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# 5555

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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 which is currently being investigated in an ongoing phase I/II clinical trial[1,2]. 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*® dimer, thereby enabling simultaneous detection of various CD137+ immune cell types as well as receptor occupancy by BT7480 in a single blood sample.

INTRODUCTION

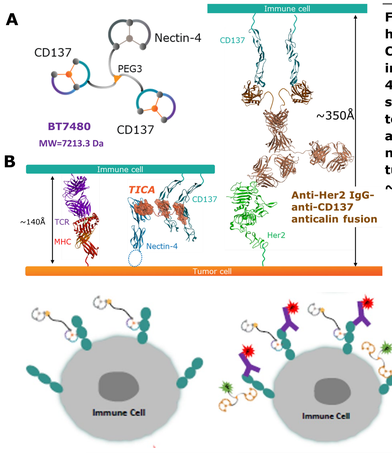

Figure 1: A) BT7480 is a fully synthetic, heterotrimeric conjugate with 1 Nectin-4 and 2 CD137 binding *Bicycles*. CD137 is expressed by immune cells in the tumor and blood and Nectin-4 is expressed on cancer cells in a variety of solid tumor types[1,3,4]. B) *Bicycles* are designed to combine advantages 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:

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

$$\Delta TE = \%CD137+Bicycle+\ full\ stain\ panel - \%CD137+Bicycle+\ FMX\ panel$$

RESULTS

Bicycle CD137 receptor occupancy flow cytometry panel development

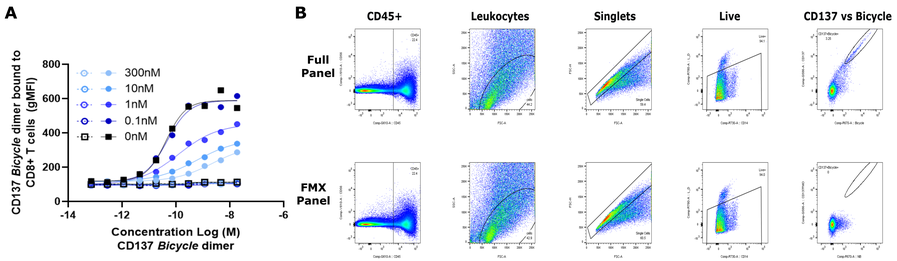

Figure 3: A) Fluorescently labelled CD137 *Bicycle*® dimer was shown to directly compete with various concentrations of CD137 *Bicycle* TICA™ for binding to CD137 on human CD137+ T cells (filled/solid lines), but not CD137- T cells (open/dashed lines). B) Gating strategy to detect CD137+ immune cells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

Panel performance testing across clinically-relevant sample matrices

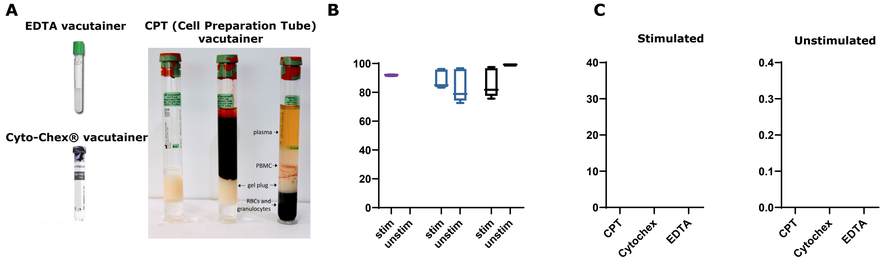

Figure 4: A) Panel performance was tested across blood-based sample matrices routinely used in the clinic including EDTA and Cyto-Chex® blood collection tubes and Cell Preparation Tubes (CPT) (n=3 each). B) Whole blood samples were stained with the 14-plex custom panel and analyzed. CPT were selected 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

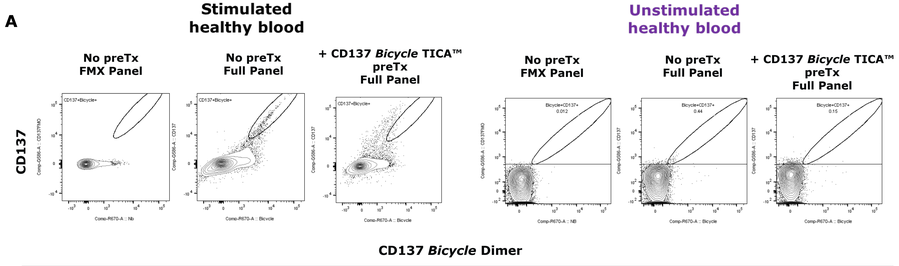

***Figure 5: A) Ex vivo* RO assessments in anti-CD3 stimulated (top) and unstimulated (bottom) healthy human blood collected in CPT demonstrated dose-dependent detection of CD137 RO by CD137 *Bicycle* TICA™ (n=5, representative donor sample pretreated with 10nM CD137 *Bicycle* TICA™ shown, following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

RESULTS

Bicycle CD137 receptor occupancy assay is suitable for clinical testing purposes

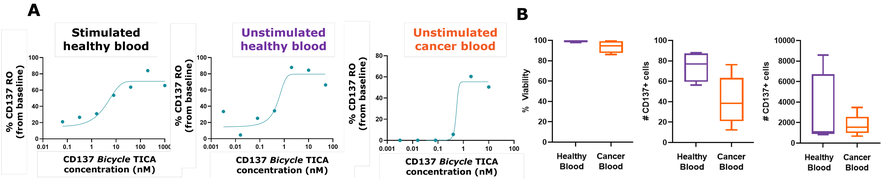

Figure 6: A) The optimized method and dose-dependent detection of CD137+ cells and RO by CD137 Bicycle TICA™ was verified in stimulated and unstimulated healthy blood, and unstimulated lung cancer patient whole blood samples collected in CPT (n=5, representative donor shown). B) Method optimization resulted 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

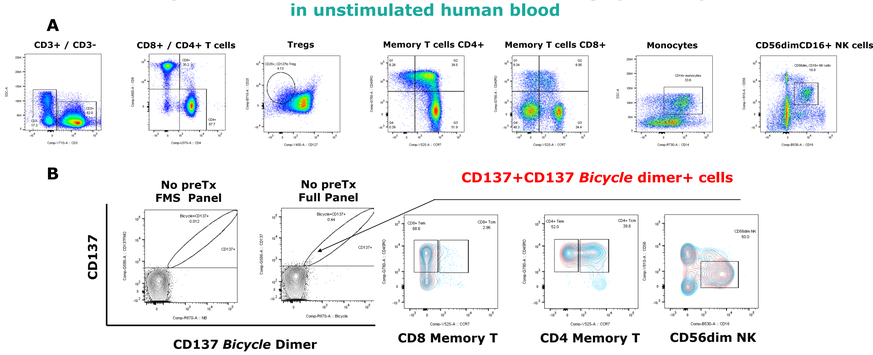

Figure 7: A) Gating strategy to detect and quantify immune cell subsets of interest circulating in blood including T cells, NK cells, and myeloid cells. B) Phenotypic analysis following gating shown in Figures 3B and 7A of immune cells that are CD137+ and that bind the CD137 *Bicycle*® dimer in unstimulated blood samples (n=5, representative donor shown).

CONCLUSION/SUMMARY

- **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]**

REFERENCES

[1] Hurov, K., et al. *Journal for Immunotherapy of Cancer*. 2021. [2] NCT05163041
[3] Challita-Eid, et al. *Cancer Research*. 2016. [4] Campbell, C. et al. AACR. 2021. [5] Keen, N. AACR. 2021.