Development of a CD137 receptor occupancy assay to support the phase I/II study of BT7480, a *Bicycle* tumor-targeted immune cell agonist® (*Bicycle* TICA®)

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ABSTRACT

Bicycles are fully synthetic constrained peptides with antibody-like affinities that target selectively, readily penetrate tumor tissue, have relatively short half-lives, and can be chemically linked together to generate multifunctional molecules. BT7480 is a *Bicycle* TICA® being developed as a first-in-class CD137 therapeutic for the treatment of human cancers associated with Nectin-4 expression and is currently being investigated in an ongoing Phase I/II clinical trial. Monitoring target engagement for a given therapeutic can be a key factor in recommending the Phase II dose. While flow cytometry-based receptor occupancy (RO) assays are commonly used to monitor target engagement in the clinic, a CD137-specific RO assay presents several important challenges that have historically hampered monitoring RO in the clinic, including the dynamic expression of CD137 on unstimulated and stimulated T cells, the low frequency of CD137+ cells in human blood, and limited reagents to confidently detect CD137+ cells in the presence of CD137-targeting drugs. To address these challenges, a fit-for-purpose, 14-plex flow cytometry panel was developed that incorporates a fluorescently labelled CD137-specific binding *Bicycle*®. This *Bicycle*® was shown to directly compete with a *Bicycle* TICA® for binding to CD137, but not with a fluorescently labelled anti-CD137 antibody, thereby enabling simultaneous detection of various CD137+ immune cell types, as well as receptor occupancy by BT7480 in a single blood sample.

INTRODUCTION

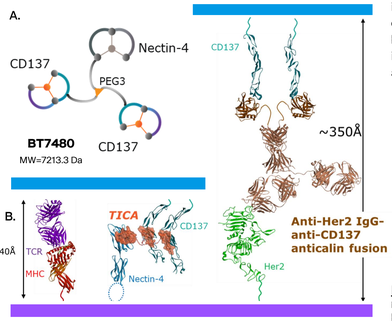

Figure 1: A) BT7480 is a fully synthetic, heterotrimeric conjugate with 1 Nectin-4 and 2 CD137 binding *Bicycles*. CD137 is expressed by immune cells in the tumor and blood and Nectin-4 is expressed on cancer cells in a variety of solid tumor types[1,3,4]. B) *Bicycles* are designed to combine advantages of both small molecules and antibodies. CD137 *Bicycle* TICAs represent a novel immuno-oncology modality to deliver tumor-targeted CD137 agonist activity and are ~30x smaller than other targeted agonists[5].

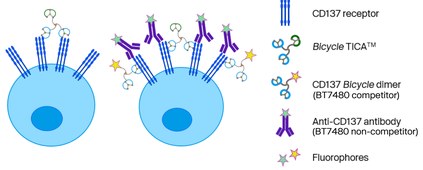

Figure 2: CD137 *Bicycle* dimer was used as a detection reagent to measure receptor occupied by CD137 *Bicycle* TICA® on human immune cells in whole blood samples. Total CD137 receptor expression was monitored using a non-competing anti-CD137 antibody.

METHODS

Using a proprietary CD137 *Bicycle*® dimer, a 14-plexed flow cytometry assay was developed to simultaneously quantify the presence of CD137+ immune cells, and receptor occupancy by BT7480 in a single blood sample. For assay development, human whole blood samples were preincubated in the presence of increasing concentrations of CD137 *Bicycle* TICA® compound followed by flow cytometry. Stimulated blood samples were generated by treating samples in the presence of CD3/CD28 Dynabeads for 72hrs prior to incubation with compound and panel testing. Data are reported as % RO and calculated using the following formula:

$$\% \text{ TE} = (1-(\Delta\text{TE post-dose}/\Delta\text{TE pre-dose}))*100$$

$$\Delta\text{TE} = \%\text{CD137+}\textit{Bicycle}\text{+ full stain panel - }\%\text{CD137+}\textit{Bicycle}\text{+ FMX panel}$$

RESULTS

Bicycle CD137 receptor occupancy flow cytometry panel development

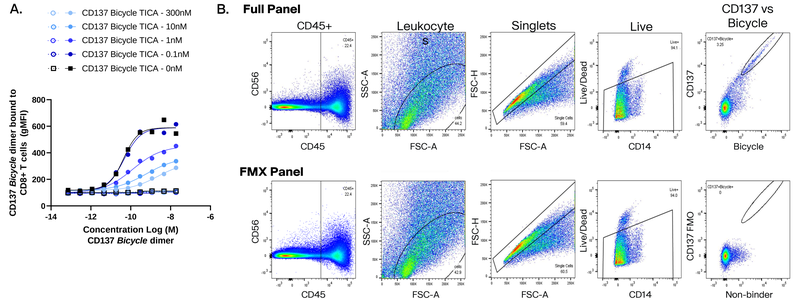

Figure 3: A) Fluorescently labelled CD137 *Bicycle* dimer was shown to directly compete with various concentrations of CD137 *Bicycle* TICA® for binding to CD137 on human CD137+ T cells (filled/solid lines), but not CD137- T cells (open/dashed lines). B) Gating strategy to detect CD137+ immune cells in human whole blood samples as well as CD137+ CD137 *Bicycle* dimer+ cells (stimulated CPT donor representative sample shown). The FMX panel excluded the CD137 antibody and used a fluorescently labeled non-binding *Bicycle* dimer in place of the CD137 *Bicycle* dimer.

Panel performance testing across clinically-relevant sample matrices

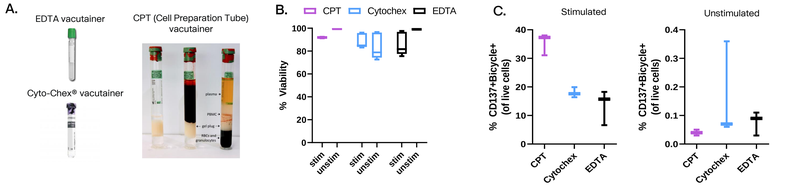

Figure 4: Panel performance was tested across blood-based sample matrices routinely used in the clinic including EDTA and Cyto-Chex® blood collection tubes and Cell Preparation Tubes (CPT) (n=3 each). B) Whole blood samples were stained with the 14-plex custom panel and analyzed. CPT were selected as the optimal sample matrix based on sample viability and highest detection of CD137 antibody+ and CD137 *Bicycle*+ cells.

Bicycle CD137 receptor occupancy assay is functional in human whole blood samples

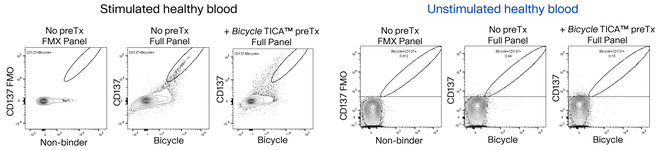

Figure 5: Ex vivo RO assessments in anti-CD3 stimulated (left) and unstimulated (right) healthy human blood collected in CPT demonstrated dose-dependent detection of CD137 RO by CD137 *Bicycle* TICA® (n=5, representative donor sample pretreated with 10nM CD137 *Bicycle* TICA® shown, following gating strategy shown in Figure 3B).

RESULTS

Bicycle CD137 receptor occupancy assay is suitable for clinical testing purposes

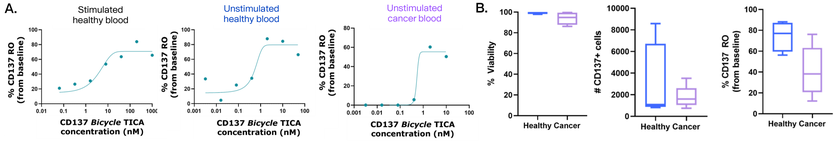

Figure 6: A) The optimized method and dose-dependent detection of CD137+ cells and RO by CD137 *Bicycle* TICA® was verified in stimulated and unstimulated healthy blood, and unstimulated lung cancer patient whole blood samples collected in CPT (n=5, representative donor shown). B) Method optimization resulted in consistent detection of CD137 RO by CD137 *Bicycle* TICA® and >1000 CD137+ cells with >70% viability in unstimulated healthy and cancer blood samples (n=5, pretreated with 10nM CD137 *Bicycle* TICA® shown).

CD137 *Bicycle* dimer® detects CD137+ cells that are largely memory T cells in unstimulated human blood

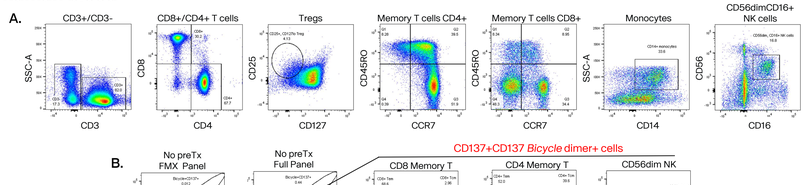

Figure 7: A) Gating strategy to detect and quantify immune cell subsets of interest circulating in blood including T cells, NK cells, and myeloid cells. B) Phenotypic analysis following gating shown in Figures 3B and 7A of immune cells that are CD137+ and that bind the CD137 *Bicycle*® dimer in unstimulated blood samples (n=10, representative donor shown).

CONCLUSIONS

- This study represents the first report of a clinic-ready CD137 RO assay
- Results demonstrate the first clinical flow cytometry assay using fluorescently labelled *Bicycle*® reagents
- Successful assay development supports the utility of the *Bicycle*® CD137 RO assay to monitor target engagement in the BT7480 first-in-human clinical trial[2]

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