A survey of EphA2 expression by immunohistochemistry (IHC) in tumor tissue microarrays (TMAs) to support BT5528 indication selection

POSTER# 5300

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ABSTRACT

- BT5528 is a *Bicycle*® Toxin Conjugate (BTC), a novel class of chemically synthesized molecules, comprising a bicyclic peptide targeting EphA2 (Erythropoietin-producing hepatocellular receptor A2) tumor antigen, linked to cytotoxin (monomethyl auristatin E [MMAE]) via a cleavable linker
- EphA2 is overexpressed in a range of solid tumors and contributes to oncogenesis, tumor-associated angiogenesis and metastasis
- Preclinical activity of BT5528 has been described previously, demonstrating increased efficacy is associated with high EphA2 tumor cell expression levels in xenograft models [1]
- An IHC assay was developed to CAP/CLIA standards to quantify EphA2 tumor expression. This assay detects the extracellular domain (ECD) of EphA2 (BT5528 binding site)
- Enrollment to a Phase I dose escalation study with BT5528 (NCT04180371) started Oct 2019 in patients with advanced solid tumors associated with EphA2 expression

INTRODUCTION

- EphA2:
  - is overexpressed in many difficult to treat tumors, such as NSCLC, TNBC, pancreatic, ovarian, gastric/upper GI, and urothelial cancers
  - is expressed at relatively low levels in normal adult tissues
  - has been targeted by other drugs such as MEDI-547 and MM-310, which failed in the clinic due to unacceptable toxicity [2]
- Multiple independent IHC assays using a variety of reagents have been used to study EphA2 expression across indications. No IHC assay that specifically detects the EphA2 ECD has been reported
- The proprietary Bicycle IHC assay was used to survey EphA2 ECD expression across a variety of tumor types to prioritize indications for clinical development and is employed for the assessment of EphA2 expression in patient tumor tissue in the BT5528-100 trial

METHODS

- An EphA2 IHC assay was developed to CAP/CLIA standards on the Dako platform using R&D Systems EphA2 primary antibody (AF3035) at 10 µg/mL and the Dako FLEX detection kit
- TMAs (US Biomax & Tristar Technology Group) of indications reported to have high EphA2 were stained and scored for EphA2 expression
- H-scores (the product of stain intensity on a scale of 0-3 and % positive tumor cells) were generated by a pathologist independently for tumor cell membrane (TM) and tumor cytoplasm (TC)
- H-scores ≥20 were considered positive for EphA2

RESULTS

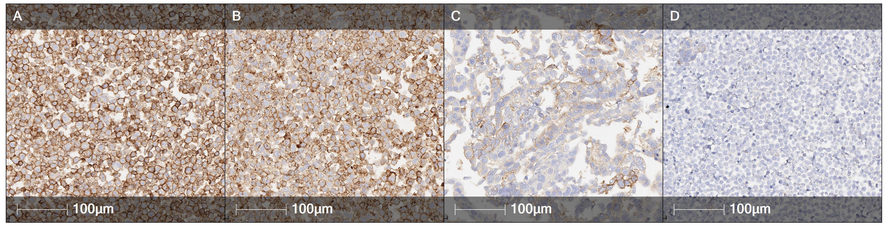

Figure 1: Cell lines controls with known EphA2 expression levels (flow cytometry, data not shown) were stained by IHC. A. HT1080 - EphA2 high B. MDA-MB-468 - EphA2 medium C. HT1376 - EphA2 medium D. NCI-H1836 - EphA2 negative

Table 1: Summary of EphA2 ECD expression by IHC in tumor membrane (TM) and tumor cytoplasm (TC) of 7 indications

TMA	Indication	Total cores (N)	TM Positive N (%)	TC Positive N (%)	TM or TC Positive N (%)
PA2081b	Pancreatic	92	48 (52)	14 (15)	48 (52)
BL2082a	Bladder	142	42 (30)	37 (26)	51 (36)
HN803f	Head & Neck	69	14 (20)	17 (25)	22 (32)
ST1001a	Stomach	53	10 (19)	15 (28)	15 (28)
LC1921b, ATGC1118, TA2820	NSCLC	221	45 (20)	52 (24)	60 (27)
BC1115c	Ovarian	83	8 (10)	13 (16)	17 (20)
BR1301	TNBC	124	3 (2)	25 (20)	25 (20)

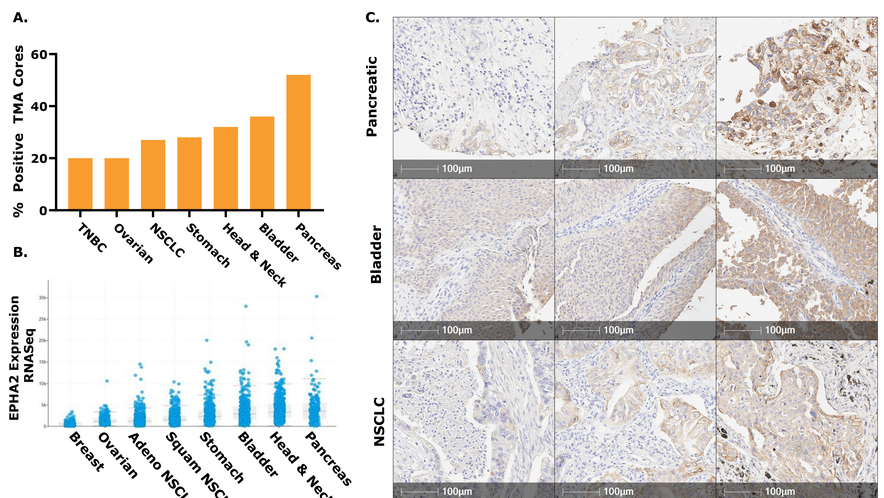

Figure 2: A. Percent positive TMA cores by indication. B. EphA2 mRNA expression from the same indications assessed by IHC [3,4] C. Images from 3 indications (pancreatic cancer, bladder cancer, and NSCLC) representing 3 bins of EphA2 H-scores: left to right TM= 0-19, TM = 20-99, TM = 100+.

RESULTS

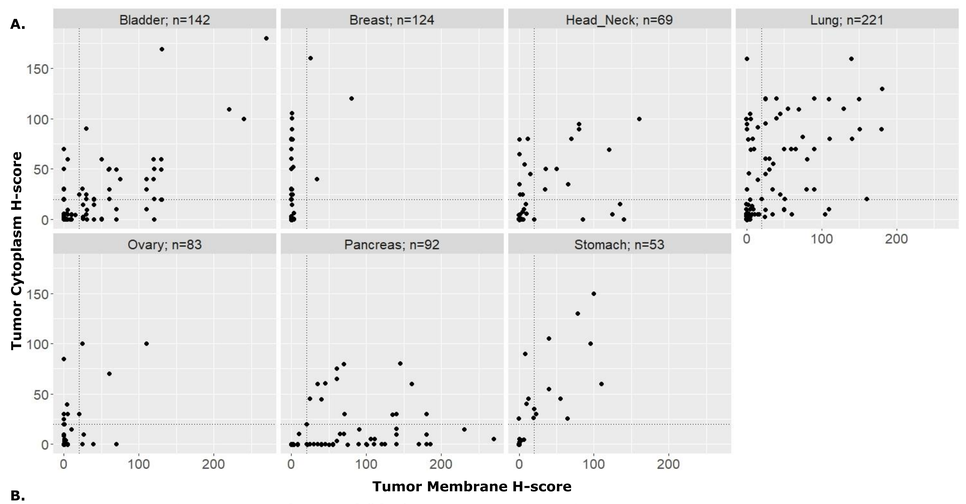

Figure 3: A. The tumor cytoplasm vs membrane H-score for each core was plotted by indication, with dotted lines marking the H-score = 20 cutoff B. Percent EphA2 positivity of adenocarcinoma and squamous cell carcinoma NSCLC plotted by tumor grade and stage.

CONCLUSIONS/SUMMARY

- An IHC assay has been established to CAP/CLIA standards to determine expression of EphA2 ECD in FFPE human tumor tissue collected in the BT5528-100 trial
  - First report of an EphA2 IHC assay and scoring paradigm which delineates tumor membrane and tumor cytoplasm H-scores independently
- Similar rank order of indications by EphA2 protein positivity via IHC or level of EphA2 mRNA expression
- The prevalence and patterns of EphA2 expression, in both tumor cytoplasm and membrane, vary across indications, with the highest frequency observed in pancreatic cancer
- In NSCLC adenocarcinoma, the frequency of EphA2 expression increases with higher grade and stage

References: [1] Bennett et al, Mol Cancer Ther. 2020 [2] Annunziata et al, Invest New Drugs. 2013 [3] Cerami et al, Cancer Discov. 2012 [4] Gao et al, Sci. Signal. 2013

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