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ABSTRACT

- BT8009 is a Bicycle® Toxin Conjugate (BTC), a novel class of chemically synthesized molecules, comprising a bicyclic peptide targeting the tumor antigen Nectin-4, linked to the cytotoxin monomethyl auristatin E (MMAE) via a molecular spacer and cleavable linker
- Nectin-4 is a clinically validated tumor target and has been reported to be highly expressed in a wide range of solid tumors [1]
- Preclinical activity of BT8009 has been described previously, demonstrating increased efficacy is associated with high Nectin-4 tumor cell expression levels in xenograft models [2]
- An immunohistochemistry (IHC) assay using a proprietary Nectin-4 monoclonal antibody was developed to CAP/CLIA standards to quantify Nectin-4 tumor expression. This assay detects the extracellular domain (ECD) of Nectin-4 (BT8009 binding site)
- A Phase I/2 dose escalation and expansion study with BT8009 (NCT04561362) began dosing in Sep 2020 in patients with advanced solid tumors associated with Nectin-4 expression

INTRODUCTION

- Nectin-4:
 - Cell adhesion molecule, widely expressed during development with restricted adult normal tissue expression (e.g. skin, esophagus)
 - Expressed in many difficult to treat solid tumors such as bladder, breast (including triple negative breast cancer [TNBC]), lung, ovarian, esophageal, head & neck (HNSCC), pancreatic, and gastric cancers
- Approved Nectin-4 targeted ADC enfortumab vedotin is highly efficacious in heavily pretreated advanced bladder cancer and like BT8009 also contains MMAE linked via a val-cit linker
- Multiple independent IHC assays using a variety of reagents have been used to study Nectin-4 expression across indications
- The proprietary Bicycle IHC assay was used to survey Nectin-4 ECD expression across a variety of tumor types to prioritize indications for clinical development and is employed for the assessment of Nectin-4 expression in patient tumor tissue in the BT8009-100 trial

METHODS

- A Nectin-4 IHC assay was developed to CAP/CLIA standards on the Leica platform using a proprietary rabbit monoclonal α -Nectin-4 primary antibody YMW-1-58 (Abcam, Burlingame CA) and the BOND Polymer Refine detection kit
- TMAs (US Biomax) from indications reported to have high Nectin-4 were stained and scored for Nectin-4 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 and tumor cytoplasm. If tumor membrane or cytoplasm is not specified, H-score refers to the greater of the two metrics.

RESULTS

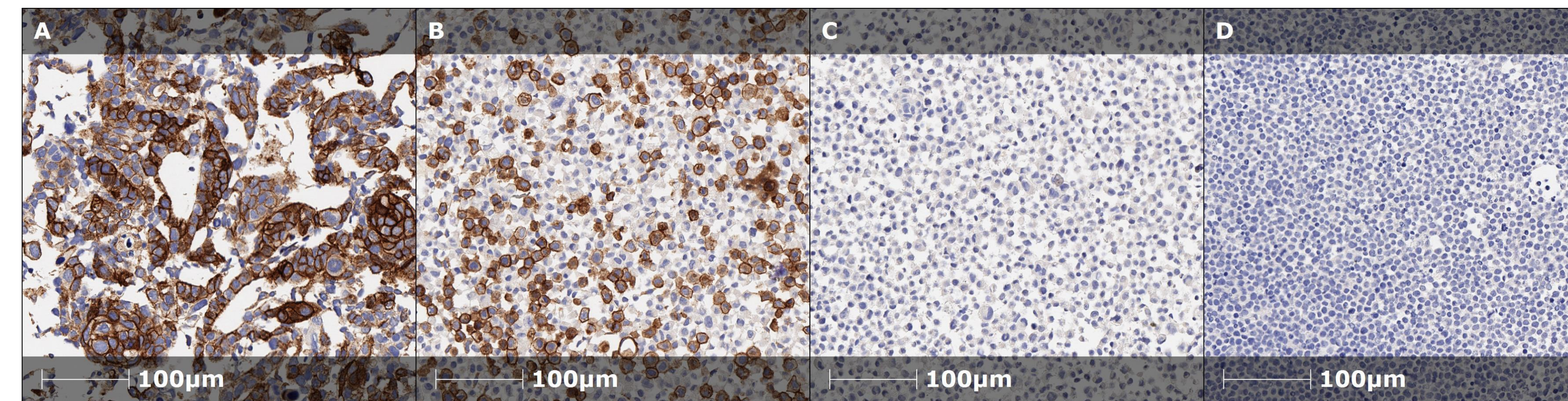


Figure 1: Cell line controls with known Nectin-4 expression levels (flow cytometry, data not shown) were stained by IHC. A. HT1376 – Nectin-4 high B. MDA-MB-468 – Nectin-4 medium C. HT1080 – Nectin-4 negative D. NCI-H1836 – Nectin-4 negative

Table 1: Summary of Nectin-4 ECD expression by IHC in 8 indications.

Indication	Total cores (N)	Percent Negative (H-score < 20)	Percent Low (H-score 20-99)	Percent Moderate (H-score 100-199)	Percent High (H-score 200+)
Breast (all)	225	20	23	45	12
TNBC	141	14	18	53	15
Bladder	142	23	22	48	8
Esophagus	140	46	22	29	4
Head & Neck	69	42	26	28	4
Lung	157	61	22	17	0
Ovarian	89	54	31	13	1
Pancreas	96	81	16	3	0
Stomach	131	96	2	2	0

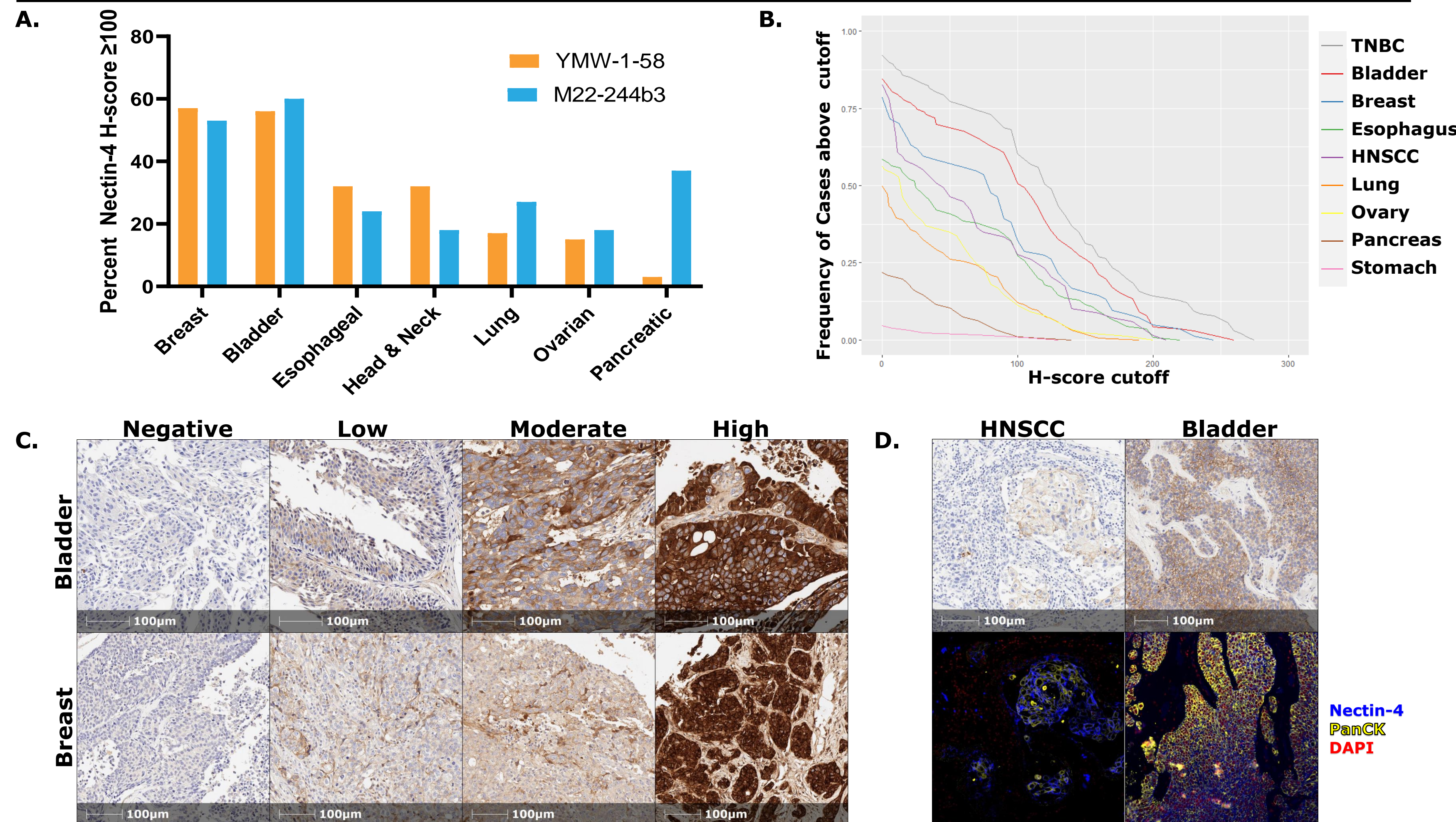


Figure 2: A. Percent of TMA cores which meet Nectin-4 H-score cutoff ≥ 100 across common indications as assessed by BicycleTx (YMW-1-58) and Challita-Eid et al (M22-244b3)[1] B. Frequency of Nectin-4 expression plotted by H-score cutoff C. Examples of images from bladder and breast cancer representing 4 bins of Nectin-4 H-scores: negative (0-19), low (20-99), moderate (100-199), and high (200-300). D. Comparison of YMW-1-58 performance when configured for IHC (top) or multiplexed immunofluorescence (bottom)

RESULTS

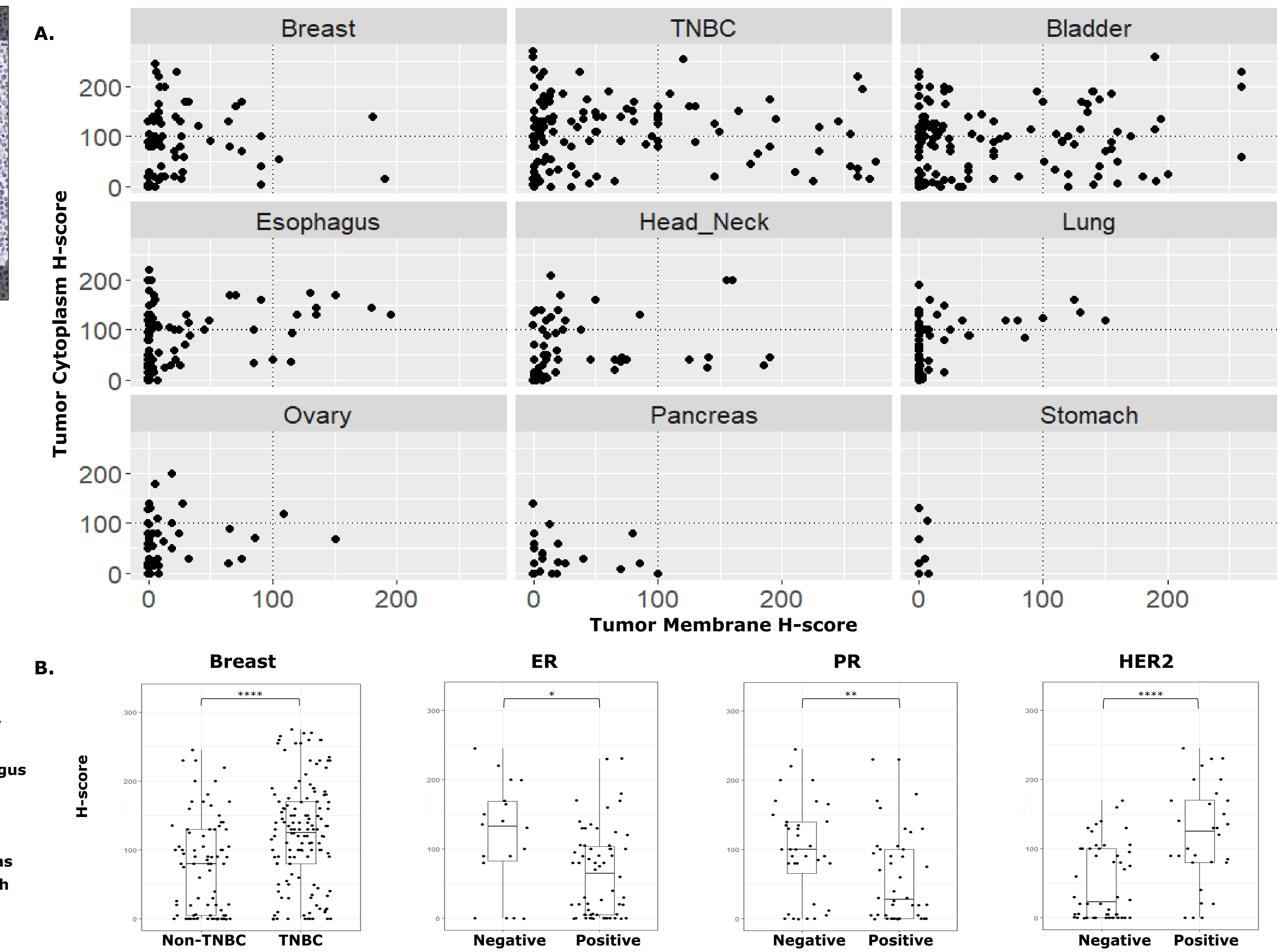


Figure 3: A. The tumor cytoplasm vs membrane H-score for each core was plotted by indication, with dotted lines marking an H-score = 100 cutoff B. Nectin-4 H-score distribution in non-TNBC vs TNBC, followed by Nectin-4 distribution in non-TNBC plotted by hormone receptor and HER2 status. Statistical significance determined by Welch's t-test, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$

CONCLUSIONS/SUMMARY

- An IHC assay has been established to CAP/CLIA standards to determine expression of Nectin-4 ECD in FFPE human tumor tissue collected in the BT8009-100 trial
- Benchmarking YMW-1-58 antibody performance against published literature shows similar degree of Nectin-4 expression across most indications tested, excluding pancreatic cancer
- The prevalence and patterns of Nectin-4 expression, in both tumor cytoplasm and membrane, vary across indications, with the highest frequency observed in bladder and breast cancer
- Consistent with the literature, Nectin-4 expression is significantly enriched in TNBC and in HR- and HER2+ breast cancer [3,4]

References: [1] Challita-Eid et al, Cancer Res. 2016 [2] Rigby et al, Cancer Res. 2019 [3] Al-Torky et al, Archives of Clin & Exp Res. 2015 [4] M-Rabet et al, Annals of Onc. 2017