Investigating soluble Nectin-4 and EphA2 as cancer biomarkers in plasma

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Abstract #
#P05

INTRODUCTION

- 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 (Ephrin type-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 [1-2].
- Several Bicycles were designed to target EphA2 and Nectin-4, including Bicycle Toxin Conjugates® (BTCs) and Bicycle Tumor-Targeted Immune Cell Agonists (Bicycle TICAs), and are being investigated in clinical trials.
- Evidence from other studies have shown EphA2 and Nectin-4 as increased in the blood of cancer patients[3-4]. Based on these findings**, we hypothesized that measurement of soluble Nectin-4 and EphA2 in plasma could be used as a biomarker of tumors expressing these tumor antigens**.
- 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.

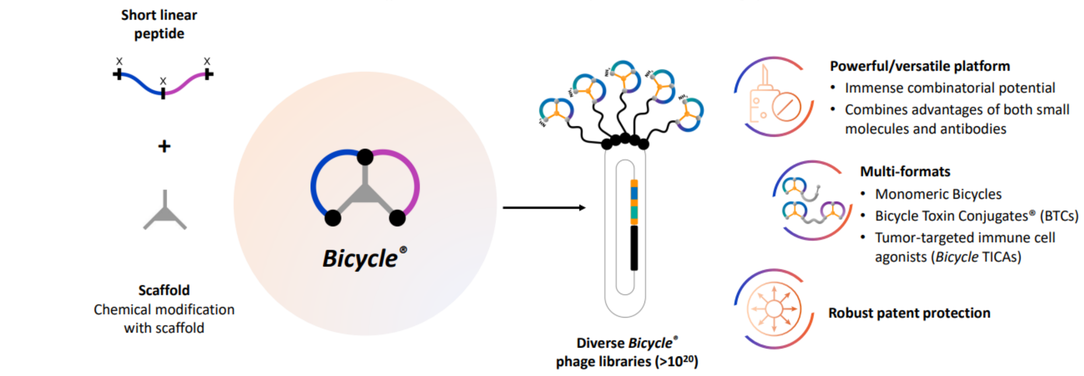


Figure 1: Bicycle® platform delivers a toolkit of building blocks to create novel medicines.

METHODS

- We developed a custom Olink® Focus panel using Olink's Proximity Extension Assay (PEA)[5] 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)[6] data on an indication-indication basis.

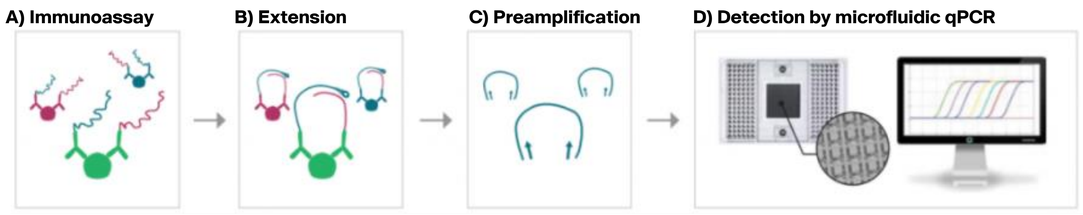


Figure 2: Overview of PEA technology for the custom Olink® Focus panel. A) Antibody pairs, labeled with DNA oligonucleotides , bind target antigen in solution. B) Oligonucleotides that are brought into close proximity hybridize and are extended by DNA polymerase. C) The newly created piece of DNA barcode is amplified by PCR. D) The amount of each DNA barcode is quantified by microfluidic qPCR.

RESULTS

		Ovarian	Lung	Breast	Head & Neck	Gastric	Bladder	Pancreatic	Healthy	p-value
# of Samples		19	24	24	23	19	20	19	43	
Sex	M	0	14	0	16	12	18	12	19	$3.9*10^{-11}$
	F	19	10	24	7	7	2	7	24	
Avg Age (SD)		65.2 (11.1)	66.2 (11.8)	60.6 (12.2)	58.5 (13.7)	63.0 (11.1)	69.9 (11.5)	65.0 (7.1)	58.4 (5.3)	$1.2*10^{-3}$
Race										
White		16	20	21	21	16	17	14	31	.08
Black or AFAM		3	3	3	2	2	1	3	4	
AAPI		0	0	0	0	1	0	0	6	
AIAN		0	1	0	0	0	2	2	0	
Other		n/a	n/a	n/a	n/a	n/a	n/a	n/a	2	

Table 1: Demographics of plasma samples. AFAM = African American, AAPI = Asian American or Pacific Islander, AIAN = American Indian or Alaskan Native. P-value for age is derived from one-way ANOVA test. P-values for race and sex are from Chi-squared test of independence. Significant differences found in sex and age.

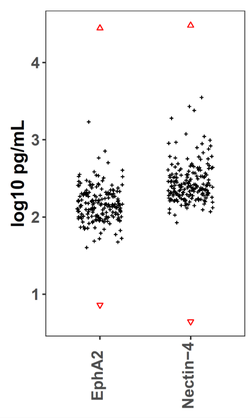


Figure 3. sEphA2 and sNectin-4 concentrations are between the limits of quantification in all tested samples. Red arrows indicate the upper and lower limits of quantification.

sEphA2 and sNectin-4 are higher in cancer compared to non-cancer

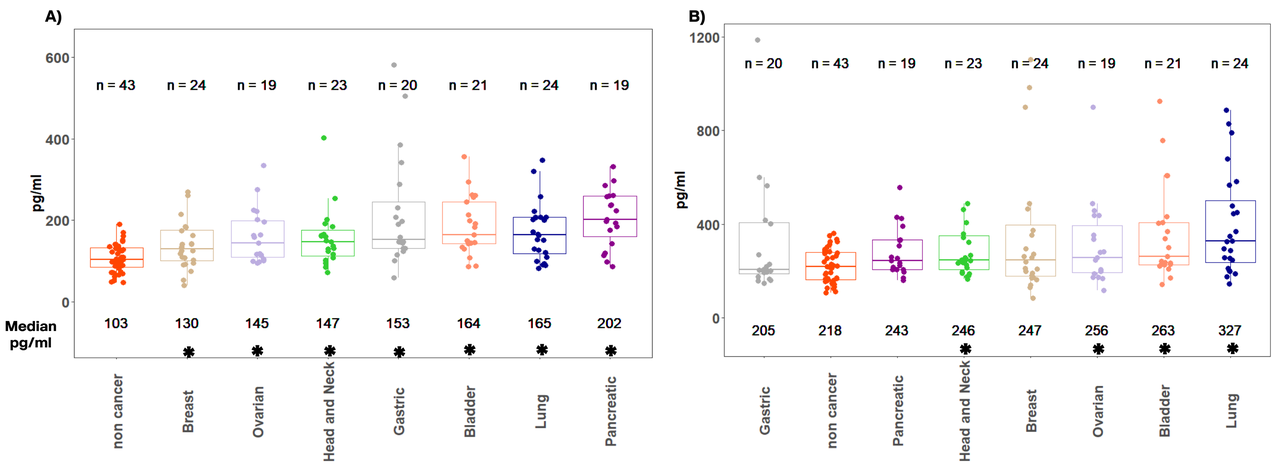


Figure 4: Measurement by Olink of **A**) sEphA2 and **B**) sNectin-4 concentration across cancer indications and non-cancer. Boxplots display the median, Q1, and Q3 concentrations of soluble proteins. Samples sizes (n) are shown at the top of each plot along with the median soluble protein concentration at the bottom for each indication. Boxplots are ordered from left to right by their median concentration. *Indications differing from non-cancer using the Wilcoxon Rank sum test with $p < 0.05$ after Bonferroni correction.

RESULTS

Regression analysis identifies age as a covariate potentially influencing sNectin-4 and sEphA2 that we recommend considering in future studies

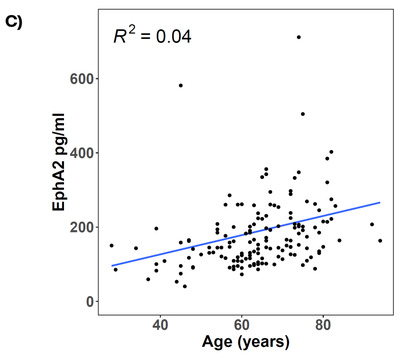
A)

EphA2	
Covariate	p-value
Cancer	
Bladder	.412
Breast	.127
Gastric	**.014**
Head and Neck	**.003**
Lung	.305
Ovarian	.305
Pancreatic	.091
Age	**.003**
Race	
Black or AFAM	.247
AAPI	.882
AIAN	.258
Other	.490
Male	.430

B)

Nectin-4	
Covariate	p-value
Cancer	
Bladder	.885
Breast	**.032**
Gastric	.248
Head and Neck	**.006**
Lung	.304
Ovarian	.582
Pancreatic	.952
Age	**.001**
Race	
Black or AFAM	.482
AAPI	.899
AIAN	.999
Other	.587
Male	.326

C)



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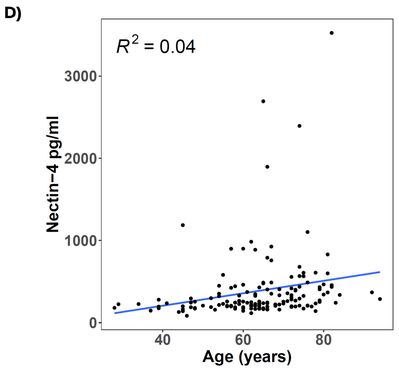
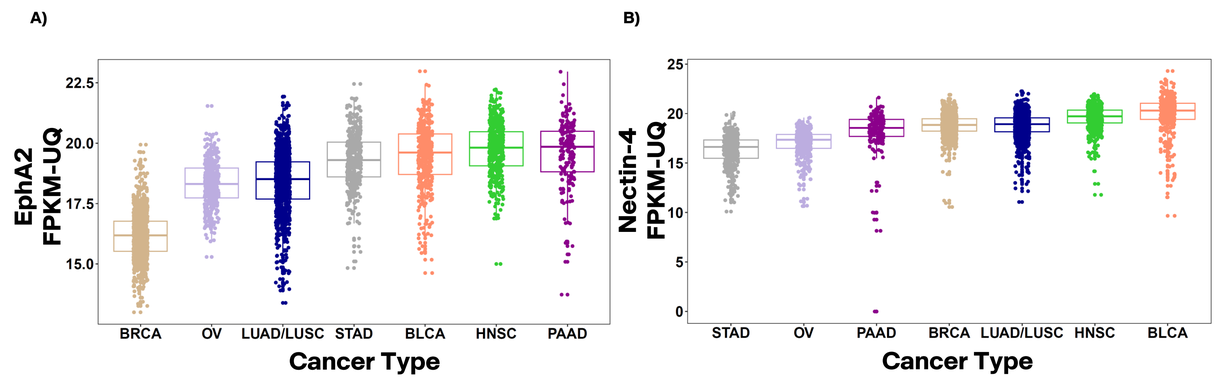
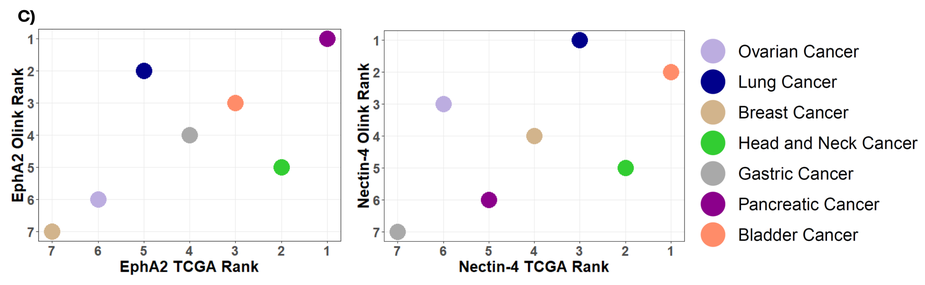


Figure 5: Regression modeling to understand covariates potentially influencing sEphA2 or sNectin-4 concentration. A multiple linear regression model using ordinary least squares (OLS) was used to predict **A)** sEphA2 or **B)** sNectin-4 and for each variable, the significant predictor p-value is displayed. Variables with a p-value <.05 are underlined. Linear regression showing weak correlation between age and **C)** sEpha2 or **D)** sNectin-4. Coefficient of determination is shown at top left.

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





- There is general concordance of EphA2 and Nectin-4 expression by Olink and TCGA, particularly in pancreatic, bladder, gastric, and breast cancer.
- We hypothesize that differences between TCGA and plasma may be influenced by factors such as conversion of mRNA into protein and amount of tumor antigen shed into plasma.

Figure 6 Comparison of EphA2 and Nectin-4 mRNA from TCGA to plasma sEphA2 and sNectin-4 measured by Olink. TCGA abbreviations for cancers: Ovarian = OV, Lung (Squamous and Adenocarinoma) = LUSC/LUAD (Combined dataset), Breast = BRCA, Head & Neck = HNSC, Gastric = STAD, Bladder = BLCA, Pancreatic = PAAD. **A)** EphA2 and **B)** Nectin-4 expression (FPKM-UQ) from the TCGA database was plotted by indication with boxplots to mark median, Q1, and Q3. **C)** Rank was assigned using median concentration of sEphA2 (left) or sNectin-4 (right) in our plasma samples or by using the median expression value for each TCGA indication. 1 = Highest expression, 7 = Lowest expression.

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.

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