2 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™)

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Background *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.

Methods TCGA RNAseq data 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 RNA expression analysis indicated co-expression of Nectin-4 and CD137 in several tumor types with >50% tumors within NSCLC, HNSCC, breast, esophageal, and ovarian cancers expressing high levels of both targets. Spatial proteomic studies in HNSCC, lung, breast and bladder cancer samples demonstrated that Nectin-4 and CD137 co-expression at the protein level (>1% positive cells) was detected in 74% samples tested. CD137+ cells in Nectin-4+ tumors were identified as CD4+ T cells (37.6%), CD8+ T cells (16.8%) and CD68+ macrophages (5.9%). A subset of CD137+ cells (32.7%) were found to be deeply tumor penetrant and within close proximity of Nectin-4+ tumor cells across all indications tested.

Conclusions 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 for the BT7480 FIH clinical trial expected to start in 2H-2021.

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