An IHC assay has been established to CAP/CLIA standards to determine expression of EphA2 ECD in tumor tissue. The prevalence and patterns of EphA2 expression, in both tumor cytoplasm and membrane, vary across 10 indications. The specific detection of the EphA2 ECD has been reported and a Preclinical activity of BT5528 has been described previously, demonstrating its potential as an anti-angiogenic agent.

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

**RESULTS**

Figure 2: A. Percent positive TMA cores by indication. B. EphA2 mRNA expression from the same indications.

Figure 3: A. The tumor cytoplasm vs membrane H-score for each core was plotted by indication, with dashed line 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 pattern 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.

**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.
- TMA (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 the percentage of 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.