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

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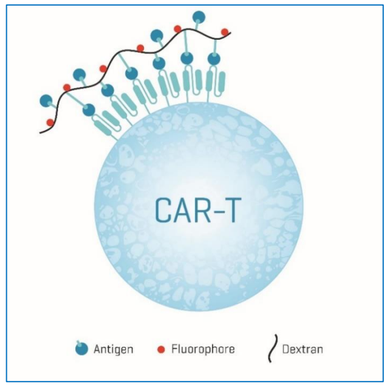
Introduction

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 often do not address CAR receptors recognition of its target. Detection methods using the targeted ligand (ie: hCD19 + fluorescent antibody) exist but they are indirect and laborious.

In this study we developed:

- a direct CD19 CAR-T cells detection method using hCD19 Dextramer® reagents.
- hCD19 Dextramer® reagents which can detect high and low affinity/expression CAR T cells.



Six Different CD19 Dextramer® prototypes

Six different hCD19 Dextramer® prototype reagents (#1 to #6) were generated. The six reagents were composed of PE fluorescent multimer and hCD19 proteins of different structural format and in different stochiometric ratios. (Table 1 and Fig. 1)

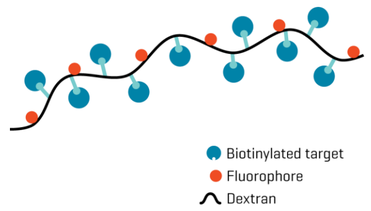


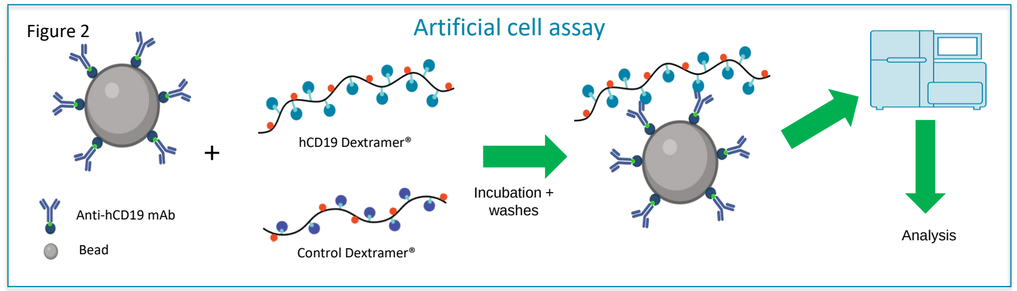
Figure 1: hCD19 Dextramer®. PE Fluorescent backbone coated with hCD19 proteins as per table 1.

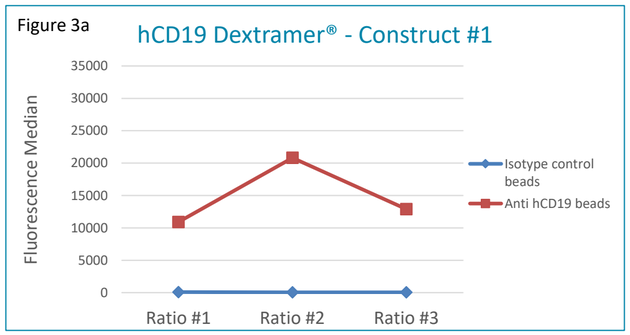
hCD19 Dextramer® reagent	hCD19 proteins construct	Dextran:CD19 ratios	Fluorochrome
Reagent #1	Construct #1	Ratio #1	PE
Reagent #2	Construct #1	Ratio #2	PE
Reagent #3	Construct #1	Ratio #3	PE
Reagent #4	Construct #2	Ratio #4	PE
Reagent #5	Construct #2	Ratio #5	PE
Reagent #6	Construct #2	Ratio #6	PE
Control reagent	Ctrl protein	Control ratio	PE

Table 1: hCD19 Dextramer® reagents prototypes and control. List of six prototype manufactured with two different sources of hCD19 and one control reagent manufactured with irrelevant ligand. Six different Dextran:hCD19 ratios were tested.

Artificial cell assay – Evaluation of hCD19 Dextramer functionality

Figure 2: Staining artificial cells with hCD19 Dextramer®. Prototypes reagents + controls were incubated together with anti CD19 antibody coated beads, washed and acquired on FACS to assess CD19 correct refolding and functionality.





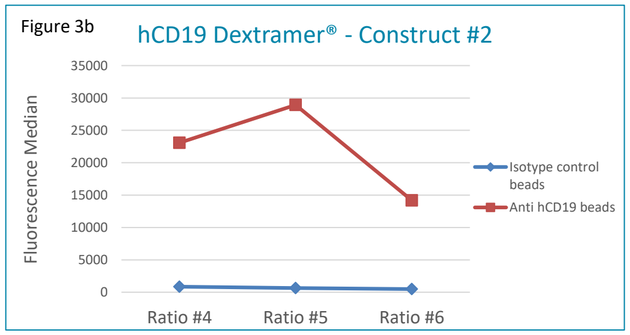


Figure 3a and 2b: hCD19 Dextramer® prototypes are functionals and bind specifically to artificial cells.
Six prototype reagents and one control were run into an artificial cell assay. Fluorescence values emitted by beads bound to the reagents were measure by flow cytometry and MFI values were plotted into charts to evaluate each prototype ability to bind the target. All 6 prototypes could specifically recognize the anti hCD19 beads and not the isotype control Ab coated beads. CAR Dextramer®, hCD19 reagent performances are different according to hCD19:Dextran ratio and hCD19 structure. Both parameters have an influence on reagent performance in this artificial setting.

CAR-T cells detection with the hCD19 Dextramer®

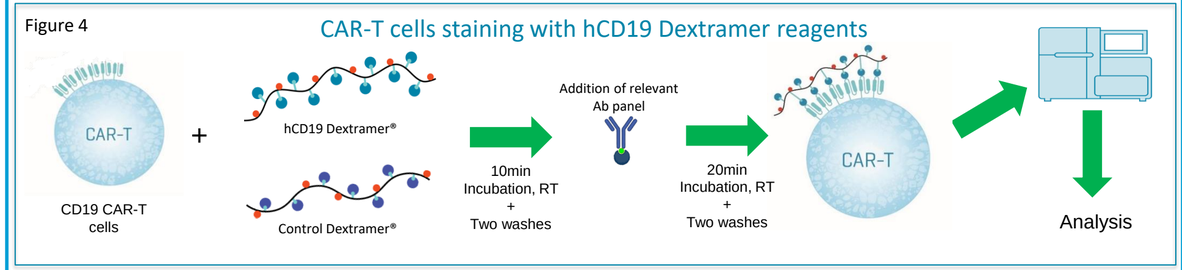


Figure 4: Staining cells with hCD19 Dextramer. A fast process. Prototypes reagents + controls and Ab were incubated (10min + 20min) with cells expressing CD19 specific CAR constructs, washed and acquired on FACS to assess protype reagents performance.

CAR Expression level	Medium	High			Low	
CAR Dextramer	**CAR construct #0**	**CAR construct #1**	**CAR construct #2**	**CAR construct #3**	**CAR construct #2**	**CAR construct #3**
Reagent #1	+	-	-	-	-	+
Reagent #2	++++	-	-	-	-	++++
Reagent #3	+++	-	-	-	+	+
Reagent #4	++++	-	+	+	+	++++
Reagent #5	++++	+	+	+	+++	++++
Reagent #6	++	-	+	-	+++	+++

Table 2: hCD19 Dextramer®, detect CD19 CAR-T cells with high and low CAR expression level. T Cells expressing CD19 CAR of different affinities and CAR expression levels were stained with the prototype CAR Dextramer® reagents. Performance were evaluated by comparing with preferred indirect CAR detection methods (hCD19 + fluorescent antibody). All reagents could detect at least one of the CAR-T cell types, but reagent #5 performance was identical or better than the indirect method with all types of CAR-T cells regardless of CAR-T avidity. *"-" CD19 Dextramer® performance inferior to the indirect method. "+" CD19 Dextramer® performance comparable to the indirect method. "++", "+++" and "++++" CD19 Dextramer® performance superior to the indirect method, respectively +10%, + 20% or >30% CAR-T cells detected.*

Conclusions

- CD19 Dextramer® allows quick, sensitive and specific detection of CD19 CAR-T cells despite overall avidity and opens new possibilities for monitoring CAR-T-cells without being dependent on tags.
- CD19 Dextramer® offers a method to evaluate cell surface expression of functional CD19 binding CAR of CAR T cells, which a tag doesn´t reveal.
- CD19 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 BCMA, etc.

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