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Impact of KRAS and BRAF mutations on treatment efficacy and survival in high-grade gastroenteropancreatic neuroendocrine neoplasms

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Abstract

High-grade gastroenteropancreatic neuroendocrine neoplasms (HG GEP-NEN) typically disseminate early. Treatment of metastatic disease has limited benefit and prognosis is generally discouraging. Data on the clinical impact of mutations in HG GEP-NEN are scarce. There is an unmet need for reliable biomarkers to predict treatment outcome and prognosis in metastatic HG GEP-NEN. Patients with metastatic HG GEP-NEN diagnosed at three centres were selected for KRAS-, BRAF mutation and microsatellite instability (MSI) analyses. Results were linked to treatment outcome and overall survival. After pathological re-evaluation, 83 patients met inclusion criteria: 77 (93%) GEP neuroendocrine carcinomas (NEC) and six (7%) GEP neuroendocrine tumours (NET) G3. NEC harboured higher frequency of mutations than NET G3. Colon NEC harboured a particular high frequency of BRAF mutations (63%). Immediate disease progression on first-line chemotherapy was significantly higher for NEC with BRAF mutation (73%) versus wild-type (27%) ($p = .016$) and for colonic primary (65%) versus other NEC (28%) ($p = .011$). Colon NEC had a significant shorter PFS compared to other primary sites, a finding independent of BRAF status. Immediate disease progression was particularly frequent for BRAF mutated colon NEC (OR 10.2, $p = .007$). Surprisingly, BRAF mutation did not influence overall survival. KRAS mutation was associated with inferior overall survival for the whole NEC population (HR 2.02, $p = .015$), but not for those given first-line chemotherapy. All long-term survivors (>24 m) were double wild-type. Three NEC cases (4.8%) were MSI. Colon NEC with BRAF mutation predicted immediate disease progression on first-line chemotherapy, but did not affect PFS or OS. Benefit of first-line platinum/etoposide treatment seems limited for colon NEC, especially for BRAF mutated cases. KRAS mutations did not influence treatment efficacy nor survival for patients receiving first-line chemotherapy. Both frequency and clinical impact of KRAS/BRAF mutations in digestive NEC differ from prior results on digestive adenocarcinoma.

KEYWORDS
neuroendocrine carcinoma, neuroendocrine neoplasms, gastroenteropancreatic, KRAS, BRAF

1 | INTRODUCTION

Gastroenteropancreatic neuroendocrine neoplasms (GEP-NEN) are a heterogeneous group with various phenotypic and molecular characteristics depending on grade, differentiation and site. High-grade (HG) GEP-NEN are characterized by high proliferation rate (Ki-67 >20%) with a well differentiated morphology as in NET G3, or with poorly differentiated morphology as in neuroendocrine carcinoma (NEC).[1] At present, as many as 30% of HG GEP-NEN cases have an immediate progression of disease with no benefit of first-line chemotherapy.[2,3] The need to improve first-line treatment is therefore urgent.

Molecular abnormalities, including mutations, copy-number- and epigenetic alterations drive cancer development.[4,5] There are limited data on molecular alterations in HG GEP-NEN. Mutations appear to be more frequent in NEC compared to NET G3, and GEP-NEC harbour frequent mutations in TP53, KRAS and BRAF.[6,7] Among digestive tumours, almost 90% of pancreatic- (PDAC) and 50% of colorectal adenocarcinomas (CRC) harbour KRAS mutations.[8] KRAS mutations have been associated with inferior survival in both metastatic colorectal cancer and PDAC.[9–11] BRAF mutations are reported in 5–20% of metastatic colorectal adenocarcinoma, but rarely described in adenocarcinomas in the remaining GI-tract.[12,13] In CRC, BRAF mutations are recognized as an unfavourable prognostic factor, associated with limited benefit of chemotherapy and a short survival after first-line treatment.[10,14–16] Targeted therapy combining BRAF- and EGFR +/− MEK-inhibition has proven effective for BRAF mutated metastatic CRC.[17] FDA recently approved tumour agnostic BRAF/MEK inhibition for BRAF V600E mutated metastatic cancer. Microsatellite instability (MSI) indicates mismatch repair deficiency and is a marker for benefit of check-point inhibition, and an approved biomarker for a tumour-agnostic treatment-approach.[18]

For HG GEP-NEN, the correlation between molecular alterations and treatment efficacy has not been extensively explored, and there are few data addressing impact on prognosis. At present the only study addressing the molecular influence on treatment-efficacy is a small study on pancreatic HG NEN, where KRAS predicted response to platinum-based chemotherapy.[19] There is an unmet need for markers to guide treatment decisions in HG GEP-NEN. The aim of our study was to expand the molecular knowledge on HG GEP-NEN and to investigate the impact of KRAS- and BRAF mutations on the effect of standard palliative chemotherapy and overall survival in metastatic HG GEP-NEN patients.

2 | METHODS

2.1 | Patient selection

HG-NEN with gastroenteropancreatic or unknown origin with a predominance of gastrointestinal (GI) metastasis was included in Nordic NEC Registries. From three Norwegian hospitals, 114 patients with metastatic disease and available tumour tissue were included. At the time of diagnosis (1999–2016), NET G3 was not an established subgroup of HG NEN. To update on the differentiation between NET G3 and NEC, all cases with non-small cell morphology and Ki-67 <55% were reassessed by a NEN experienced pathologist at the respective participating centres. Ki-67 was evaluated in hot-spots by eyeballing. Clinical information was available through the Nordic NEC Registries. Treatment effect was radiologically evaluated by RECIST. Direct progression on first-line chemotherapy was defined as either confirmed radiological ($n = 17$) or clinical progression ($n = 1$) at first evaluation or confirmed NEC-specific death within 2 months of last treatment in cases not subjected to radiology ($n = 6$). With the exception of one patient receiving four cycles of chemotherapy, evaluation was done after 1–3 cycles. No cases were overlapping with our recent molecular publication.[5]

2.2 | Molecular analysis

Tumour DNA was extracted from formalin fixed, paraffin-embedded tissue samples. KRAS and BRAF mutational analyses were done according to the method of choice at the hospital of inclusion. All included cases were analysed by real time PCR methods (Therascreen/Entrogen/Panagene and/or Sanger sequencing), with the exception of 17 cases subjected to next generation sequencing (NGS) for mutation calling. Details concerning methods applied are accounted for in Supplementary methods in Data S1. Microsatellite instability (MSI) was analysed by either the Promega MSI Analysis system (Promega) or by an in-house protocol as specified in Supplementary methods in Data S1. Normal tissue for comparison was extracted from tumour free site on tissue sample as indicated by pathological expertise. MSI-low and microsatellite stable (MSS) tumours were regarded as stable.

2.3 | Ethics

Ethical approvals were granted by local authorities (2007/165 and 2012/940/REK Vest) and the study was conducted in accordance with the Declaration of Helsinki. For patients included before 2013 the need for written consent was waived for terminal ill or diseased patients.

2.4 | Statistical analysis

Descriptive statistics were expressed as absolute- and relative frequencies, median, percentiles and range. Exact chi-square was used for group comparison of categorical variables. Logistic regression was used to assess categorical variables in both multivariate and univariate analyses. OS was defined as time from metastases to time of death or last known follow up, and progression-free survival as time from first treatment to time of progression or date of last known follow up. Time-to-event analyses were analysed using log rank test, and survival curves were estimated by Kaplan Meier. To evaluate the

TABLE 1 Baseline characteristics, treatment and molecular alterations in patients with high grade gastroenteropancreatic neuroendocrine neoplasms ($n = 83$).

Characteristic	Valid cases 83	Subgroup	NET G3, $n = 6$	NEC, $n = 77$
Gender	83	Male	5 (83%)	47 (61%)
Age, median (range)	83		62 (31–71)	63 (31–90)
Performance status	76	0–1	5 (83%)	54 (77%)
		≥2	1 (17%)	16 (23%)
Primary tumour site	83	Oesophageal		6 (8%)
		Gastric		7 (9%)
		Pancreas	3 (50%)	16 (21%)
		Colon		21 (27%)
		Rectum		9 (12%)
		Unknown[a]	1 (17%)	15 (19%)
		Other GI[b]	2 (33%)	3 (4%)
Resection of primary tumour	78		4 (67%)	25 (32%)
Liver metastases	83		6 (100%)	55 (71%)
NEC morphology	75	Small cell		33 (44%)
		Large cell		42 (56%)
Ki-67 median (range)	83		33 (25–57)	75 (21–100)
CgA staining	80	Strongly positive	6 (100%)	45 (61%)
Octreotide scintigraphy[c]	24	Positive > liver	1 (50%)	8 (36%)
ALP > UNL[c]	75		3 (75%)	44 (62%)
LDH > UNL[c]	77		1 (17%)	37 (52%)
Palliative chemotherapy				
First-line	70		5 (83%)	65 (91%)
		Platinum/etoposide	3 (60%)	59 (91%)
Second-line	70		1 (33%)	35 (52%)
Third-line	63		1 (33%)	17 (28%)
Molecular alterations				
KRAS mutation	83		1 (17%)	16 (21%)
BRAF V600 mutation	80			13 (18%)
MSI[c]	67			3 (4.8%)

Abbreviations: ALP, alkaline phosphatase; CgA, chomogranin A; GI, gastrointestinal; LDH, lactate dehydrogenase; UNL, upper normal limit.
[a]Unknown with dominance of GI metastases.
[b]Other GI; NET G3: 1 small intestine, 1 gallbladder/duct. NEC: 2 anal, 1 small intestinal.
[c]Percentage as fractions of examined cases.

predictive role of variables, a Cox regression model was used. Variables with an impact on the efficacy of first-line chemotherapy or OS in univariate analyses were included in multivariate analyses together with known prognostic variables from previous studies. All *p*-values were two-sided with values of < .05 considered statistically significant. Statistical analyses were performed using STATA, version 16.1.

3 | RESULTS

Of an initial cohort of 114 patients, 31 were excluded due to inadequate tumour DNA ($n = 11$), unconfirmed metastatic disease ($n = 5$), insufficient clinical information ($n = 1$), doublets ($n = 3$), non-GEP primary tumour ($n = 2$), reclassification as low-grade (G1-G2) NET ($n = 4$), goblet- or Merkel cell-carcinoma ($n = 2$) and if categorized as MiNEN ($n = 3$). Of the remaining 83 HG GEP-NEN patients, 77 (93%) were reclassified as NEC and six (7%) as NET G3. The majority of NEC cases were pancreatic, colonic or of unknown primary with a predominance of GI metastases (Table 1). Pancreas was the most common site for NET G3 (50%). All patients included had available KRAS tumour status, whereas BRAF and MSI were available for 80 (97%) and 67 (81%). KRAS was annotated as codon specific mutations with some missing information at the nucleotide level, due to varying methods applied. With the exception of two BRAF V600 cases missing

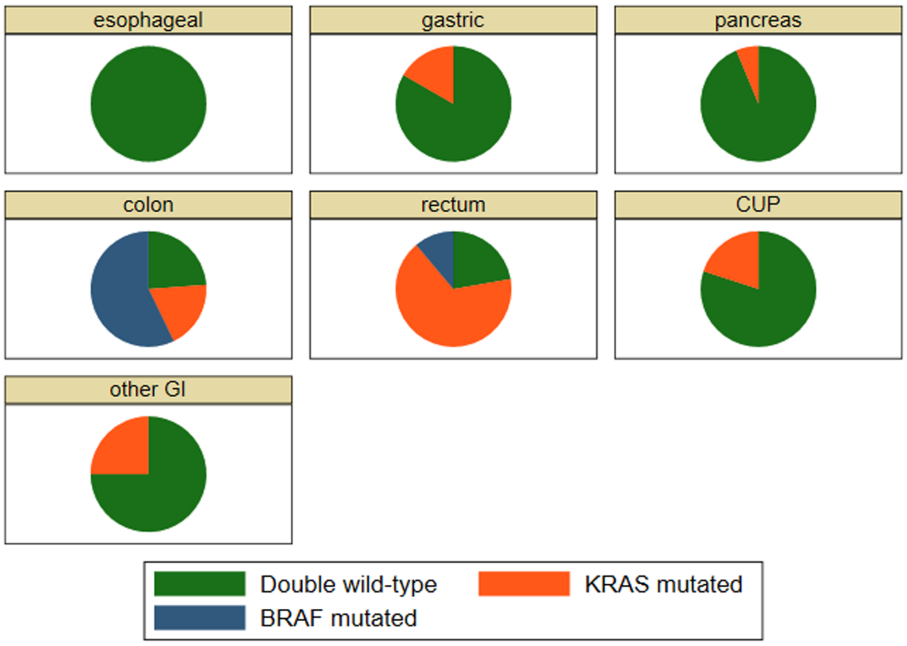

FIGURE 1 The distribution of KRAS and BRAF mutations by primary tumour for 77 neuroendocrine gastroenteropancreatic carcinomas (NEC).

nucleotide calling, all were V600E mutations. Only one NET G3 harboured a KRAS mutation, and none had BRAF mutations or MSI. For NEC, KRAS and BRAF mutations were found in 16 (21%) and 14 (19%), respectively. One pancreatic NEC had a BRAF P450fs mutation, a frameshift causing loss of function, presumed non-pathogenic and not included in the further BRAF specific analysis.[20] All other BRAF mutations were restricted to colorectal NEC, and found in 12 (63%) colon and one (13%) rectal NEC. KRAS mutations were found in six (67%) rectal NEC, varying from 6% to 25% in remaining GI cases, with the exception of oesophageal NEC harbouring neither KRAS nor BRAF mutations (Figure 1). KRAS and BRAF mutations were mutually exclusive. The incidence of KRAS mutations were higher in non-small cell (NSC) than in small cell (SC) NEC, found in 12 (29%) of NSC-NEC and in three (9%) of SC-NEC ($p = .036$). Among colon NEC, BRAF mutations were found in 2/4 (50%) of SC-NEC and 10/14 (71%) of NSC-NEC ($p = .423$). Of 62 NEC-cases examined for microsatellite instability (MSI), three (4.8%) were MSI: one CUP and two colonic (one with co-occurring BRAF mutation).

3.1 | Treatment and response to treatment

Of 65 NEC patients receiving first-line chemotherapy, 91% received cis/carboplatin and etoposide. Of these, 56 cases were evaluated according to RECIST, 20 (36%) obtained partial response and 19 (34%) experienced disease stabilization. Nine NEC cases were not radiologically evaluated; one had clinical progression after three chemotherapy courses, the remaining eight received 1–2 courses of chemotherapy and deceased within 2 months from last treatment, of which six due to confirmed NEC related cause. Combining radiological progression and obvious clinical progression, 24 NEC cases (38%) experienced immediate progression after first-line chemotherapy. Such direct treatment failure was particularly evident for colon NEC. All colon NEC received cis/carboplatin and etoposide combinations as first-line treatment, and 11 (65%) experienced immediate progression, as opposed to 13 (28%) of all other NEC combined (OR 4.65, 95% CI: 1.42–15.2, $p = .011$). Assessing BRAF mutations among colon NEC, 8/10 (80%) of BRAF-mutated progressed immediately, versus 2/5 (40%) of BRAF wild-type (OR 6, CI: 0.56–64, $p = .138$) (Table 2). Specifically, the OR for immediate progression was 10.2 for BRAF-mutated colon-NEC ($p = .007$) and 1.9 for BRAF wild-type colon NEC ($p = 0.43$) when compared to all other NEC combined ($n = 46$). The number of patients receiving second-line chemotherapy ($n = 35$) was not affected by neither KRAS mutations, colonic primary site, nor colon BRAF mutation status ($p = .864$, .742 and .486, respectively). As second line treatment the majority of BRAF mutated colon-NEC received CapTem (5/6, 83%). There was no difference in PFS after second-line treatment when comparing BRAF mutated versus wild-type colonic NEC, nor when comparing BRAF mutated NEC versus wild-type. Three patients received "adenocarcinoma-like treatment" (FLIRI) as third-line treatment, whereby one had partial response and two experienced disease progression as best response. No cases received targeted treatment towards BRAF mutations or immunotherapy. Of the five NET G3 cases given chemotherapy, three (60%) received cis/carboplatin and etoposide treatment, whereof one obtained partial response.

3.2 | Progression-free survival

Median progression-free survival (PFS) was significantly longer for NET G3 (12 months) compared to NEC (5.4 months) ($p = .047$) (Figure 2A). Among NEC, colonic primary ($n = 19$) had shortest PFS of 2.2 months, significantly shorter than 6 months observed for extra-colonic NEC

TABLE 2 Immediate disease progression (PD) on first-line chemotherapy for metastatic NEC. Univariate analyses.

	Immediate PD	OR	CI	*p*-value
Colonic primary	**65%**	4.65	1.43–15.2	**.011**
Pancreatic primary	21%	0.36	0.90–1.47	.156
BRAF mutation	**73%**	6.04	1.40–26.01	**.016**
KRAS mutation	35%	2.27	0.61–8.46	.223
LDH (>UNL vs. normal)	41 vs. 40%	0.95	0.34–2.67	.916
ALP (>UNL vs. normal)	35 vs. 38%	1.14	0.39–3.38	.811
Performance status ≥2 vs. <2	36 vs. 50%	1.78	0.45–6.98	.410
Other chemotherapy vs. platinum/etoposide	39 vs. 33%	0.80	0.13–4.71	.801
Celltype NSC vs. SC	44 vs. 31%	1.73	0.60–4.95	.308
Mutated vs. wild-type[a]	**61** vs. 25%	4.67	1.55–14.04	**.006**

Abbreviations: ALP, alkaline phosphatase; LDH, lactate dehydrogenase; NSC, non-small cell; SC, small cell; UNL, upper normal limit.
[a]Either BRAF or KRAS mutation vs. double wild-type.

($n = 46$) ($p = .002$) (Figure 2B). KRAS mutation did not significantly influence PFS 5.4 months for KRAS wild-type ($n = 54$) and 2.2 months for KRAS mutated ($n = 11$), ($p = .31$). Colon NEC demonstrated a short PFS independent of BRAF mutational status (BRAF wild-type 2.3 months, BRAF mutated 1.9 months) (Figure 2C).

3.3 | Overall survival

Considering the whole population, median overall survival (OS) for NEC ($n = 77$) was significantly shorter than for NET G3 ($n = 6$) (9.5 vs. 16.2 months, $p = .02$), and KRAS mutation predicted shorter survival for NEC (HR 2.02, 95% CI: 1.14–3.56, $p = .015$), also after adjusting for primary site. For the population receiving first-line chemotherapy, survival difference between NEC ($n = 65$) and NET G3 ($n = 5$) was less substantial, 11.3 versus 16.2 months (HR 2.39, 95% CI: 0.86–6.71, $p = .097$). As data on biomarkers predicting benefit and survival after palliative chemotherapy in GEP-NEC patients is scarce, all further analyses were done on the NEC cohort treated with chemotherapy. When compared to wild-type, KRAS mutations were associated with a non-significant shorter survival among NEC (OS 6.5 vs. 11.8 months, HR 1.75, 95% CI: 0.90–3.41, $p = .099$) (Figure 3A), Survival for colon NEC did not differ comparing BRAF mutated cases versus non mutated cases (OS 8.9 vs. 4.9, HR 0.96, 95% CI: 0.35–2.64, $p = .940$) (Figure 3B). Pancreatic NEC ($n = 14$) had a significant longer survival (15.7 months) than extra-pancreatic NEC ($n = 51$) (10.5 months), (HR 0.42, CI: 0.22–0.81, $p = .009$) (Figure 3C). Poor performance status (PS) clearly predicted shorter survival, OS was 6 months for PS ≥2 ($n = 10$) versus 12.2 months for PS 0–1 ($n = 52$) (HR 3.16, 95% CI: 1.53–6.53, $p = .002$). Colon NEC had a OS of 7.5 months, as opposed to 12 months for all other NEC combined (HR 1.45, 95% CI: 0.83–2.49, $p = .185$). Mutational status did not impact on OS for colon NEC, with OS of 4.9, 4.1 and 8.9 months for double wild-type, KRAS- and BRAF mutated, respectively, with no significant differences in hazard of death between the groups (HR 1.34 and 1.59, 95% CI: 0.30–6.13 and 0.48–5.23 for KRAS- and BRAF-mutated, compared to double wild-type). In multivariate analyses, poor PS, colonic primary and elevated ALP (alkaline phosphatase) were poor prognostic factors, whereas pancreatic primary was associated with longer survival (Table 3B). Long-term survival (>24 months) was achieved by 10 (13%) NEC patients. All of these received chemotherapy (90% cis/carboplatin and etoposide), with a significant difference in mutational status as all long-term survivors were double wild-type, harbouring neither KRAS nor BRAF mutation ($p = .031$). The 12 NEC cases receiving only best supportive care (BSC) had an OS of 0.6 months, compared to 11.3 months for NEC given first-line chemotherapy (HR 11.4 95% CI: 5.31–24.28, $p < .001$). Performance status was significantly worse for the BSC group compared to those given chemotherapy (67% vs. 16% had PS ≥2, $p = .001$). There was no significant difference in mutational status between cases given only BSC and cases given chemotherapy.

4 | DISCUSSION

Compared to prior reports on digestive adenocarcinoma, we found a lower incidence of KRAS mutations in pancreatic NEC and a higher frequency of BRAF mutations in colon NEC. BRAF mutation in colon NEC predicted failure to first-line cis/carboplatin and etoposide treatment, without affecting PFS or OS. Benefit of first-line cis/carboplatin and etoposide seems limited for colon NEC, especially for BRAF mutated cases, suggesting that other first-line options should be considered for these patients. Our findings are of clear contrast to prior results in digestive adenocarcinomas, indicating that GEP-NEC molecularly is a different disease, and that the clinical influence of activating mutations in KRAS and BRAF seems to differ between digestive NEC and digestive adenocarcinoma.

We found KRAS mutations among 21% of GEP-NEC. Prior studies have reported a 10%–22% incidence, differing according to primary site.[6,7] In pancreatic NEC, KRAS mutations have been reported in 23%–49% of cases,[7,19,21] significantly higher than the one in 16 (6%) observed in this study. In pancreatic adenocarcinoma (PDAC),

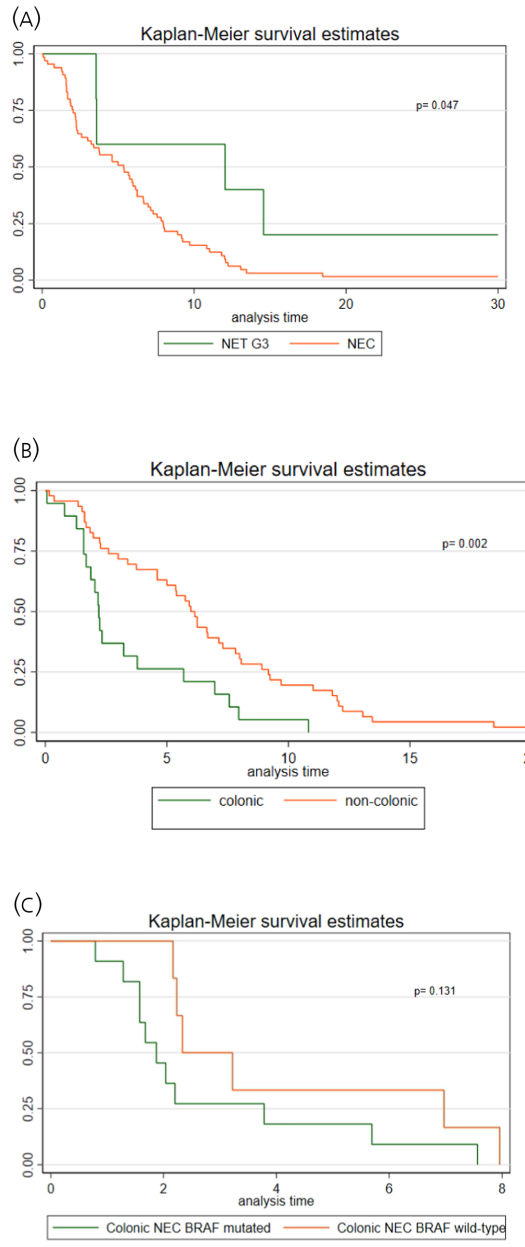

FIGURE 2 Progression free survival (PFS) after first-line chemotherapy for metastatic gastroenteropancreatic neuroendocrine neoplasms according to subgroups. (A) NET G3 ($n = 4$) vs NEC ($n = 64$). (B) Colon NEC ($n = 19$) vs other NEC combined ($n = 45$). (C) BRAF mutated colon NEC ($n = 11$) vs BRAF wild-type colon NEC ($n = 6$).

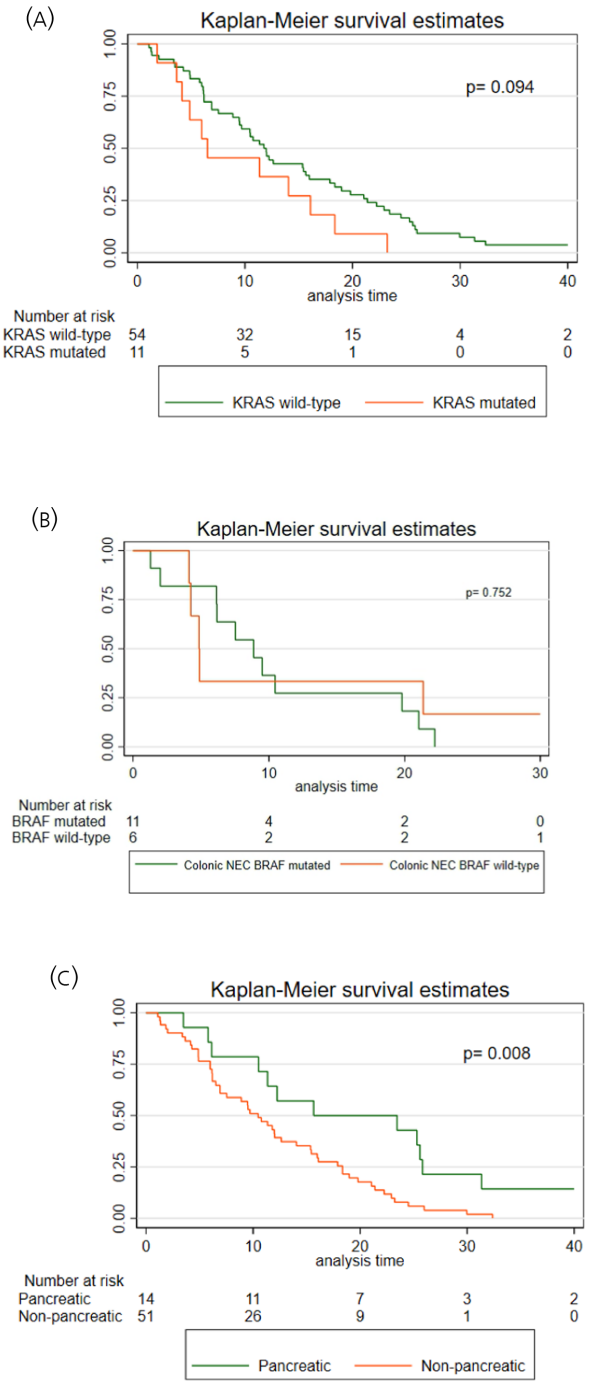

FIGURE 3 Overall survival after first-line chemotherapy for metastatic gastroenteropancreatic neuroendocrine carcinoma (NEC) according to subgroups. (A) According to KRAS mutational status ($N = 65$). (B) According to BRAF mutational status for colon NEC ($N = 17$). (C) According to primary pancreatic NEC vs extra-pancreatic NEC ($N = 65$).

KRAS mutations are present in >90%.[22] Among our NEC cases, 12 (18%) harboured BRAF mutation, with the highest frequency (63%) in colon NEC, higher than in previous publications reporting frequencies in the range of 28%–49%.[7,23] As most BRAF mutations are located in the right colon, the distribution of primary colorectal site will affect the frequency. We do not possess information on sidedness. A recent study on 30 colorectal NEC found BRAF mutations in 23% and KRAS mutations in 53%, with no effect on OS.[24] In contrast to our study, this study had 47% rectal primaries and only 37% had metastatic disease. In metastatic colorectal adenocarcinoma, BRAF mutations are found in 8%–20% of cases with an incidence of 20%–28% in colon (predominantly right) and 3%–5% in rectum.[12,25]

TABLE 3 Prognostic factors for overall survival for 65 metastatic gastroenteropancreatic neuroendocrine carcinoma (NEC) patients receiving first-line chemotherapy.

	HR	CI	*p*-value
(a) Univariate analyses			
Celltype NSC vs. SC	1.34	0.80–2.22	.265
KRAS mutated	1.75	0.90–3.41	.099
BRAF mutated	1.44	0.75–2.761	.273
MSI	1.51	0.47. 4.91	.488
Performance status ≥2 vs. <2	3.16	1.53–6.53	**0.002**
Pancreatic primary	0.42	0.22–0.81	**.009**
Colonic primary	1.45	0.84–2.49	.185
LDH > UNL	1.63	0.98–2.73	.062
ALP > UNL	1.43	0.85–2.41	.166
KRAS or BRAF V600 mutation[a]	1.81	1.05–3.12	**.032**
(b) Multivariate analyses			
Celltype NSC vs. SC	1.71	0.83–3.55	.143
KRAS mutated	1,25	0.44–3.58	.677
BRAF mutated	0.51	0.18–1.44	.204
Performance status ≥2 vs <2	4.55	1.76–11.78	**.002**
Pancreatic primary	0.27	0.12–0.61	**.001**
Colonic primary	2.77	1.01–7.55	**.047**
LDH > UNL	1.06	0.51–2.20	.873
ALP > UNL	2.32	1.10–4.90	**.027**

Abbreviations: ALP, alkaline phosphatase; LDH, lactate dehydrogenase; NSC, non-small cell; SC, small cell; UNL, upper normal limit.
[a]Either BRAF or KRAS mutation vs. double wild-type.

Molecular similarities have previously been described between NEC and adenocarcinoma of both colorectal and pancreatic origin suggesting a joint origin/ precursor.[26,27] Our findings, however, suggest that colorectal-NEC differs molecularly from colorectal adenocarcinoma.

In a study on 169 GEP-NEC cases, the hazard of death was similar for patients obtaining partial response and disease stabilization as best response to chemotherapy.[28] As such, selecting those at risk for immediate progression at first evaluation is critical to improve outcomes for NEC patients. Immediate progression on first-line chemotherapy is described as particularly high for colorectal NEC, reported in up to 60%.[29] In our study, the majority (65%) of colon NEC progressed directly on cis/carboplatin and etoposide treatment, with a PFS of 2.2 months. The frequency of immediate progression was high also for extra-colonic NEC (28%), with a PFS of 6 months. The benefit of first-line chemotherapy seems less for GEP-NEC than for digestive adenocarcinoma. In CRC-studies, immediate progression on first-line palliative chemotherapy has been reported for 5%–10% of cases[30,31] and 18% in population based cohorts.[12] The large proportion of treatment failure for NEC, and in particular for colonic primary, clearly points to the urgency of exploring alternative first-line treatment regimens.

The benefit of cis/carboplatin and etoposide chemotherapy seems especially low for BRAF mutated colon NEC. In our study, BRAF mutated colon NEC predicted immediate disease progression on first-line chemotherapy, but did not affect PFS, OS nor the numbers of cases receiving second-line chemotherapy. This is in striking contrast to prior results on colorectal adenocarcinoma, where BRAF mutations do not affect the efficacy of first-line chemotherapy, but predicts failure to later lines of treatment and a much shorter OS.[16] We could not explain the lack of OS correlation by a better effect or longer PFS in later lines of treatment. A possible explanation could be that lack of efficacy to cis/carboplatin and etoposide in first-line is counteracted by a better response with longer duration to "adenocarcinoma-like" chemotherapy in later lines. However, in our data irinotecan was only given as third-line treatment to three patients. As such, we could not test for this hypothesis. Benefit of irinotecan and oxaliplatin-based chemotherapy have been reported for NEC.[32–34] An ongoing randomized trial will hopefully clarify if an adenocarcinoma-like treatment is a better option for GEP-NEC.[35]

Metastatic GEP-NEC and particularly colon NEC seem to have a much shorter survival compared to adenocarcinoma counterparts. Comparing our colon NEC to population based data on colon adenocarcinoma, PFS (2.2 vs. 7.9 m) and OS (6 vs. 11 m) is considerably shorter.[12] Our results indicate that the short survival in colon NEC is not explained by BRAF mutations.

Further molecular research is needed to decipher the aggressive behaviour of NEC and the prognostic diversity of BRAF mutations within the same primary site. Both preclinical- and clinical studies gives hope for an effective future targeted treatment approach for BRAF mutated GEP-NEC.[23,36] Encorafenib in combination with cetuximab has approval for treatment of BRAF V600E mutated metastatic colorectal cancer without limiting the indication to adenocarcinoma cases. FDA recently granted accelerated approval for dabrafenib combined with trametinib for unresectable metastatic solid tumours with BRAF V600E mutations.

We found no predictive role of KRAS per se to treatment effect. Although PFS was more than twice as long for KRAS wild-type compared to KRAS mutated NEC (5.3 vs. 2.2 months), the difference was not significant. In contrast, for colorectal adenocarcinoma KRAS mutations are associated with inferior response to chemotherapy with a shorter survival.[37] The impact of mutations on treatment-efficacy for HG GEP-NEN is, to our knowledge, only explored for pancreatic cases, reporting superior response of platinum-based treatment for KRAS-mutated HG-NEN, although not significant for NEC.[19] A subgroup-analysis on the same data, found increased response to platinum-treatment for NEC with Rb loss and/or KRAS mutations.[38] As our study only included one pancreatic NEC with KRAS mutation, we could not analyse this. We found a significant association between KRAS mutations and inferior survival for the whole NEC cohort, but for those given chemotherapy the difference did not reach significance. The prognostic role of KRAS mutations for other types of cancers are debated, and potential correlations might be dependent on codon-specific aberrations and co-mutations not included in our data.[39,40] In our study all long-term GEP-NEC survivors were double-wild type for BRAF/KRAS, similar to results in metastatic colorectal adenocarcinoma.[41–43] In multivariate

analyses, colonic primary, poor PS and elevated ALP, were poor prognostic factors for GEP-NEC in our study, as shown in prior studies. Pancreatic NEC primary were associated with superior survival, in line with previous studies.[29,44]

A huge challenge in the daily work with GEP-NEC patients is the often rapid disease-progression with corresponding deterioration in PS contraindicating palliative chemotherapy. In our study, as many as 12/77 NEC cases (16%) were not eligible for palliative chemotherapy, mainly due to poor PS. Based on other cancer studies, we expected higher frequencies of KRAS and BRAF mutations among these cases, but this was not found. Median survival was only three weeks among the untreated metastatic NEC patients, comparable to the four weeks observed in the Nordic NEC study.[29] This underlines the importance of rapid referral of patients with metastatic GEP-NEC for consideration of palliative chemotherapy.

Our study has a limited number of cases and for some cases, characteristics or molecular data were missing. Results from all small size studies must be interpreted with caution. Our study did not possess nucleotide specific information on all mutations. For colon NEC, we lack information on sidedness. We sought to strengthen the quality of our study by reassessing all NSC-NEC with Ki-67 < 55%, to assure correct stratification to NET G3 and NEC. The chosen cutoff was done according to the findings in a recent study.[6] The majority of cases did not undergo re-evaluation and a centralized pathology revision was not performed. However, NEN dedicated pathologists at the participating centers were responsible for the initial diagnosis, and NET G3 cases with Ki-67 >55% are extremely rare. Although our study has several limitations, it serves to enlighten a yet unknown field of the predictive and prognostic impact of molecular aberrations for GEP-NEC.

5 | CONCLUSION

Here, we report clear differences in both frequency and clinical impact of KRAS and BRAF mutations when comparing digestive NEC to prior studies on its adenocarcinoma counterparts, suggesting that they are separate entities with different mutational phenotypes. BRAF mutational status predicted limited effect of first-line chemotherapy, without affecting PFS or OS. Benefit of first-line platinum/etoposide treatment seems minor for colon NEC, especially for BRAF mutated cases. The high amount of colonic BRAF mutations represent potential targets for tailored treatment approaches.

AUTHOR CONTRIBUTIONS

Hege Elvebakken: Data curation; formal analysis; methodology; writing – original draft; writing – review and editing. **Geir Hjortland:** Data curation; writing – review and editing. **Herish Garresori:** Data curation; writing – review and editing. **Per Arne Andresen:** Data curation; formal analysis; writing – review and editing. **Emiel AM Janssen:** Data curation; formal analysis; writing – review and editing. **Olav Karsten Vintermyr:** Data curation; formal analysis; writing – review and editing. **Inger Marie Bowitz Lothe:** Data curation; formal analysis; writing – review and editing. **Halfdan Sorbye:** Conceptualization; data curation; formal analysis; methodology; supervision; writing – original draft; writing – review and editing.

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PEER REVIEW

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DATA AVAILABILITY STATEMENT

The datasets generated or analysed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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