Quantitation of CD137 and Nectin-4 expression across multiple tumor types to support indication selection for BT7480, a *Bicycle* tumor-targeted immune cell agonist™ (*Bicycle* TICA™)

ABSTRACT# 2

Heather Cohen[1], Carly Campbell[1], Kristen Hurov[1], Johanna Lahdenranta[1], Tara Gelb[1], David Galbraith[3], Dan Rozelle[3], Mate L Nagy[4], Qingyan Au[4], Erinn A Parnell[4], Phil Brandish[1], Sebastien Hazard[1], Dominic Smethurst[2], Nicholas Keen[1], Stephen J Blakemore[1]

[1]Bicycle Therapeutics, Lexington, MA; [2]Bicycle Therapeutics, Cambridge, UK; [3]Rancho Biosciences, San Diego, CA; [4]Neogenomics, Aliso Viejo, CA

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™ that binds both CD137 on immune cells and Nectin-4 on cancer cells to deliver a potent anti-tumor immune signal in Nectin-4 expressing tumors. Nectin-4 has been reported to be highly expressed in a wide range of human solid tumors, however the expression of CD137, abundance and localization of CD137+ immune cells in Nectin-4+ tumors are unknowns. A translational and informatics pipeline was established to interrogate the human tumor microenvironment to identify patient populations most likely to benefit from BT7480, which is being developed as a potential first-in-class molecule for the treatment of high unmet need cancers associated with Nectin-4 expression.

INTRODUCTION

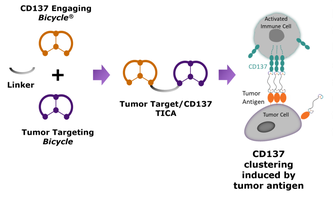


Figure 1: BT7480 is a fully synthetic *Bicycle*® TICA™ that delivers CD137 immune agonist activity to Nectin-4-expressing tumors[1]. CD137 is a costimulatory receptor that drives T cell function and survival and is also expressed on NK and myeloid cells. Nectin-4 is a cell adhesion molecule that is highly expressed in a wide range of solid tumor indications[2,3]

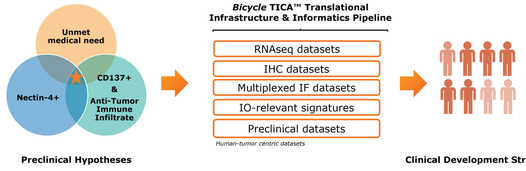


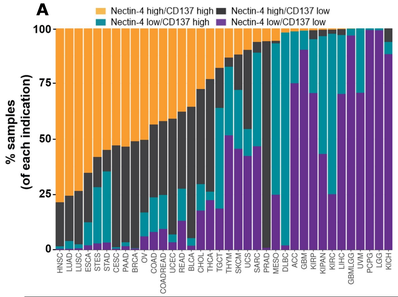
Figure 2: A *Bicycle* TICA™ translational and informatics pipeline was established to identify patients most likely to benefit from BT7480, specifically those with Nectin-4 expressing cancers that co-express CD137, are infiltrated with anti-tumor immune cells, and are of high unmet medical need.

METHODS

TCGA RNAseq data[4] for Nectin-4 and CD137 were analyzed from ~10,000 samples across 36 human cancers. Using a proprietary Nectin-4 mAb and MultiOmyx™ technology, a 19-plexed immunofluorescence assay was developed to simultaneously quantify the presence of Nectin-4+ and CD137+ cells, identify immune cell subsets and their spatial topography in 43 human tumor FFPE samples from HNSCC, lung, bladder, and breast cancers. Each FFPE slide was presented to a pathologist for tissue annotation and selection of regions of interest for image analysis. Proprietary deep learning-based workflows were applied to identify stroma and tumor regions, individual cells and perform cell classification for phenotypes of interest.

RESULTS

CD137 AND NECTIN-4 TRANSCRIPTS ARE CO-EXPRESSED ACROSS MULTIPLE SOLID TUMOR TYPES



B

Indication	Total samples (N)	% Nectin-4/CD137+ (of samples with > average expression)
Head & Neck	520	78.5
Lung, adeno	517	75.5
Lung, squam	501	73.3
Esophagus	184	65.2
Stomach-esophageal	599	60
Stomach	415	54.7
Cervical	304	52.8
Pancreatic	178	52.3
Breast	1093	51
Ovarian	307	50.3

Figure 3: A) Transcript co-expression analysis across TCGA. B) Frequency of samples within the top 10 indications expressing high levels of CD137 and Nectin-4 (> average expression across TCGA) are shown.

SPATIAL PROTEOMIC PROFILING OF NECTIN-4+ AND CD137+ CELLS USING MULTIOMYX™ TECHNOLOGY

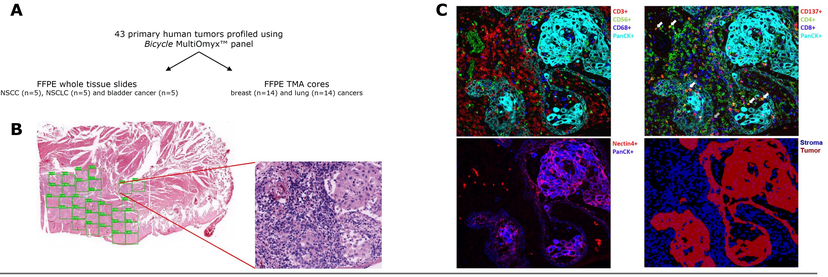
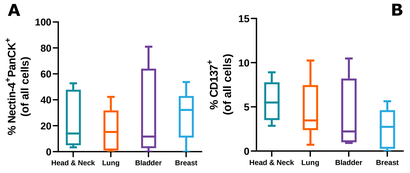


Figure 4: A) 43 FFPE tumor samples were profiled for target expression, immune cell infiltrate and spatial proteomic analysis using a proprietary *Bicycle* MO panel. B) 30 ROIs were selected from whole tissue slides (example HNSCC sample is shown) or 1 ROI from each TMA core was selected for image analysis. C) A single ROI from a representative HNSCC sample is shown. T cells (CD3+, red), macrophages (CD68+, blue), NK cells (CD56+, green), and tumor cells (PanCK+, cyan) detected throughout tumor (top left). Examples of CD137+ CD4 and CD8 T cells are shown and represented by white and gray arrows respectively (top right). Co-expression of Nectin-4 (red) and PanCK (blue) on tumor cells (bottom left). Tumor and stroma regions were identified using a PanCK and DAPI mask respectively (bottom right, in red and blue respectively).

CO-EXPRESSION OF CD137 AND NECTIN-4 PROTEINS DETECTED IN >50% CANCER SAMPLES TESTED



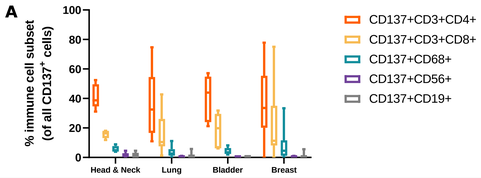
B

Indication	Total samples (N)	% Nectin-4/CD137+ (of samples with > 1% target+ cells)
Head & Neck	5	100
Lung (all)	19	73.7
Lung adeno	8	75
Lung squam	10	70
Bladder	5	60
Breast	14	57.1

Figure 5: A) Proteomic analysis of Nectin-4 and CD137 expression across 43 human tumor samples. Tumor Nectin-4 expression where total Nectin-4+PanCK+ cells are normalized to total cells (left) and CD137+ immune infiltrate where total CD137+ cells detected are normalized to total cells (right). B) Frequency of samples co-expressing Nectin-4 and CD137 at the protein level (>1% positive cells) is shown.

RESULTS

MAJORITY OF CD137+ IMMUNE CELLS IN NECTIN-4 EXPRESSING TUMORS ARE T CELLS AND MACROPHAGES



B

Indication	Total samples (N)	% CD137+CD3+CD4+ (of all CD137+ cells)	% CD137+CD3+CD8+ (of all CD137+ cells)	% CD137+CD68+ (of all CD137+ cells)	% CD137+CD56+ (of all CD137+ cells)	% CD137+CD19+ (of all CD137+ cells)
Head & Neck	5	41.5	15.8	6.7	1.6	1.6
Lung (all)	19	37.2	16.6	3.7	0.3	1.2
Lung adeno	8	32.7	18.0	2.2	0.4	1.0
Lung squam	10	46.7	15.3	5.1	0.1	1.1
Bladder	5	40.3	18.3	4.2	0.6	0.23
Breast	14	36.6	22.6	8.1	0.1	0.9

Figure 6: A) Subset analysis of CD137+ immune infiltrate detected across samples are shown and include T cells (CD3+CD4+ and CD3+CD8+), macrophages (CD68+), NK cells (CD56+), and B cells (CD19+). Data are total cells per phenotype normalized to total CD137+ cells detected across samples within each indication. B) Average frequency of CD137+ immune cell subsets across each indication is shown.

A SUBSET OF CD137+ IMMUNE CELLS CO-LOCALIZE WITH NECTIN-4+ TUMOR CELLS IN HEAD & NECK, LUNG, AND BLADDER CANCERS

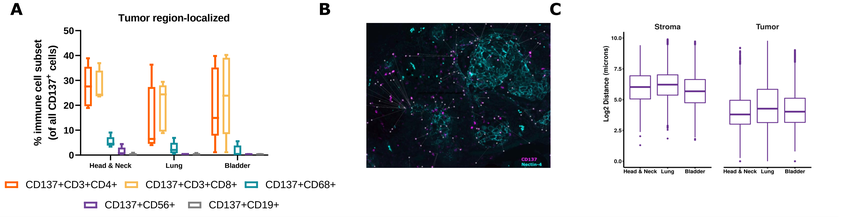


Figure 7: A) Frequency of CD137+ cell subsets detected deep within tumor bed using a PanCK mask to identify the tumor region in each sample. Data shown are from 5 samples per indication. B) Example MultiOmyx™ generated nearest-neighbor (KNN) graph from the same ROI in Figure 4 is shown. C) Single cell analysis of the distance between a CD137+ cell detected in the stroma (left) or in the tumor bed (right) to the nearest Nectin-4+ is shown. CD137+ immune cells were detected within 150 microns of Nectin-4+ tumor cells across indications analyzed. Average number of cells analyzed per sample was >2000. Data shown are from 5 samples per indication.

CONCLUSION/SUMMARY

Results from this study support prioritization of indications for BT7480 clinical development and the utility of the MultiOmyx™ assay to monitor Nectin-4 and CD137 expression and to demonstrate proof-of-mechanism in the BT7480 FIH clinical trial expected to start in 2H-2021.

REFERENCES

[1] Hurov, K., et al. *Journal for ImmunoTherapy of Cancer*. In press.
[2] Challita-Eid, P. et al. *Cancer Research*. 2016; 76(10):3003-13.
[3] Campbell, C. et al. AACR Annual Meeting 2021. Poster #1197.
[4] Data generated by the TCGA Research Network:: https://www.cancer.gov/tcga.