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 (Figure 1).

**EphA2 (Eph type 2):** A receptor 2, a member of the Ephrin superfamily of receptor tyrosine kinases, and Nectin-4, a cell adhesion molecule from the Nectin-like family, are overexpressed on the surface of some tumor cells. Several Bicycles were designed to target EphA2 and Nectin-4, including Bicycle Toxin Conjugates (BTCs) and Bicycle Tumor-Targeted Immune Cell Agonists (Bicycle TICA). And are being investigated in clinical trials.

Using Olink® technology (methods), 191 plasma samples from cancer and healthy patients were tested using a custom designed Olink® Focus panel to measure soluble Nectin-4 (sNectin-4) and soluble EphA2 (sEphA2). The concentrations of these tumor antigens were then compared in healthy patients and across cancer indications.

**METHODS**

- We developed a custom Olink® Focus panel using Olink’s Proximity Extension Assay (PEA)® to determine the absolute quantification in pg/mL of sEphA2 and sNectin-4 in plasma.
- We collected 43 plasma samples from healthy patients via BioIVT, and 148 cancer patient plasma samples from Discovery Life Sciences (DLS), representative of seven cancer indications (Table 1).
- Using our Olink® panel to measure sEphA2 and sNectin-4, we determined sEphA2 and sNectin-4 concentrations were measurable within the limits of quantification in all tested plasma samples (Figure 3). We compared sEphA2 and sNectin-4 concentration by indication to healthy samples and investigated demographic variables potentially influencing sEphA2 or sNectin-4 using regression analysis. Median rank for protein concentration was then compared to median expression rank in The Cancer Genome Atlas (TCGA) data on an indication-indication basis.

**RESULTS**

**Concordance of EphA2 and Nectin-4 expression in Olink and TCGA datasets**

**SUMMARY**

- sEphA2 and sNectin-4 are higher in several cancer indications than in non-cancer samples.
- OLS identified age as a significant predictor of sEphA2 and sNectin-4. We recommend continued monitoring of age or covariates that may be associated with age in future analyses as a factor influencing expression.
- Expression of sEphA2/sNectin-4 aligns with prediction based on TCGA mRNA expression of the indications surveyed, suggesting that soluble protein expression from a liquid biopsy should be representative of expression from a tumor biopsy.
- Results from this study suggest that sEphA2/sNectin-4 in plasma may be used as a biomarker to help identify patients with tumors expressing these antigens.

**REFERENCES**

1. Tandon et al, Expert Opin Ther Targets (2022)