Using CAR Dextramer® platform to develop universal anti-CD20 CAR-T cells detection reagents.

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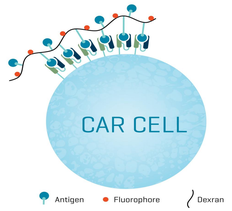
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Introduction

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®, CD20 reagents.



CAR reagent development platform

CAR Dextramer®, CD20 reagents were developed using a CAR Dextramer® reagent development platform (Fig. 1). During development, CD20 specifications as well as its identity (Fig. 3 and 4) are tested and evaluated using an artificial bead assays.

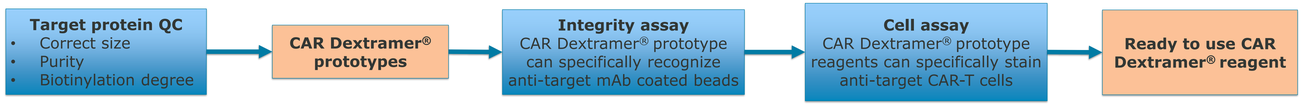


Figure 1: CAR Dextramer® reagent development platform.

CAR Dextramer® structure

CAR Dextramer® prototypes were composed of PE or APC fluorescent multimer and CD20 proteins in different stochiometric ratios.

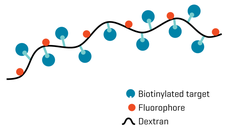


Figure 2: CAR Dextramer®. PE or APC Fluorescent backbone coated with target proteins.

Target	CAR Dextramer®	Stoichiometry
CD20 Protein #1	Prototype #1	CD20:Dextran #1
	Prototype #2	CD20:Dextran #2
	Prototype #3	CD20:Dextran #3
	Prototype #4	CD20:Dextran #4
	Prototype #5	CD20:Dextran #5
	Prototype #6	CD20:Dextran #6
	Prototype #7	CD20:Dextran #7
	Prototype #8	CD20:Dextran #8
CD20 Protein #2	Prototype #9	CD20:Dextran #1
	Prototype #10	CD20:Dextran #2
	Prototype #11	CD20:Dextran #3
	Prototype #12	CD20:Dextran #4
	Prototype #13	CD20:Dextran #5
	Prototype #14	CD20:Dextran #6
	Prototype #15	CD20:Dextran #7
	Prototype #16	CD20:Dextran #8

Table 1: CAR Dextramer®, CD20 prototypes. Prototypes were made with different stoichiometries, fluorescent backbones (APC, PE) and protein sources (Protein #1, Protein #2)

CAR Dextramer® a flexible platform – Prototypes development

Two CD20 proteins fulfilling the quality requirements were used to manufacture CAR Dextramer ®, CD20 prototypes. The prototypes had different stoichiometries, fluorescent backbones (APC, PE) and protein sources (Protein#1 or Protein #2) . They were analyzed in an artificial cell assay to evaluate (i) target integrity: CAR Dextramer, CD20 ability to recognize the antibody coated beads, (ii) influence of stoichiometry on signal intensity and (iii) influence of protein source. Results: Prototypes manufactured with protein #1 could specifically detect the anti CD20 beads and not the isotype control Ab coated beads while prototypes made with protein #2 demonstrated no specific labelling of anti-CD20 coated beads (Fig 4a and 4b vs 4c). Most optimal staining was observed with prototype #3 (largest signal to noise ratio) for both PE (Fig 4a) and APC (Fig 4b) labelled CD20 CAR Dextramer reagents.

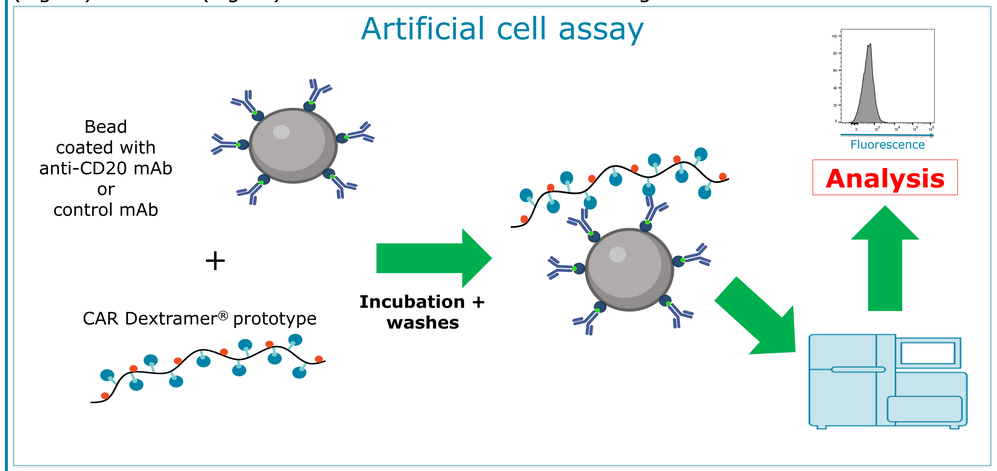


Figure 3: Staining artificial cells with CAR Dextramer® prototypes. CAR Dextramer® prototype reagents + controls were incubated together with anti target antibody coated beads, washed and acquired on FACS to assess target correct refolding and functionality.

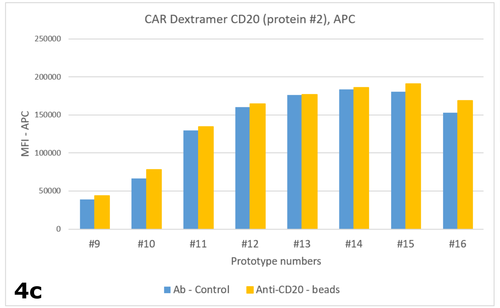
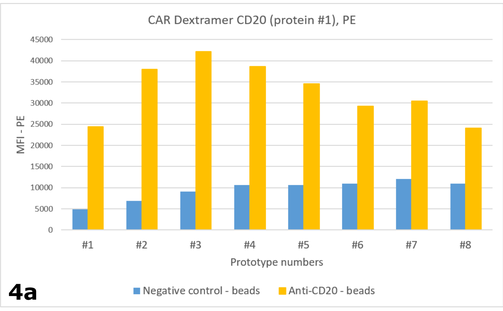
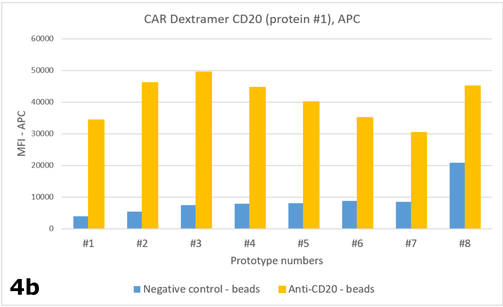


Figure 4a, 4b and 4c: CAR Dextramer®, CD20 prototypes are functional and bind specifically to artificial cells. Prototype reagents and controls were run into an artificial cell assay. Fluorescence values emitted by reagent bound to the beads were measured by flow cytometry and MFI values were plotted into charts to evaluate each prototype ability to bind the coated beads.





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 CD20 CAR-T cells despite overall avidity and opens new possibilities for monitoring CAR-T-cells without being dependent on tags.
- CAR Dextramer ® offers a method to evaluate cell surface expression of functional CD20 binding CAR of CAR T cells, which a tag doesn´t reveal.
- 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.
- The CAR Dextramer® technology is flexible and can be adapted to CAR-T cells having other specificities like Her-2, etc.

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