Dextramer® reagent development platform (Fig. 1). During development, antigen specifications as well as target protein identity (Fig. 3 and 4) and ability to detect CAR T-cells (Fig 5) are tested and evaluated using different assays.

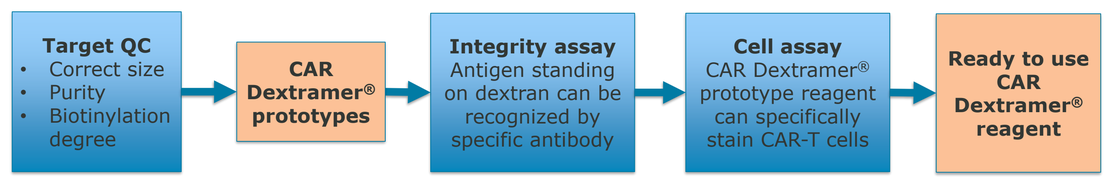


Figure 1: CAR Dextramer® reagent development platform.

CAR Dextramer® structure

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

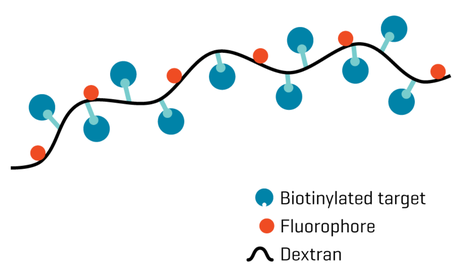


Figure 2: CAR Dextramer®. PE Fluorescent backbone coated with target proteins.

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

Table 1: CAR Dextramer® prototypes.

CAR Dextramer® a flexible platform – Prototypes development

Targets fulfilling the quality requirements were used to manufacture CAR Dextramer ® prototypes. The prototypes had different specificities (CD19, BCMA or MSLN) 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 3: 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.

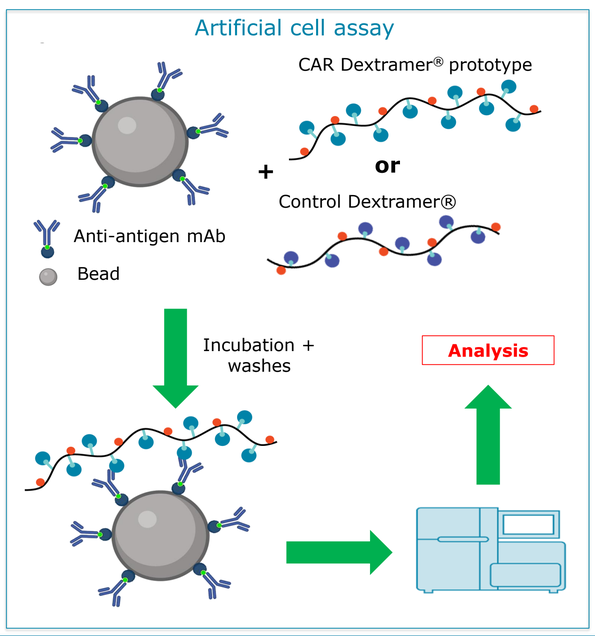
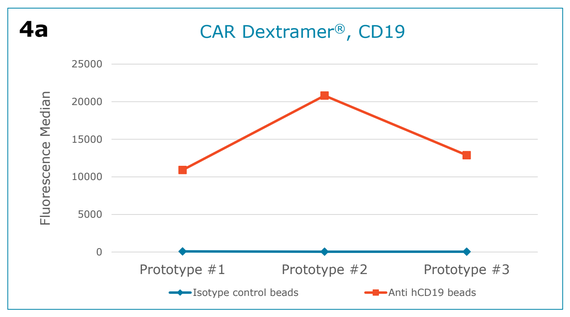
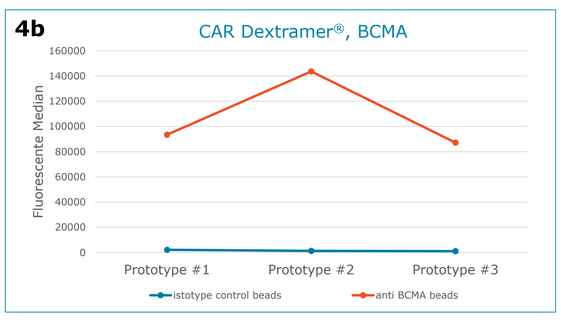
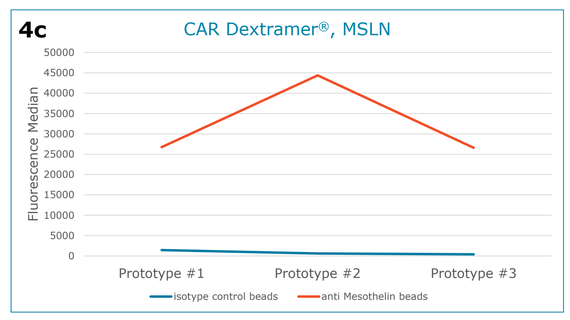


Figure 4a, 4b and 4c: CAR Dextramer® prototypes are functional and bind specifically to artificial cells.
Three prototype reagents and one control were run into an artificial cell assay. Fluorescence values emitted by beads bound to the reagents were measured by flow cytometry and MFI values were plotted into charts to evaluate each prototype ability to bind the target. All prototypes could specifically recognize the anti CD19, MSLN 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

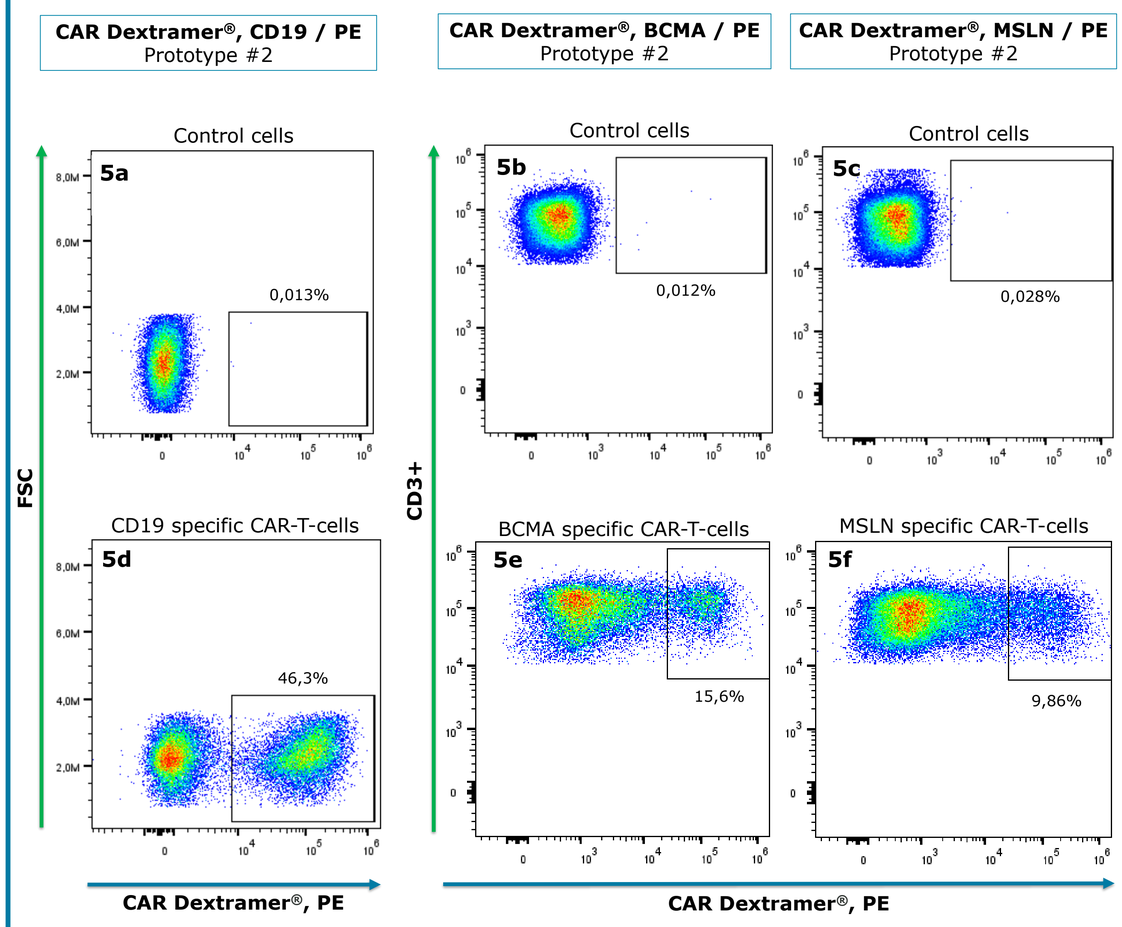


Figure 5: CAR Dextramer® reagents PE fluorescent can specifically detect CAR T-cells. Screened CAR Dextramer® prototypes were used to stained (i) Control non-transduced primary T-cells (5a, 5b and 5c) (ii) Primary CAR T cells specific for CD19 (5d) , BCMA (5e) or MSLN (5f). None of the reagents showed background on control cells. All prototypes could specifically detect CAR T-cells.

CAR Dextramer® - Detect CAR-T cells in whole blood

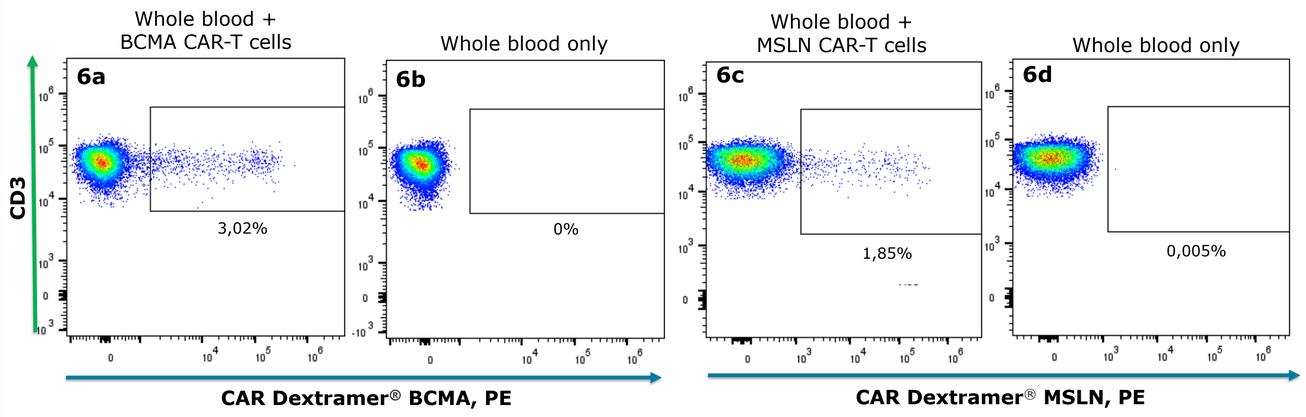


Figure 6: CAR Dextramer® reagents PE fluorescent can specifically detect BCMA or MSLN specific CAR T-cells spiked in whole blood. CAR-T cells specific for BCMA or MSLN were titrated and spiked in whole blood. CAR Dextramer® BCMA or MSLN reagent were used to stained whole blood containing CAR-T cells specific for BCMA (6a), MSLN (6c) or whole blood only (6b and d). The CAR Dextramer® could specifically detect the CAR-T cells when present. Whole blood only condition (6b and 6d) stained with CAR Dextramer® displays no background staining.

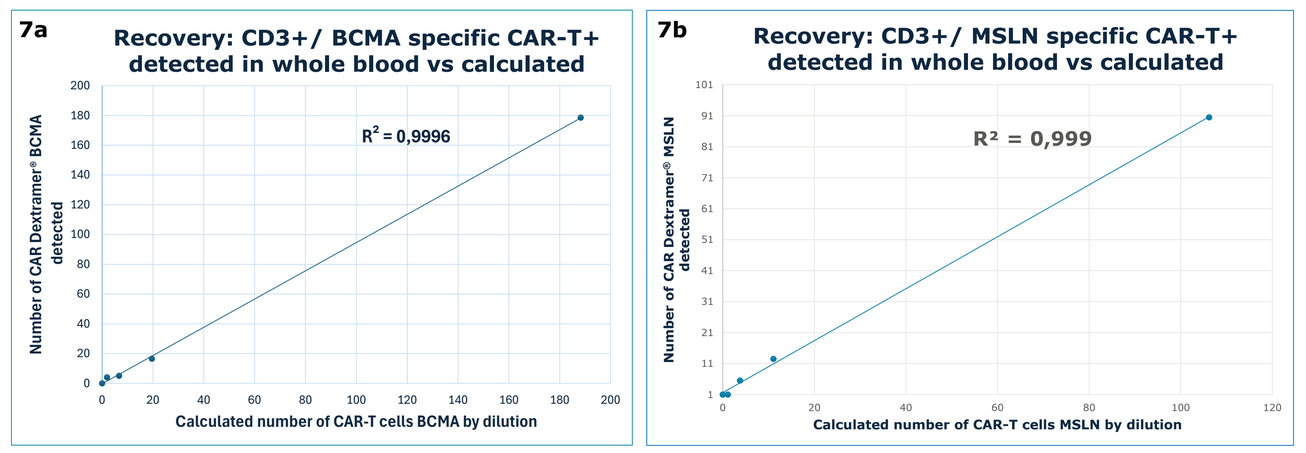


Figure 7: CAR Dextramer® BCMA and MSLN reagents PE fluorescent can accurately detect CAR-T cells spiked in whole blood. Number of events detected by flow cytometry following CAR Dextramer® staining was compared to the number of calculated CAR-T cells. Data show a strong correlation between the expected number of CAR-T cells and the events detected, proving CAR Dextramer® ability to accurately and specifically detect all spiked in cells as low as 4-6 events.

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 (Fig. 3 and 4).
- 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 (Fig. 5).
- CAR Dextramer® accurately and specifically detects CAR-T cells spiked in whole blood with high sensitivity (Fig. 6 and 7) and can be used to monitor CAR-T cell persistency in whole blood
- 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.

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