Background: Neurotrophic tyrosine receptor kinases (NTRK) are a gene family encoding receptor tyrosine kinases expressed across a spectrum of normal and neoplastic tissues. Fusion of any of these genes with various upstream partners leads to oncogenic protein expression and uncontrolled proliferation. Identification of tumors driven by NTRK fusions is clinically relevant because they can be targeted with small molecule or antibody inhibitors. Here, we report the development of a testing strategy for detection of NTRK fusions in a wide range of tumor types.

Methods: An IHC staining protocol was developed using the pan-NTRK (EPR17341) antibody in combination with VENTANA OptiView DAKO IHC Detection kit (Ventana) on the previously validated Benchmark/ultra (DAKO) platform. This protocol was tested on 101 tissue samples, across 12 different tumor types. A comprehensive panel of 120 positive and 120 negative tissue samples was used in developing the assay. Analytical testing to determine specificity and sensitivity of the assay was performed on 32 tissue microarrays. A cross-sectional evaluation of IHC positivity was performed in 164 cases of tumor types with ISH

Results: In cancer, fusion of one of these genes with various upstream partners leads to aberrant protein expression, leading to an expressed fusion transcript and a single or multiple kinase domains.

Conclusions: In solid tumors with low prevalence and intensity of IHC expression, the pan-NTRK IHC assay may identify tumors for further evaluation. However, in tumors with high prevalence and intensity of IHC expression, the pan-NTRK IHC assay may be a tool to further distinguish tumors harboring fusions.

**HIC staining by ISH across tumor types**

**Materials and Methods**

**ICHC staining by ISH across tumor types**

**References and Acknowledgements**