Molecular and Immune Landscape of Early-Onset versus Average-Onset Well-Differentiated Enteropancreatic Neuroendocrine Tumors

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INTRODUCTION

- The incidence of neuroendocrine tumors (NETs) has increased over the past few decades, especially among younger patients.
- There are likely biological differences between younger and older patients who develop well-differentiated NETS, but these differences have not been investigated in detail.
- Data from other gastrointestinal malignancies suggest early-onset may be associated with biological differences.
- A preliminary analysis of small-volume NETs from the Surveillance, Epidemiology, and End Results (SEER) database found that age was a statistically significant correlate for outcomes, further supporting the possibility of biological differences between younger and older-onset well-differentiated NETs.
- We sought to compare the landscape of DNA mutations, RNA expression, and immune profiling in early onset (EO) versus average onset (AO) pancreatic and intestinal NETS to identify biological differences between the two populations.

METHODS

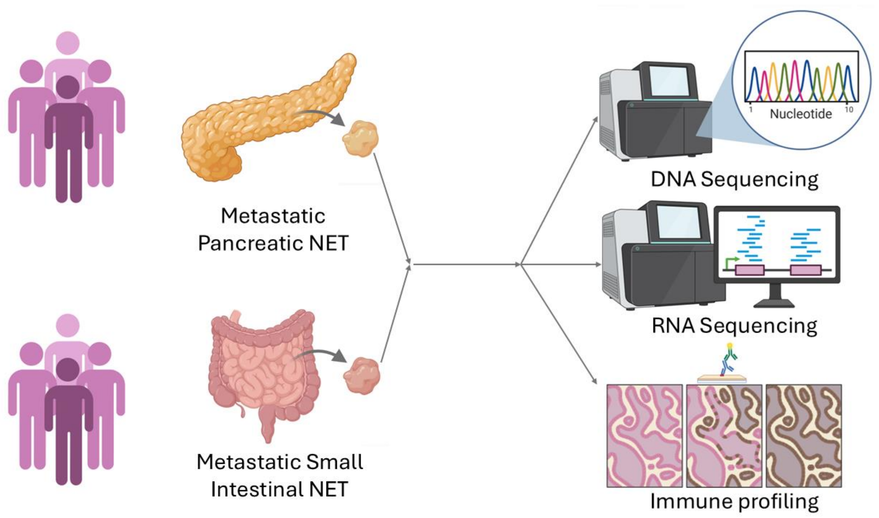


Figure 1. Molecular profiling. Patients with a curated diagnosis of either PNETs (N=317) or SNETs (N=185) who were sequenced with the Tempus xT assay were selected from the Tempus multi-modal database. DNA sequencing was performed to identify somatic alterations and whole transcriptome RNA-seq data on a subset of patients were normalized to log2(TPM+1) with assay batch correction. Differential expression between EO (<50 years of age at diagnosis) and AO-NETS (≥50 years) was assessed using Wilcoxon rank sum tests with Benjamini-Hochberg correction. Pathway enrichment was assessed via single sample gene set enrichment analysis (ssGSEA). Immune profiling analysis included tumor mutational burden (TMB), microsatellite instability (MSI), PD-L1 status, and immune infiltration estimated via quanTIseq.

RESULTS

Characteristic	Overall N = 317	Average onset: 50-90+ N = 223	Early onset: 0-49 N = 94	p-value
KRAS	**34 (11%)**	**32 (14%)**	**2 (2.1%)**	**0.001**
LRP1B	**11 (3.5%)**	**3 (1.3%)**	**8 (8.5%)**	**0.003**
TP53	**78 (25%)**	**65 (29%)**	**13 (14%)**	**0.004**
SMAD4	**30 (9.5%)**	**27 (12%)**	**3 (3.2%)**	**0.013**
RB1	**38 (12%)**	**32 (14%)**	**6 (6.4%)**	**0.046**
ZFHX3	8 (2.5%)	3 (1.3%)	5 (5.3%)	0.053
MTAP	22 (6.9%)	19 (8.5%)	3 (3.2%)	0.088
TSC2	28 (8.8%)	22 (9.9%)	6 (6.4%)	0.3
CDKN2A	54 (17%)	41 (18%)	13 (14%)	0.3
ARID1A	20 (6.3%)	16 (7.2%)	4 (4.3%)	0.3
MEN1	83 (26%)	56 (25%)	27 (29%)	0.5
PTEN	27 (8.5%)	20 (9.0%)	7 (7.4%)	0.7
CDKN2B	44 (14%)	32 (14%)	12 (13%)	0.7
KMT2D	18 (5.7%)	13 (5.8%)	5 (5.3%)	0.9
ATRX	29 (9.1%)	20 (9.0%)	9 (9.6%)	0.9
DAXX	38 (12%)	27 (12%)	11 (12%)	>0.9
SETD2	23 (7.3%)	16 (7.2%)	7 (7.4%)	>0.9

Table 1. Somatic mutations by onset in EO and AO Pancreatic NETs. Showing genes with at least 5% mutation prevalence in EO or AO. P-values calculated using chi-squared or Fisher's exact tests.

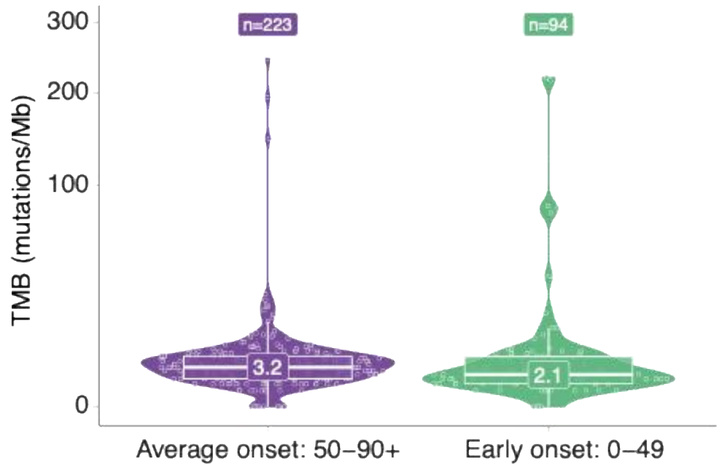
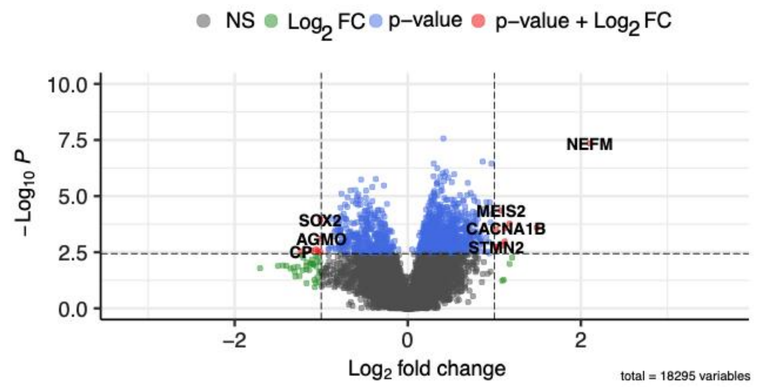


Figure 2. Violin/box plot with tumor mutational burden (TMB) value in EO and AO Pancreatic NETs. Values in box plots are median TMB. P=0.014, Wilcoxon rank sum test.

A



B

Characteristic	Overall N = 254	Average onset: 50-90+ N = 176	Early onset: 0-49 N = 78	p-value	q-value
Myogenesis	0.18 (0.16, 0.22)	0.18 (0.15, 0.21)	0.20 (0.17, 0.23)	<0.001	0.003
Hedgehog Signaling	0.31 (0.27, 0.35)	0.30 (0.26, 0.34)	0.33 (0.30, 0.37)	<0.001	0.004
E2F	0.32 (0.24, 0.42)	0.35 (0.25, 0.46)	0.28 (0.22, 0.34)	<0.001	0.004
Apical Junction	0.27 (0.24, 0.31)	0.26 (0.23, 0.30)	0.28 (0.26, 0.32)	<0.001	0.006
MYC	0.38 (0.34, 0.43)	0.40 (0.34, 0.45)	0.36 (0.33, 0.41)	<0.001	0.006
G2M Checkpoint	0.34 (0.26, 0.44)	0.36 (0.26, 0.47)	0.31 (0.25, 0.36)	0.001	0.012
UV Response	0.37 (0.33, 0.41)	0.37 (0.33, 0.40)	0.39 (0.35, 0.43)	0.005	0.04
VEGF	0.43 (0.41, 0.46)	0.43 (0.40, 0.45)	0.45 (0.41, 0.47)	0.014	0.079
DNA Repair	0.39 (0.37, 0.41)	0.39 (0.37, 0.41)	0.38 (0.36, 0.40)	0.015	0.079

Figure 3. A) Volcano plot with differentially expressed genes between EO and AO pancreatic NETS. **B)** Gene set enrichment analysis. Values are enrichment scores and interquartile range, with higher values indicating higher expression. Table only contains 9 statistically significant pathways out of 54 pathways tested. Statistical significance was defined as p<0.05 (Wilcoxon rank sum test) and q<0.10 for GSEA.

CONCLUSIONS

This is the largest molecular analysis comparing EO and AO-enteropancreatic NETs. More age-based differences were seen in PNETs than SNETS. AO cases attained more alterations typically found in neuroendocrine carcinoid (NECs) and grade 3 NETs. Our results suggest that age at diagnosis may be an important determinant of tumor biology and clinical management.