Comprehensive Genomic Profiling of Gastroenteropancreatic Neuroendocrine Neoplasms (GEP-NENs)

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ABSTRACT

Purpose: GEP-NENs are rare malignancies with increasing incidence. Their molecular characteristics are still undefined. We explored the underlying biology of GEP-NENs and the differences between gastrointestinal (GI) and pancreatic (PNEN), high-grade (HG), and low-grade (LG) tumors.

Experimental Design: GEP-NENs were analyzed using next-generation sequencing (NGS; MiSeq on 47 genes, NextSeq on 592 genes), IHC, and *in situ* hybridization. Tumor mutational burden (TMB) was calculated on the basis of somatic nonsynonymous missense mutations, and microsatellite instability (MSI) was evaluated by NGS of known MSI loci.

Results: In total, 724 GEP-NENs were examined: GI ($N = 469$), PNEN ($N = 255$), HG ($N = 135$), and LG ($N = 335$). Forty-nine percent were female, and median age was 59. Among LG tumors, the most frequently mutated genes were *ATRX* (13%), *ARID1A* (10%), and *MEN1* (10%). HG tumors showed *TP53* (51%), *KRAS* (30%), *APC* (27%), and *ARID1A* (23%). Immune-related biomarkers yielded a lower prevalence in LG tumors compared with HG [MSI-H 0% vs. 4% ($P = 0.04$), PD-L1 overexpression 1% vs. 6% ($P = 0.03$), TMB-high 1% vs. 7% ($P = 0.05$)]. Compared with LG, HG NENs showed a higher mutation rate in *BRAF* (5.4% vs. 0%, $P < 0.0001$), *KRAS* (29.4% vs. 2.6%, $P < 0.0001$), and *PI3KCA* (7% vs. 0.3%, $P < 0.0001$). When compared with GI, PNEN carried higher frequency of *MEN1* (25.9% vs. 0.0%, $P < 0.0001$), *FOXO3* (8.6% vs. 0.8%, $P = 0.005$), *ATRX* (20.6% vs. 2.0%, $P = 0.007$), and *TSC2* (6.3% vs. 0.0%, $P = 0.007$), but lower frequency of mutations in *APC* (1.0% vs. 13.8%, $P < 0.0001$).

Conclusions: Significant molecular differences were observed in GEP-NENs by tumor location and grade, indicating differences in carcinogenic pathways and biology.

Introduction

Neuroendocrine neoplasms (NEN) comprise a heterogeneous group of tumors (1). Pancreatic and gastrointestinal tract (GEP)-NENs are rare diseases but the incidence and prevalence are increasing, likely owing to improvements in detection and diagnosis (2). Molecular data that may explain their clinical heterogeneity, from indolent to highly aggressive, and divergent treatment responses, are lacking (3).

In the era of precision medicine where elucidating the molecular pathways to carcinogenesis could guide targeted therapies' development (4), the pathogenesis of GEP-NENs is largely unknown and only a few studies have attempted to characterize the molecular features of this group of diseases (5). In addition, they may be part of the spectrum of some hereditary syndromes such as MEN1, Familial Adenomatous Polyposis (FAP), or Lynch syndrome. Previous reports on molecular characterization of GEP-NENs have been conducted on cohorts with small sample sizes ($N < 160$; refs. 6–11). However, a recent study has demonstrated that small intestinal neuroendocrine tumors (NET) can be classified into three groups based on molecular profiling, with different survival outcomes after resection of the primary tumor (12). This suggests that novel molecular profiling may be useful in the clinical setting to facilitate personalized management and improve prognostic classification for patients (13–15).

Immune checkpoint inhibitors have dramatically changed the standard of care in many types of cancers and PD-L1 expression has proven to be a positive predictive biomarker of response in many different tumor types (16). In metastatic GEP-NENs, the expression of PD-L1 is associated with higher WHO tumor grade (G3), and has both predictive and prognostic value for survival of patients (17). These data deserve further validation to better understand whether patients with GEP-NENs may benefit from this treatment strategy and whether MSI-H status predicts Lynch syndrome.

Compelling evidence suggests that tumor mutational burden (TMB) may be a useful biomarker to select patients who could respond to immunotherapy, independently from the microsatellite instability

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Translational Relevance

Gastroenteropancreatic neuroendocrine neoplasms (GEP-NEN) are rare diseases, but the incidence and prevalence are increasing. Molecular data that may explain their clinical heterogeneity, from indolent to highly aggressive, and divergent treatment responses are lacking. Here, we present data from one of the largest cohorts of GEP-NENs that underwent extensive molecular analyses using next-generation sequencing. We show that significant molecular differences are present in GEP-NENs by tumor location and grade. Moreover, we provide novel insights into several mutations in targetable genes that may pave the way to novel therapeutic options. Our results suggest that deep molecular profiling of GEP-NENs is paramount to molecular-based classification of these tumors and biomarker-driven clinical trial design and novel targeted agent development, together with hereditary syndrome prediction.

(MSI) status of the tumor. However, little is known about the prevalence of TMB in GEP-NENs.

The incorporation of signaling, metabolic, and molecular information to improve the classification of GEP-NENs might enable the rational design of clinical trials to exploit the efficacy of specific agents, according to the precision medicine paradigm. In addition, molecular profiling is anticipated to improve prognostication and treatment selection, inform patient follow up, and enhance patient outcomes (18). Moreover, these data may have direct implications for germline testing: the diagnosis of hereditary syndromes is crucial to prevent second tumors in patients and also because specific surveillance programs may also prevent cancer-related deaths in their relatives.

As recently shown for pancreatic neuroendocrine tumors (PanNEN; ref. 19), a comprehensive molecular analysis can identify several novel candidate carcinogenetic mechanisms that may be used to develop new biomarkers and targeted therapies.

On the basis of these data, we can hypothesize that a deep molecular profiling of GEP-NENs will provide new insights into biology of these rare diseases, which may lay the bases to a new molecular-based classification of these tumors, biomarker-driven clinical trial design, and novel targeted agents' development.

Materials and Methods

A cohort of 724 GEP-NENs that underwent comprehensive genomic profiling by Caris Life Sciences were identified from a retrospective database from February 2013 to December 2017. All samples were analyzed as part of standard of care (SOC). Molecular characteristics, microsatellite instability (MSI) status, TMB, as well as protein expression by IHC were analyzed for differences based on the tumor's primary location (GI vs. pancreas) and grade (HG vs. LG). Tumors were selected from our database based on their pathology report: the histologic diagnosis and accompanying diagnostic IHC workup performed at the referring pathology laboratories were reviewed in all cases by a board-certified pathologist at Caris Life Sciences. Specimens included were taken from any biopsy site including both local lesions or metastatic deposits. Tumors were included if the primary location was noted to be from the GI tract [including esophageal/gastroesophageal junction (GEJ), gastric, duodenum, small intestine, large intestine, colon, pancreas, or biliary tract; unknown primary site cases were excluded). To limit samples with a mixed histology, cases that had terms that would suggest a mixed histology such as "adenocarcinoma" or "carcinoma, NOS" were excluded. Grading was determined on the basis of terminologies included in the pathology notes that accompanied each specimen upon arrival at Caris Life Sciences: (i) high-grade (HG) cohort included samples with any of the following terms found in the available clinical information (in either the diagnosis or histology fields): poorly differentiated, grade 3, G3, HG, small-cell carcinoma, large-cell carcinoma; while (ii) the low-grade (LG) cohort included the following terms: well differentiated, moderately differentiated, moderately well differentiated, grade 1, grade 2, intermediate grade, LG.

IHC

IHC was performed on formalin-fixed paraffin-embedded (FFPE) sections. Protein staining was scored for intensity (0 = no staining; 1+ = weak staining; 2+ = moderate staining; 3+ = strong staining) and staining percentage (0%–100%) by pathologists. PD-L1 testing was performed using the SP142 anti-PD-L1 clone (Ventana).

Next-generation sequencing

Next-generation sequencing (NGS) was performed in a CAP/CLIA/ISO-certified commercial laboratory on genomic DNA isolated from FFPE tumor samples using the NextSeq platform (Illumina, Inc.). A custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets or 44-gene oncogenic hotspot targets (Agilent Technologies). All variants were detected with >99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of 750 and an analytic sensitivity of 5%. Prior to molecular testing, tumor enrichment was achieved by harvesting targeted tissue using manual microdissection techniques. Genetic variants identified were interpreted by board-certified molecular geneticists and categorized as "pathogenic," "presumed pathogenic," "variant of unknown significance," "presumed benign," or "benign," according to the American College of Medical Genetics and Genomics (ACMG) standards. When assessing mutation frequencies of individual genes, "pathogenic," and "presumed pathogenic" were counted as mutations, whereas "benign," "presumed benign" variants, and "variants of unknown significance" were excluded.

Microsatellite instability

Microsatellite instability (MSI) was examined by counting number of microsatellite loci that were altered by somatic insertion or deletion for each sample. The threshold to determine MSI by NGS was determined to be 46 or more loci with insertions or deletions to generate a sensitivity of >95% and specificity of >99%.

Tumor mutational burden

TMB was measured by counting all nonsynonymous missense mutations found per tumor that had not been described previously as germline alterations [592 genes and 1.4 megabases (MB) sequenced/tumor]. Potential germline mutations are excluded by comparing data against dbSNP 137 full and 1000 Genomes Phase III. The threshold to define TMB-high (TMB-H) was greater than or equal to 17 mutations/MB and was established by comparing TMB with MSI by fragment analysis in colorectal cancer cases, based on reports of TMB having high concordance with MSI-H in colorectal cancer. Differences in mean TMB was assessed using Student t test.

Statistical analysis

χ^2 test was performed for comparative analysis using SPSS v23 (IBM SPSS Statistics), and a statistical significance was defined as $P < 0.05$.

Ethics statement

All human subjects' data were deidentified prior to analysis. Thus, this research was determined to be exempt from the requirement for informed consent per the Western Institutional Review Board (WIRB).

Results

Patient and tumor characteristics

The analyzed cohort consisted of 724 GEP-NENs; mean age of the patients was 59 (range 19–90), 49% were female ($n = 358$), and 51% were male ($n = 366$). The tumor primary site was in the gastrointestinal tract (GI) in 469 patients (64%), and in the pancreas in 255 patients (36%). Age was significantly higher in GI-NENs compared with PNENs (mean of 59.7 vs. 56.8; $P < 0.001$). Tumor grading was available for 470 out of 724 samples; in the cohorts 135 HG tumors were identified: 94 in GI-NENs and 41 in PNENs. The remaining 335 samples were classified as LG: 222 GI-NETs and 113 PNET (**Fig. 1**).

Molecular landscape of GEP-NENs

Across the whole cohort the most frequently mutated genes, identified by mean of NGS, were: *TP53* ($n = 574$, 18%), *ATRX* ($n = 83$, 12%), *KRAS* ($n = 578$, 11%), *MEN1* ($n = 214$, 11%), *ARID1A* ($n = 63$, 11%), and *APC* ($n = 578$, 10%). Less frequently mutated genes included *CDKN2A* ($n = 198$, 4%), *RB1* ($n = 555$, 3%), *BRAF* ($n = 576$, 2%), *PIK3CA* ($n = 572$, 2%), and *BRCA2* ($n = 352$, 2%; **Fig. 2**).

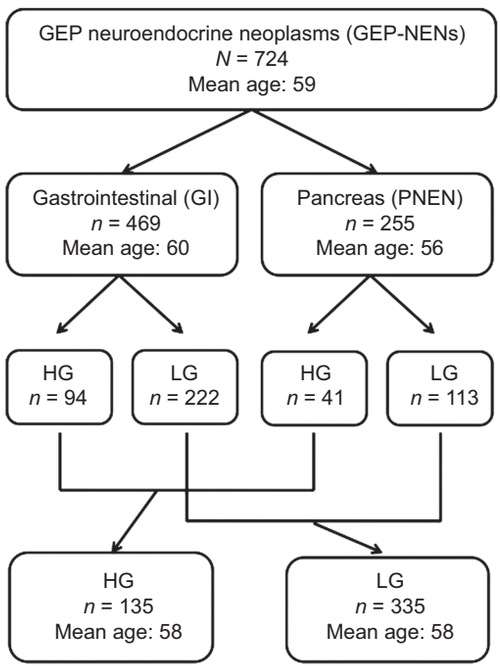


Figure 1.

Patient demographics. NEN from GI ($n = 469$), PNEN ($n = 255$), HG ($n = 135$), and LG ($n = 335$). Female-to-male ratio was 49%/51%, and the median age was 59 years.

Amplifications events, defined by copy number variation (CNV), were rare; the most frequently amplified genes were *MYC* ($n = 202$, 2%), *FGF6* ($n = 185$, 2%), *CCND2* ($n = 2\%$), and *FOXA1* ($n = 185$, 2%).

IHC staining was used to evaluate several biomarkers; the most frequently identified were TUBB3 expression ($n = 547$, 68%), MGMT methylation ($n = 595$, 37%), TOP2A expression ($n = 660$, 36%), PGP expression ($n = 380$, 20%), PR expression ($n = 402$; 15%), EGFR expression ($n = 165$, 12%), and ER expression ($n = 403$, 9%).

Less frequently observed were ALK translocations by IHC ($n = 97$, 5%) and cMET expression ($n = 391$, 3%). *ALK* fusions were in frame and potentially targetable via tyrosine kinase inhibitors (TKIs) treatment. No *NTRK* 1/2/3 fusion event was observed.

Given the molecular landscape of the whole GEP-NENs cohort, statistical analysis was performed to assess whether grading and tumor primary sites are correlated with significant molecular differences.

Molecular differences between GI versus PNEN

Compared with GI, PNEN carried a significant higher frequency of tumor mutation in *MEN1* (25.9 vs. 0%, $P < 0.001$), *FOXO3* (8.6 vs. 0.8%, $P < 0.005$), *ATRX* (20.6 vs. 2%, $P = 0.007$), *TSC2* (6.3 vs. 0%, $P = 0.007$), but lower frequency in *APC* mutations (1% vs. 13.8%, $P < 0.001$).

PNEN also showed a higher expression of PR (38.8 vs. 3.6%, $P < 0.001$) but a lower expression of ER (2.4 vs. 12.4%, $P = 0.001$) and of MGMT methylation (31.7 vs. 40.7%, $P = 0.038$; **Fig. 3**). Within the GI cohort, we investigated the molecular differences between upper versus lower GI: upper GI compared with lower showed a higher rate of *BRCA2* (7.5% vs. 0%, $P < 0.0001$), *TP53* (33% vs. 16%, $P = 0.002$), and *CTNNB1* mutations (4.7% vs. 0%, $P < 0.0001$), and lower rate of *APC* (4.7% vs. 16%, $P = 0.018$).

Molecular differences between GI versus PNET among LG tumors

LG-PNET carried significantly higher frequency of *MEN1* (24.3% vs. 0%, $P < 0.001$), *ATRX* (33.3% vs. 0%, $P = 0.001$), *FOXO3* (12.2% vs. 0%, $P = 0.005$), and *PTEN* ($P = 0.069$) mutations. Conversely, LG GI-NET had a higher, but not significant, mutation rate in *APC* (1.6% vs. 0%, $P = 0.211$). LG-NETs also carried a different pattern of ER and PR expression: LG-PNETs carried a higher expression of PR (56.6% vs. 3%, $P < 0.001$) and a lower expression of ER (0% vs. 17%, $P < 0.001$) when compared with LG GI-NETs. These results are similar to those of the whole cohort, but with higher percentage of ER and PR expression (**Fig. 4**).

Molecular differences between LG versus HG GEP-NENs

Tumor grade was associated with significant molecular differences irrespective of the different tumor site. HG GEP-NENs carried a higher frequency of *TP53* (51% vs. 3.4%, $P < 0.001$), *KRAS* (29.4% vs. 2.6%, $P < 0.001$), *APC* (27% vs. 1.69%, $P < 0.001$), *RB1* (11% vs. 0%, $P < 0.001$), *BRAF* (5.4% vs. 0%, $P < 0.001$), and *PI3KCA* (7% vs. 0.3%, $P < 0.001$) mutations. On the other hand, LG GEP-NENs showed a higher frequency of *MEN1* alterations (9.8% vs. 0%, $P = 0.032$), other frequently mutated genes were *ATRX* (13%) and *ARID1A* (10%). LG GEP-NENs also showed a higher expression of ER (17.9% vs. 0%, $P = 0.01$) and PR (19.2% vs. 7.6%, $P = 0.029$; **Fig. 5A**).

Immune-related biomarkers

Immune-related biomarkers were evaluated between HG versus LG GEP-NENs: The cohort of patients with HG-NENs, irrespective of tumor site, had a higher rate of expression of PD-L1 (6% vs. 1%,

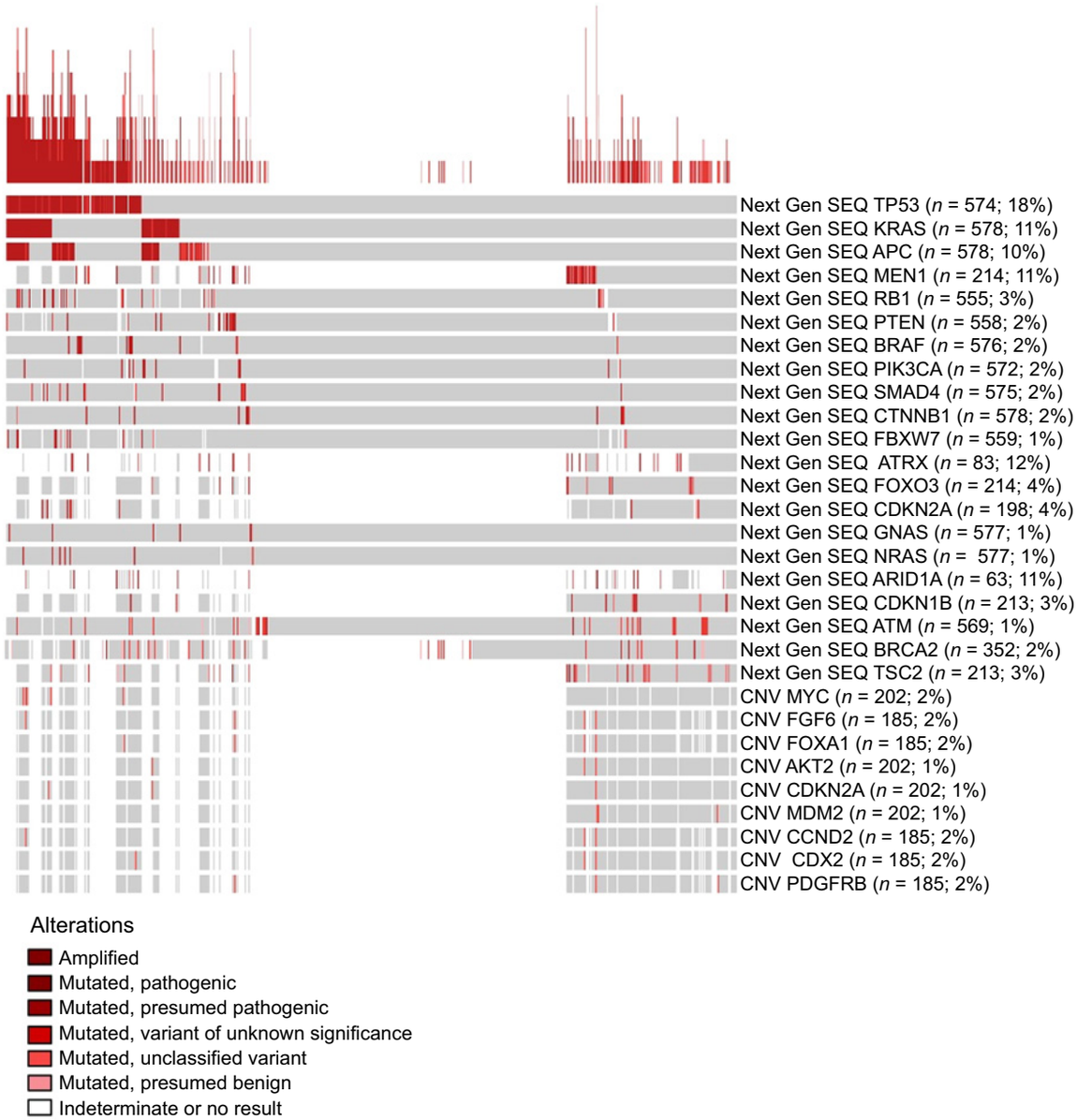


Figure 2.

Oncoprint. Comprehensive molecular profile of 724 GEP-NENs. Overall, the most frequently mutated genes were *TP53* (18%), *ATRX* (12%), *KRAS* (11%), *MEN1* (11%), *ARID1A* (11%), and *APC* (10%). Gene amplification events, as determined by NGS, were rare across the cohort. The most frequently amplified genes are *MYC* (2%), *FGF6* (2%), *CCND2* (2%), and *FOXA1* (2%). Each column is a single patient; gray boxes are those in which no alteration was detected. Samples were arranged by those that harbored a mutation in the genes listed as rows top to bottom (those with *TP53* mutation show up on left, then by *KRAS* mt, then by *APC*, etc.). The stacked graph at the top is a representation of the number of alterations that case had (the higher the line the more alterations). Mutation frequencies were determined on a per gene level excluding cases from that analysis where a particular gene was determined to be indeterminate. The variation of sample size per gene comes from the fact that not all genes within a single case are evaluable.

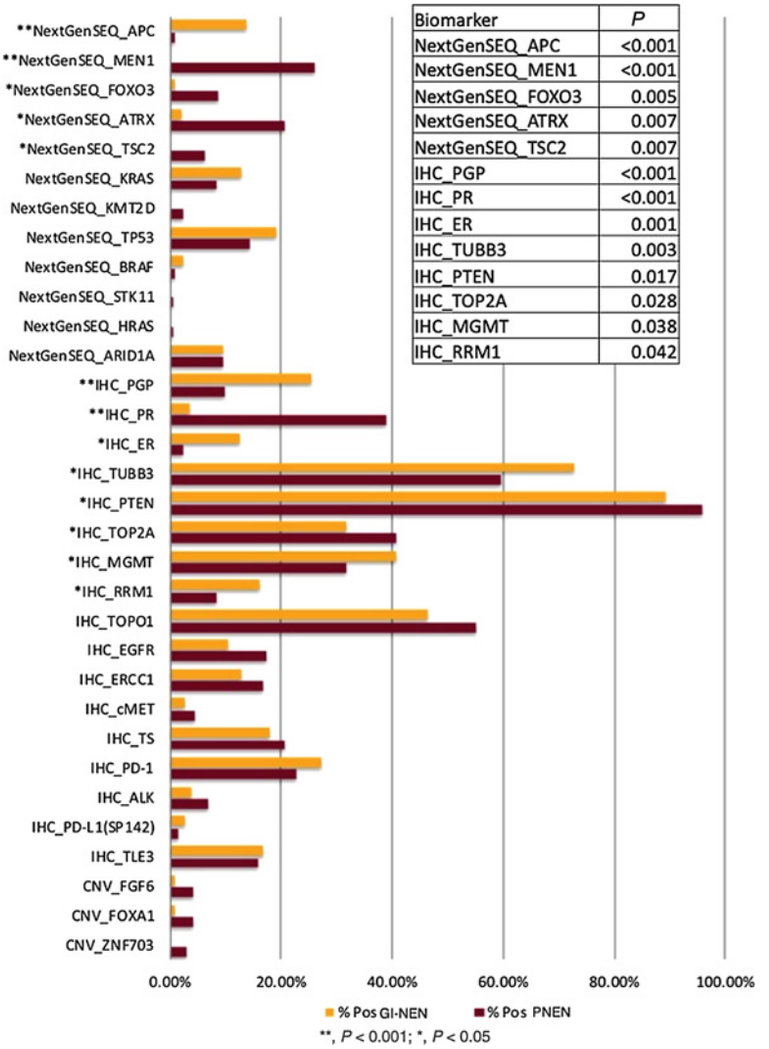
$P = 0.03$), higher mean TMB (9.5 mut/MB vs. 5.1, $P < 0.0001$), and higher MSI-H status (4% vs. 0%, $P = 0.04$; **Fig. 5B**).

Discussion

This is one of the largest cohorts of GEP-NENs underwent extensive molecular analyses using NGS. Because of the rarity and heterogeneity of NENs, treatment options had been slow until recent years. Lately, there have been advances both in the characterization of the disease and in the available therapeutic strategies: TKIs, somatostatin analogue (SSA) therapy, mTOR inhibitors, chemotherapy and Peptide Receptor Radionuclide Therapy (PRRT) have substantially improved the management in the advanced disease setting (20). Nonetheless, treatment decisions remain largely based on tumor stage and grade, despite the observation of significant heterogeneity in tumor biology. There is a well-acknowledged unmet clinical need for novel biomarkers to enable

Figure 3.

Molecular differences between PNEN and GI-NEN. Compared with GI, PNEN carried significantly higher frequency of *MEN1* (25.9% vs. 0.0%), *FOXO3* (8.6% vs. 0.8%), *ATRX* (20.6% vs. 2.0%), and *TSC2* (6.3% vs. 0.0%), but lower frequency of mutations in *APC* (1.0% vs. 13.8%). PNEN had a significantly higher expression of PR (38.9% vs. 3.7%), but interestingly, GI-NEN had higher expression levels of ER (12.5% vs. 2.4%).



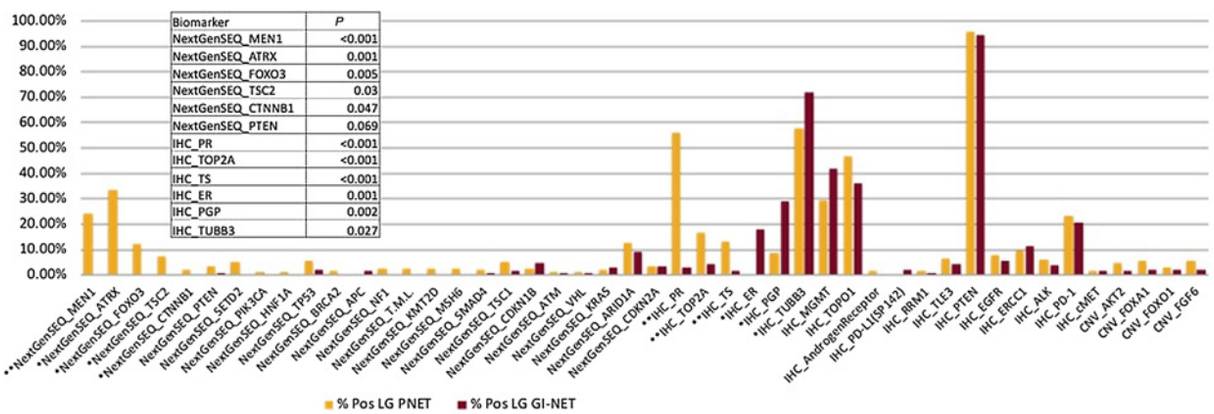
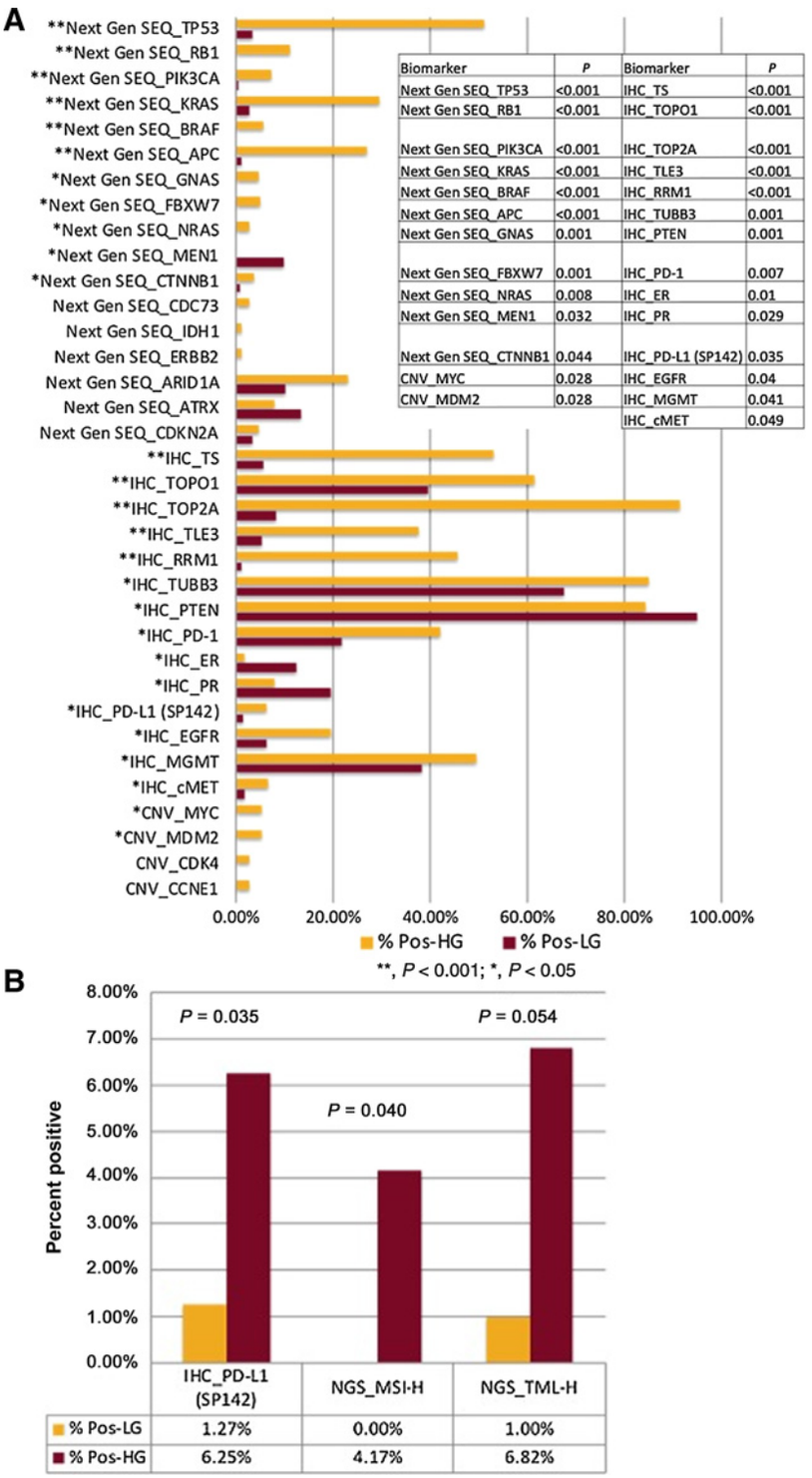


Figure 4.

Differences in LG PNET and LG GI-NET. Molecular differences between LG PNET and LG GI-NET. Compared with LG GI-NET, LG PNET carried significantly higher frequency of *MEN1* (24.3% vs. 0.0%), *ATRX* (33.3% vs. 0.0%), and *FOXO3* (12.2% vs. 0.0%). LG GI-NET had a higher mutation rate in *APC* (1.6% vs. 0.0%) and *CDKNB1* (4.9% vs. 2.5%), although neither was significant. Molecular profiles of LG tumors were similar to those of the entire cohort for each primary location.



Biomarker	P	Biomarker	P
Next Gen SEQ_TP53	<0.001	IHC_TS	<0.001
Next Gen SEQ_RB1	<0.001	IHC_TOPO1	<0.001
Next Gen SEQ_PIK3CA	<0.001	IHC_TOP2A	<0.001
Next Gen SEQ_KRAS	<0.001	IHC_TLE3	<0.001
Next Gen SEQ_BRAF	<0.001	IHC_RRM1	<0.001
Next Gen SEQ_APC	<0.001	IHC_TUBB3	0.001
Next Gen SEQ_GNAS	0.001	IHC_PTEN	0.001
Next Gen SEQ_FBXW7	0.001	IHC_PD-1	0.007
Next Gen SEQ_NRAS	0.008	IHC_ER	0.01
Next Gen SEQ_MEN1	0.032	IHC_PR	0.029
Next Gen SEQ_CTNNB1	0.044	IHC_PD-L1 (SP142)	0.035
CNV_MYC	0.028	IHC_EGFR	0.04
CNV_MDM2	0.028	IHC_MGMT	0.041
		IHC_cMET	0.049

Figure 5.

A, Molecular differences between HG and LG GEP-NEN. Among LG tumors, the most frequently mutated genes were *ATRX* (13%), *ARID1A* (10%), and *MEN1* (10%). Among HG, *TP53* (51%), *KRAS* (30%), *APC* (27%), *ARID1A* (23%), and *RB1* (11%). Compared with LG, HG NENs showed a higher mutation rate in *BRAF* (5.4% vs. 0%), *KRAS* (29.4% v 2.6%), and *PIK3CA* (7% vs. 0.3%), among others. **B,** Differences in immune markers between HG versus LG GEP-NEN. Immune-related biomarkers showed lower prevalence in LG tumors compared with HG [lower mean TML (5.1 mut/MB vs. 9.5, $P < 0.0001$), MSI-H 0% vs. 4% ($P = 0.04$), PD-L1 expression 1% vs. 6% ($P = 0.03$)].

individualized therapeutic strategies (21). Thus, better understanding of the underlying biology is paramount.

GEP-NENs include two genetically different entities: well-differentiated neuroendocrine tumors (NET) and poorly differentiated neuroendocrine carcinomas (NEC; ref. 22). In addition, well-differentiated NETs may be HG (G3 defined as having a mitotic rate >20 per 2 mm^2 or Ki67 >20%), but these neoplasms remain well-differentiated genetically and distinct from poorly differentiated NECs. Mutations in *MEN1, DAXX*, and *ATRX* are entity-defining for well-differentiated NETs, whereas NECs usually have *TP53* or *RB1* mutations (22). According to these data, in our cohort, *TP53* and *RB1* as well as *KRAS, APC, BRAF*, and *PI3KCA* were frequently mutated in

HG but not in LG. On the other hand, LG tumors harbored mutations in *MEN1* and *ATRX*. This underlines the reliability and robustness of our results and the applicability to the most updated classification of NENs to our cohort: the group defined LG may represent the NETs and the HG group may account for NECs. We recognize as a limitation that within NETs, we were neither able to distinguish between G1–G2 and G3, nor could we distinguish between G3 NET versus G3 NEC according to the novel nomenclature of NENs (23).

GEP-NENs are usually divided in GI and pancreatic (P) NENs because of the different clinical behavior, biology, and treatment strategies associated with the two groups of tumors. According to this, our data show that GI and PNET harbor a different molecular profile. Among the LG cohort, PNET carried mutations in *MEN1, ATRX, FOXO3*, and *PTEN* which were not found in GI NETs. On the other hand, GI NETs harbored mutations in *APC*, which was not present in PNET. These findings corroborate the fact that these tumors are completely different entities for which different therapeutic approaches are needed, as well as different hereditary syndromes may be predicted.

We showed that HG GEP-NENs carried a higher frequency of *TP53, KRAS, APC, RB1, BRAF*, and *PI3KCA* mutations compared with LG GEP-NENs. According to this, NECs commonly have mutations in *TP53* and *RB1* and may share mutations in *KRAS* and *SMAD4*, genes commonly involved in the pathogenesis of adenocarcinomas, as it has been shown in other series (24–26). Usually NECs show poor prognosis and platinum-based chemotherapy regimens represent the only treatment usually proposed for these patients. However, considering of the lack of tailored treatments for patients with NECs, and of the heterogeneity of response rate to standard chemotherapy, novel potential therapeutic targets may pave the way for more personalized treatment strategies (27).

To date, immunotherapy approaches have led to disappointing results in GEP-NENs. Monotherapy with anti-PD-1 (e.g., pembrolizumab) showed no signs of activity in these patients (28–32). Here, we observed higher expression of PD-L1, higher mean TMB, and higher MSI-H status in HG NENs, compared with LG, regardless of the site of tumor origin. These findings may help to explain why patients with HG tumors benefit more from immunotherapy combination that those with LG tumors. Ipilimumab plus nivolumab demonstrated a 44% overall response rate (ORR) in patients with nonpancreatic HG neuroendocrine carcinoma, with 0% ORR in low/intermediate grade disease (33). These results are encouraging and may potentially lead to a novel treatment strategy for HG NEN patients. However, caution should be taken when interpreting these data since the DART SWOG 1609 study was a prospective, open-label, multicenter phase II clinical trial of ipilimumab plus nivolumab across multiple rare tumor cohorts, reporting results from 32 enrolled patients with the (nonpancreatic) neuroendocrine tumors (33). Thus, several additional studies are warranted to investigate the molecular biology and eventually predictive biomarkers for immunotherapy in patients with GEP-NENs.

We observed that PNET carried a higher expression of PR and a lower expression of ER when compared with GI-NETs, especially in LG tumors. This is in accordance with other previous studies (34) showing that PR expression might also be a prognostic marker in patients with PNET (35, 36). Although we could not confirm the prognostic role of PR expression, we observed that HG tumors, which have a worse prognosis, also express lower levels of PR and ER. HR positivity is used as predictive biomarker for endocrine therapy in breast and other cancers, thus our findings may lead to a novel treatment strategy for patients with GEP-NETs. In fact, a single-arm phase II trial (NCT03870399) is currently evaluating the effect of tamoxifen in patients with well differentiated NET and hormone receptor–positive expression.

We acknowledge that there are several limitations to our study including the retrospective nature of the analysis, the absence of clinical data correlating our findings to outcomes, the heterogeneity of the study population unselected for tumor stage and the difficulties in grouping samples based on pathology reports and different nomenclatures. Especially regarding nomenclature, as stated above, we were not able to distinguish G3 NET from G3 NEC, which are considered two entities both genetically and clinically, thus further studies to distinguish these populations of patients are warranted.

Studies on biologically targeted therapies in GEP-NETs have, to date, focused primarily on inhibitors of VEGF or mTOR signaling pathways, even though the continuous discoveries in molecular pathways involved in tumor genesis and metastatization are paving the way to the introduction of new drugs (20). Recently, encouraging results have been shown in patients with *NTRK* fusion–positive solid tumors, included in neuroendocrine tumors, treated with tropomyosin receptor kinase (TRK) inhibitors (37, 38). Notably, no cases of *NTRK* 1/2/3 fusion were detected in the patients evaluated in this study.

Finally, we reported that up to 25% of PNEN harbor mutations in *MEN1*, which is similar to previous reports (15, 19, 39), strengthening our data. However, data on germline mutations are not available, therefore we could not evaluate the rate of germline mutations compared with somatic. However, we underline the importance of genetic testing and counseling in these patients because of the crucial clinical implications in case of hereditary syndrome diagnosis.

The demand of patients for a personalized management and therapy represent a challenging and compelling task for NEN oncologists and scientists. Therefore, most attention and efforts are needed in linking management and therapy to molecular profiling.

Conclusion

Our findings demonstrated that several molecular differences are present based on tumor location and grade in a large cohort of GEP-NENs. In addition, immune-related biomarkers showed lower prevalence in LG compared with HG tumors.

On the basis of these data, we can hypothesize that a deep molecular profiling of GEP-NENs provide new insights, which may lay the basis to a new molecular-based classification of these tumors, biomarker-driven clinical trial design and novel targeted agents' development, together with hereditary syndrome prediction.

Disclosure of Potential Conflicts of Interest

K. Poorman reports personal fees from Caris Life Sciences (full-time employee) during the conduct of the study and personal fees from Caris Life Sciences (full-time employee) outside the submitted work. M.E. Salem reports other from Caris Life Sciences (travel support), Taiho Oncology (speaking/consulting), Merck, (speaking/consulting), BMS (speaking/consulting), and AstraZeneca (speaking/consulting) outside the submitted work. R.M. Goldberg reports personal fees from Amgen (honorarium for a lecture and travel expenses), Bristol Myers Squibb (consulting, drug development advice), Genentech (consulting, expert testimony), Novartis (consulting, drug development), and Taiho (consulting on drug development and expert testimony) outside the submitted work. A.F. Shields reports personal fees and nonfinancial support from Caris Life Sciences (speakers bureau, travel, research support) outside the submitted work. J. Xiu reports personal fees from Caris Life Sciences (employment) outside the submitted work. J.J. Hwang reports personal fees from Celgene, Ipsen, Bristol Myers Squibb, Lilly, Amgen, Bayer, Eisai, Boehringer Ingelheim, Genentech/Roche, and Taiho outside the submitted work. S. Sciallero reports nonfinancial support from Novartis (congress travel & registration), Ipsen (congress travel & registration), Celgene (congress travel & registration), Sanofi (congress travel & registration), Amgen (congress travel), and personal fees from Amgen (lectures), Servier (lectures), Merck (lectures), and AstraZeneca (roundtable)

outside the submitted work. W.M. Korn reports personal fees from Caris Life Sciences (employment), other from Caris Life Sciences (ownership interest), and personal fees from Merck, Sharp & Dohme outside the submitted work. J.L. Marshall reports other from Caris (served on the Caris SAB) outside the submitted work and served as an interim CMO of Indivumed. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

A. Puccini: Conceptualization, resources, investigation, visualization, methodology, writing-original draft, project administration, writing-review and editing. **K. Poorman:** Data curation, software, formal analysis, validation, methodology, writing-review and editing. **M.E. Salem:** Writing-review and editing. **D. Soldato:** Writing-original draft, writing-review and editing. **A. Seeber:** Writing-review and editing. **R.M. Goldberg:** Writing-review and editing. **A.F. Shields:** Writing-review and editing. **J. Xiu:** Formal analysis, methodology, writing-review and editing. **F. Battaglin:** Writing-review and editing. **M.D. Berger:** Writing-review and editing. **R. Tokunaga:** Writing-review and editing. **M. Naseem:** Writing-review and editing. **A. Barzi:** Writing-review and editing. **S. Iqbal:** Writing-review and editing. **W. Zhang:** Writing-review and editing. **S. Soni:** Writing-review and editing. **J.J. Hwang:** Writing-review and editing. **P.A. Philip:** Writing-review and editing. **S. Sciallero:** Supervision, writing-review and editing. **W.M. Korn:** Resources, software, formal analysis, supervision, investigation, methodology, writing-review and editing. **J.L. Marshall:** Writing-review and editing. **H.-J. Lenz:** Conceptualization, resources, data curation, supervision, funding acquisition, validation, investigation, project administration, writing-review and editing.

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