# B-1

## Spatial profiling of neuro-immune interactions in gastroenteropancreatic NETs

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### BACKGROUND

Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are heterogeneous malignancies that arise from complex cellular interactions within the tissue microenvironment. Until recently, the absence of reliable methods to unmix tumor- and stroma-derived signals has precluded a comprehensive understanding of how GEP-NETs arise in different tissues. Here, we sought to decipher tumor-derived signals from the surrounding microenvironment by applying Nanostring Digital Spatial Profiling (DSP) to hormone-secreting and non-functional GEP-NETs. By combining this approach with in vitro studies in human-derived organoids, we demonstrate the convergence of cell autonomous immune and pro-inflammatory signals that suggests their role in neuroendocrine differentiation and tumorigenesis.

### METHODS

DSP was used to evaluate the expression of 40 neural and immune-related proteins in surgically resected duodenal and pancreatic NETs (n=20) primarily comprised of gastrinomas (18/20). A total of 279 regions of interest were examined between tumors, adjacent normal and abnormal-appearing epithelium, and the surrounding stroma. The results were stratified by tissue type and *Multiple Endocrine Neoplasia I (MEN1)* status. Immunohistochemical (IHC) staining of the tumors (n=30) confirmed neuro-immune protein expression and cellular reprogramming of preneoplastic tissues. Cell autonomous inflammatory features were further evaluated by IHC and RNAscope, while functional pro-inflammatory signaling was tested using patient-derived duodenal organoids.

### RESULTS

Duodenal gastrinomas (DGASTs) showed significant immune exclusion compared to pancreatic NETs. Compared to non-MEN1 tumors, MEN1 DGASTs and preneoplastic lesions exhibited reduced immune infiltrates coincident with neural reprogramming and a senescent phenotype. Despite a paucity of immune cells, DGASTs showed strong intratumoral expression of the pro-inflammatory and pro-neural factor IL-17B (4/6 patients), whereas expression of this cytokine was restricted to the stroma in pancreatic NETs (3/3). Treatment of human duodenal organoids with IL-17B activated NF-kB and STAT3 signaling and induced the expression of neuroendocrine markers.

### CONCLUSIONS

Multiplexed spatial protein analysis identified tissue-specific neuro-immune signatures in GEP-NETs. DGASTs are characterized by an immunologically cold microenvironment that permits cellular reprogramming and neoplastic transformation of the preneoplastic epithelium.

Moreover, DGASTs cell autonomously express pro-inflammatory factors consistent with a senescent phenotype, including tumor-derived IL-17B, that stimulate the neuroendocrine phenotype.

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