Peptide Receptor Radionuclide Therapy in Gastroenteropancreatic NEN G3: a multicenter cohort study

Esben Andreas Carlsen[1,2], Nicola Fazio[3], Dan Granberg[4], Simona Grozinsky-Glasberg[5], Hojjat Ahmadzadehfar[6], Chiara Maria Grana[7], Wouter T. Zandee[8], Jaroslaw Cwikla[9], Martin A. Walter[10], Peter Sandor Oturai[1], Anja Rinke[11], Andrew Weaver[12], Andrea Frilling[13], Sara Gritti[3], Anne Kirstine Arveschoug[14], Amichay Meirovitz[15], Ulrich Knigge[16] and Halfdan Sorbye[17]

Author affiliations

[1] Dept. of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet, Denmark

[2] Cluster for Molecular Imaging, Dept. of Biomedical Sciences, University of Copenhagen, Denmark.

[3] Division of Gastrointestinal Medical Oncology and Neuroendocrine Tumors, IEO, European Institute of Oncology IRCCS, Milan, Italy.

[4] Dept. of Medical Sciences, Uppsala University, Sweden.

[5] Neuroendocrine Tumor Unit, Dept. of Endocrinology & Metabolism, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

[6] Dept. of Nuclear Medicine, University Hospital Bonn, Germany.

[7] Division of Nuclear Medicine, IEO, European Institute of Oncology IRCCS, Milan, Italy.

[8] Erasmus Medical Center, Rotterdam, The Netherlands.

[9] Medical School, University of Warmia and Mazury, Olsztyn, Poland.

[10] Dept. of Nuclear Medicine, University Hospital of Geneva, Switzerland.

[11] Dept. of Gastroenterology, University Hospital Gießen and Marburg, Marburg, Germany.

[12] Dept. of Oncology, Churchill Hospital, Oxford, United Kingdom.

[13] Dept. of Surgery and Cancer, Imperial College London, United Kingdom.

[14] Dept. of Nuclear Medicine and PET, Aarhus University Hospital, Denmark.

[15] Dept. of Oncology and Radiation Therapy Unit, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

[16] Dept. of Surgical Gastroenterology and Dept. of Clinical Endocrinology, Rigshospitalet, University of Copenhagen, Denmark.

[17] Dept. of Oncology, Haukeland University Hospital, Bergen, and Dept. of Clinical Science, University of Bergen, Norway.

Corresponding author: Esben Andreas Carlsen. Address: Rigshospitalet, Dept. of Clinical Physiology, Nuclearmedicne & PET, Blegdamsvej 9, 2100 Copenhagen, Denmark. Email: esben.a.carlsen@gmail.com. Telephone: +45 35457179.

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Abstract

Peptide receptor radionuclide therapy (PRRT) is an established treatment of metastatic neuroendocrine tumors grade 1-2 (G1-G2). However, its possible benefit in high-grade gastroenteropancreatic (GEP) neuroendocrine neoplasms (NEN G3) is unknown. We therefore aimed to assess the benefits and side effects of PRRT in patients with GEP NEN G3. We performed a retrospective cohort study at 12 centers to assess efficacy and toxicity of PRRT in patients with GEP NEN G3. Outcomes were response rate, disease control rate, progression-free survival (PFS), overall survival (OS) and toxicity. We included 149 patients (primary tumor: pancreatic n=89, gastrointestinal n=34, unknown n=26). PRRT was 1st-line (n=30), 2nd-line (n=62) or later line treatment (n=57). Of 114 patients evaluable, 1% had complete response, 41% partial response, 38% stable disease and 20% progressive disease. Of 104 patients with documented progressive disease before PRRT, disease control rate was 69%. The total cohort had median PFS of 14 months and OS 29 months. Ki-67 21-54% (n=125) vs. Ki-67≥55% (n=23): PFS 16 vs. 6 months ($p<0.001$) and OS 31 vs. 9 months ($p<0.001$). Well (n=60) vs. poorly-differentiated NEN (n=62): PFS 19 vs. 8 months ($p<0.001$) and OS 44 vs.19 months ($p<0.001$). Grade 3-4 hematological or renal toxicity occurred in 17% of patients. This large multicenter cohort of patients with GEP NEN G3 treated with PRRT demonstrates promising response rates, disease control rates, PFS and OS as well as toxicity in patients with mainly progressive disease. Based on these results, PRRT may be considered for patients with GEP NEN G3.

Introduction

Neuroendocrine neoplasms (NEN) are a very heterogeneous entity classified according to primary tumor location, stage, proliferation rate and differentiation. The 2010 World Health Organization (WHO) Classification grades NEN according to the proliferation index Ki-67; ≤ 2% (Grade 1, G1), 3-20% (G2) and > 20 % (G3) (Bosman, et al. 2010). G1-G2 were collectively referred to as neuroendocrine tumors (NET) and G3 as neuroendocrine carcinoma (NEC). The classification is strongly prognostic, but is also used to guide treatment decisions. In 2017, WHO refined the classification of pancreatic NEN; G3 tumors are further classified as well (NET G3) and poorly differentiated (NEC) (Kloppel, et al. 2017), and a similar expansion to gastrointestinal (GI) G3 tumors is anticipated in the next WHO classification. The NET category is now only used for well-differentiated tumors regardless of their proliferation index (G1-G3), whereas the NEC category is used for poorly differentiated high-grade neuroendocrine carcinomas (G3). The terminology of NEN G3 relates to all high-grade (G3, Ki-67 >20%) neuroendocrine malignancies; i.e. both NET G3 and NEC.

Gastroenteropancreatic (GEP) NENs G3 are rare, highly malignant, with poor prognosis and limited therapeutic options (Garcia-Carbonero, et al. 2016; Ilett, et al. 2015; Sorbye, et al. 2014). The majority of patients have metastases at the time of diagnosis and median overall survival (OS) is less than 6 months including all patients (Dasari, et al. 2018). Platinum-based chemotherapy is the standard treatment in metastatic disease with response rates of 30-35%, progression-free survival (PFS) of 4-5 months and OS 11-14 months (Heetfeld, et al. 2015; Sorbye, et al. 2013; Walter, et al. 2017; Yamaguchi, et al. 2014).

In metastatic GEP NET G1-G2, peptide receptor radionuclide therapy (PRRT) targeting somatostatin receptors has been used with excellent results for the last two decades in

Europe and Israel (Bodei, et al. 2011; Imhof, et al. 2011; Kwekkeboom, et al. 2008; Pfeifer, et al. 2011; Romer, et al. 2014). The recent NETTER-1 phase 3 trial of patients with somatostatin receptor imaging (SRI) positive NET G1/G2 supports this approach (Strosberg, et al. 2017). In contrast, PRRT has generally not been recommended for GEP NEN G3 based on expectance of low expression of somatostatin receptors and rapid growth behavior. According to guidelines, PRRT can be considered in SRI-positive NET G3, but data are lacking (Garcia-Carbonero et al. 2016). PRRT could, however, be a relevant therapeutic option for NEN G3 since SRI positivity has been reported for both NET G3 and NEC (Heetfeld et al. 2015; Raj, et al. 2017; Sorbye et al. 2013; Velayoudom-Cephise, et al. 2013), as well as having expression of somatostatin receptor 2A on immunohistochemistry (Konukiewitz, et al. 2017).

Randomized large studies to assess the benefit of specific treatments are often not feasible to perform in very rare diseases. Large retrospective datasets may then initially be the only way on which to base treatment decisions. In a large multicenter international cooperation, we therefore collected retrospectively the outcomes after PRRT in patients with GEP NEN G3.

Methods

Patients

At 12 university hospitals, we retrospectively included patients that fulfilled the following criteria: 1) GEP NEN or NEN of unknown primary with dominance of abdominal metastases, 2) Ki-67 > 20%, and 3) treated with PRRT. Data on demographics, diagnosis, previous treatments, PRRT, outcome and toxicity were registered. SRI (^{68}Ga-somatostatin analogue positron emission tomography [PET]/computer tomography [CT], or ^{111}In-octreotide or ^{99m}Tc-tektrotyd scintigraphy) results were reported as tumor uptake in relation to liver uptake (none, < liver, = liver or > liver)

and used as a surrogate for somatostatin receptor density. ^{18}F-Flour-Deoxy-Glucose (FDG) PET/CT results were reported as tumor uptake present or not (positive or negative by qualitative assessment). Histological examination included chromogranin A (CgA) and synaptophysin staining, Ki-67% in hot-spots, and tumor differentiation (poor, intermediate and well). Most of the centers have specific NET pathologists and in cases where differentiation was lacking in the original pathology report, a reclassification was done if sections were available. Plasma values of chromogranin A, lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) obtained at the time of first PRRT were reported.

Patients were grouped according to Ki-67 index (21-54% and ≥ 55%) based on the Nordic NEC study and other reports (Garcia-Carbonero et al. 2016; Sorbye, et al. 2018; Sorbye et al. 2013; Thang, et al. 2018). Furthermore, patients were grouped by combined Ki-67% and differentiation: Ki-67: 21-54% and well-differentiated tumor (NET G3) vs. Ki-67: 21-54% and poorly differentiated tumor (NEC; Ki-67 21-54%) vs. Ki-67 ≥ 55% and poorly differentiated tumors (NEC; Ki-67 ≥ 55%) (Milione, et al. 2017).

Ethical committee approval was obtained in accordance with regional guidelines (either approval of the study or exempt of application due to the retrospective design). Regional ethics committees for participating centers are Rigshospitalet (Videnskabsetisk Komité, Region Hovedstaden) and Aarhus University Hospital (Videnskabsetisk Komité, Region Midt), Denmark; University Hospital Bonn (Ethikkommission an der Medizinischen Fakultät der RheinischenFriedrich-Wilhelms-Universität Bonn) and University Hospital Gießen and Marburg (Ethics Committee of the Philipps-University Marburg, Medicine), Germany; Hadassah-Hebrew University Medical Center (Hadassah-Hebrew University Medical Center Institutional Ethical Committee), Israel; European Institute of Oncology (Ethics Committee), Italy; Erasmus Medical Center (Medical Research and Ethics Committee, Rotterdam), The Netherlands; MSWiA Hospital

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Treatment

Patients received PRRT according to local guidelines at their respective institution. In general, treatment was given intravenously and consisted of a radioisotope (^{177}Lutetium, ^{90}Yttrium or ^{111}Indium) conjugated with a somatostatin analogue (octreotide or octreotate). Patients were planned to a series of PRRT, typically consisting of four cycles each and separated by approximately 8 weeks. The intended cumulative activity was calculated by taking renal function and bone marrow irradiation into account. To reduce renal irradiation, patients were pretreated with an intravenous amino-acid solution. Planned PRRT cycles were discontinued in case of progression of disease or adverse effects limiting further cycles.

Outcomes

Response rate (RR) was defined as complete response (CR) or partial response (PR) according to the response evaluation criteria in solid tumors (RECIST 1.1) (Eisenhauer, et al. 2009). Disease control rate (DCR) was defined as CR or PR in all patients or stable disease (SD) in patients with progressive disease (PD) at the start of PRRT. PFS was time from first cycle of PRRT to disease progression radiologically by RECIST 1.1, or clinically assessed by a physician [i.e. worsening of performance status due to NEN]. If no progression was documented, date of death or date last

follow-up if alive was used. OS was time from first cycle of PRRT to death or date of last follow-up if still alive. Toxicity was reported as acute if occurring during PRRT and as long-term if occurring after PRRT and within 1 year of PRRT. Toxicity was graded according to the Common Terminology Criteria for Adverse Events v.4, reporting grade 3-4 only.

Statistics

Continuous variables are reported as median and range. By means of Kaplan-Meier estimation, PFS and OS was calculated and reported as median with 95 % confidence intervals (CI). Log-rank test was used to compare PFS and OS estimates between groups. Cox regression analysis was performed for PFS and OS with covariates: age, gender, performance status, SRI tumor uptake, Ki-67 (dichotomized), primary tumor site, tumor morphology (well vs. poorly differentiated, excluding the intermediate group due to few cases), plasma LDH and plasma ALP. P-values < 0.05 were considered statistically significant. All analyses were performed using SPSS statistics 25.

Results

Patients

From August 1999 to May 2017, 149 patients with GEP NEN G3 received PRRT at 12 centers (Table 1). The primary tumor site was predominantly in the pancreas (n=89) or unknown (n=26). Other sites included the esophagus (n=2), stomach (n=4), gallbladder/common bile duct (n=2), small bowel (n=18), colon (n=3), rectum (n=3) and other abdominal sites (n=2), here collectively referred to as GI (n=34). All but two patients had metastatic disease. The median Ki-67 was 30%, ranging from 21-100%. Ki-67 21-54% was found in the majority of patients (n=125) vs. $\geq$ 55%

(n=23), missing for one patient. Tumor morphology was equally distributed among poorly (n=62) and well differentiated (n=60) with only few cases of intermediate differentiation classification (n=9). Seventeen of 20 patients (85%) with Ki-67% ≥ 55% vs. 44 of 110 patients (40%) with Ki-67 21-54% had poorly differentiated tumor morphology. All patients with SRI showed tumor uptake, predominantly > liver uptake.

Treatment

At the start of PRRT, 104 patients (70%) had radiologically progressive disease (determined by RECIST in 67 patients), which also was the main indication for PRRT (65%) (Table 2). The median time from diagnosis to first PRRT was 8 months (range 0-174). PRRT was frequently given as 2nd-line (n=62) or a later line of treatment (n=57). Patients received a median of 4 cycles PRRT (range 1-15) with a median cumulative activity of 18 gigabecquerel (range 4-85). Radioisotopes ^{177}Lutetium and/or ^{90}Yttrium were used for PRRT in all patients other than a single patient who received ^{111}Indium. Concurrent chemotherapy was applied for six patients. Overall, 98 patients (65.8%) completed their planned protocol of PRRT cycles, while 51 patients did not (Table 2). The main reasons for not completing the planned PRRT cycles were progressive disease (n=19), clinical deterioration (n=6) or toxicity (n=6). Data on treatment after PRRT was available for 118 patients (79.2%). Chemotherapy (n=65) and somatostatin analogs (n=67) were frequently used, while surgery on the primary tumor or metastases (n=8), liver embolization (n=12) and external radiotherapy (n=19) were less frequently used.

Response and survival analysis

Of 114 patients evaluable by RECIST, 1 (1%) had CR, 47 (41%) PR, 43 (38%) SD and 23 (20%) PD. An example of a PR is shown in Figure 1. Disease control was seen in 79 patients (69%) responding to PRRT. RR did not differ among subgroups, including differentiation (42% vs. 43% for well and poorly differentiated, respectively) and Ki-67 index (42% vs. 43% for Ki-67 21-54% and Ki-67 $\geq$ 55%, respectively) (Table 3). We observed similar RR with use of ^{177}Lu or ^{90}Y PRRT and for patients from the 12 centers (data not shown). Median follow-up was 23 months (range 0-210) and during follow-up 107 patients died. The cause of death was NEN in 91 of 94 cases with available data. The median PFS was 14 months (95%CI 10.4-17.6) and median OS was 29 months (95%CI 23.3-34.7) for all patients. Median PFS and OS were significantly longer for patients with a Ki-67 21-54% ($p < 0.001$), well differentiated tumor ($p < 0.001$), PS < 2 ($p < 0.001$), normal plasma levels of LDH ($p < 0.001$) and ALP ($p < 0.001$) (Figures 2-3). PFS and OS were independent of the amount of SRI tumor uptake, primary tumor site and line of treatment. In univariate analyses of PFS and OS, Ki-67 index, differentiation, PS as well as plasma LDH and ALP were statistically significant predictors (Table 4). In multivariate analysis (n=75), PS, plasma LDH and ALP were statistically significant predictors for PFS and OS, and age was significant for PFS and differentiation for OS (Table 5). Excluding plasma LDH and ALP from the multivariate analysis resulted in 106 patients in the model; differentiation and PS were statistically significant predictors for PFS and OS (data not shown).

Toxicity

Acute grade 3-4 toxicity occurred in 19 patients (13%), most frequently hematological (n=9) or renal (n=3) (Table 2). In four patients, the acute hematological toxicity persisted beyond the time of PRRT and was thus included as long-term toxicity as well. Another 15 patients without any acute

severe toxicity developed long-term hematological (n=11), renal (n=3) or not specified (n=1) grade 3-4 toxicity. For first, second and later line of treatment 5 (17%), 16 (26%) and 13 (23%) patients had grade 3-4 toxicity, respectively. With ^{177}Lu 24 (24%), ^{90}Y 7 (21%) and combined ^{177}Lu/^{90}Y 3 (25%) patients had grade 3-4 toxicity, respectively. Renal grade 3-4 toxicity occurred in two patients (6%) treated with ^{90}Y and four patients (4%) treated with ^{177}Lu.

Discussion

To the best of our knowledge, this is the largest study to assess the outcome after PRRT in patients with advanced high-grade GEP NEN. The majority of the patients had radiological progressive disease at the start of PRRT; RR was 42% and DCR was 69% for evaluable patients. A promising median PFS of 14 months and median OS of 29 months was found. Hematological or renal grade-3-4 toxicity occurred in 17% of patients, not more than observed for other patient groups given PRRT. These results suggest that PRRT can be effective and tolerable in high-grade GEP NEN patients.

Comparison with standard treatment

The current recommendations for first-line treatment of advanced GEP NEC is systemic platinum-based chemotherapy giving a RR of 30%, PFS 4-5 months and OS 11 months (Heetfeld et al. 2015; Sorbye et al. 2013; Walter et al. 2017; Yamaguchi et al. 2014). Second-line treatment for NEC is usually of short benefit with an estimated PFS of 3-4 months (Hadoux, et al. 2015; Hentic, et al. 2012; Olsen, et al. 2014; Olsen, et al. 2012; Walter et al. 2017; Welin, et al. 2011). The Nordic NEC study showed a poorer RR to platinum-based chemotherapy in patients with Ki-67 < 55% (RR: 15%) compared to patients with a Ki-67 ≥ 55% (RR: 42%) (Sorbye et al. 2013). Data for advanced

NET G3 are generally scarce; however, RR to platinum-based chemotherapy is low (0-17%) with a short PFS (2.4 months)(Sorbye et al. 2018). Median survival is reported to be more than 40 months but as data is presented as a mixture of stages, results are difficult to interpret (Heetfeld et al. 2015; Hijioka, et al. 2017; Sorbye et al. 2018; Velayoudom-Cephise et al. 2013). In a high-grade GEP-NEN population of 136 patients, median survival from time of first diagnosis was best for NET G3 (43.6 months), intermediate for NEC with a Ki-67 21-54% (24.5 months) and 5.3 months for NEC cases with a Ki-67 ≥ 55% (Milione et al. 2017). A combination of capecitabine and temozolomide has been suggested for patients with well differentiated tumor morphology and a Ki-67 21-54%, but data are scarce (Garcia-Carbonero et al. 2016; Heetfeld et al. 2015; Sorbye et al. 2018). In our cohort half the patients were treated with somatostatin analogs (SSA) either before and/or after PRRT. SSA is not recommended for high-grade NEN, but may be explained by the selection of patients with a positive SRI or use of SSA after PRRT in general.

Cross-trial comparisons are difficult as well as evaluation of the benefit of PRRT without a control arm. However, a RR of 42% and DCR of 69% indicate that PRRT has an effect in our cohort. No differences in RR were observed in subgroups according to both well vs. poor differentiation and Ki-67 21-54% vs. Ki-67 ≥ 55%, as RR was approximately 40% in all subgroups. It may be that the efficacy of PRRT mediated by radiation is less sensitive to the degree of differentiation and rate of proliferation as long as the somatostatin receptor target is present on the tumor cells. The benefit of platinum based chemotherapy seems to be more dependent on a high degree of proliferation, as evident in the Nordic NEC study (Sorbye et al. 2013). As most of our patients had radiologically progressive disease at the start of PRRT, a PFS of 14 months indicates that PRRT seems to benefit many patients. Interestingly, no differences in RR, PFS and OS were evident in our cohort in regard to the line of treatment. Differentiation, Ki-67, PS, LDH and ALP were all significantly correlated to OS, as shown in previous studies (Lamarca, et al. 2017; Sorbye et al. 2013). However, the true

benefit of PRRT for PFS and especially OS is not possible to decide without a prospective randomized trial, which will be difficult to perform in such a rare disease. As implementation of PRRT may seem more likely in NET G3, such a randomized trial could compare PRRT vs standard chemotherapy regime (platinum-based or temozolomide/capecitabine) in a GEP NET G3 population. Data to clarify whether concurrent chemotherapy to PRRT should be considered is awaited (ClinicalTrials.gov: NCT02736448). Safety of PRCRT has been reported for 65 patients with 5-year follow-up, showing modest reversible hematological toxicity and comparable to PRRT (Kesavan, et al. 2014).

Comparison with previous PRRT data in NEN G3 and classification

Three single-center retrospective studies recently reported the outcome of PRRT in NEN with a high Ki-67 and SRI tumor uptake > liver.

An Australian study (Thang et al. 2018) assessed 28 patients with NEN and Ki-67 > 20% (median Ki-67: 32.5%). The majority received PRRT with concurrent chemotherapy. The RR was 35%, PFS 9 months and OS 19 months for all patients. According to Ki-67 index PFS (12 vs. 4 months) and OS (46 vs. 7 months) differed for Ki-67 ≤55% and Ki-67 > 55%.

A German study (Zhang, et al. 2018) assessed 69 patients with GEP NEN and Ki-67 index >20 % (median Ki-67 30 %). In their study, approximately one third received concurrent chemotherapy – the effect hereof was uncertain. The RR was 31 %, DCR 78 %, PFS 10 months and OS 20 months (rounded values). According to Ki-67 index PFS (11 vs. 4 months) and OS (22 vs. 7 months) differed for Ki-67 ≤55% and Ki-67 > 55%.

An Italian study (Nicolini, et al. 2018) assessed 33 patients with GEP NEN and Ki-67 index of 15-70% (median Ki-67: 25%). The RR was 6%, PFS 23 months and OS 52.9 months.

298 Overall, in our study we found similar results: PFS (16 vs.6 months) and OS (31 vs.9 months)
299 differed significantly in patients with Ki-67 < 55% vs. Ki-67 ≥ 55%.

300 In general, the likelihood of somatostatin receptor expression on neuroendocrine cells
301 decreases with increasing grade of tumor, whereas the opposite applies for FDG uptake (Binderup,
302 et al. 2010; Hicks, et al. 2017). NET G3 seems to have a positive SRI uptake in 70% of cases,
303 whereas for NEC the figure is more likely 30% (Heetfeld et al. 2015; Raj et al. 2017; Sorbye et al.
304 2018; Sorbye et al. 2013; Velayoudom-Cephise et al. 2013). Preliminary studies have also shown
305 the effectiveness of PRRT in patients with a more aggressive grade NEN with ^{18}F-FDG-avid and
306 SRI uptake (Kashyap, et al. 2015). Patients with concordant ^{18}F-FDG and SRI avid lesions may be
307 more radiosensitive by having a high proliferative fraction. Few of the patients in our cohort had
308 ^{18}F-FDG PET/CT data available limiting further analysis.

309 As previously reported (Basturk, et al. 2015), the grading of NEN according to Ki-67
310 may be optimized by further subclassification of patients with Ki-67 > 20%. In the current study of
311 patients graded as NEN G3 based on Ki-67, nearly half the patients had well-differentiated tumor
312 morphology. The majority of patients with well-differentiated tumors also had Ki-67 21-54%. There
313 was a marked difference in outcomes in our cohort when comparing subgroups based on tumor
314 morphology: PFS (19 vs.8 months) and OS (44 vs.19 months) differed significantly comparing
315 well differentiated vs. poorly-differentiated neoplasms.

316

317 **Toxicity**

318 In our study, 26 patients (17%) had either acute or long-term grade 3-4 renal or hematological
319 toxicity. This is similar to that reported in other larger retrospective analysis of patient groups given
320 PRRT (Imhof et al. 2011; Kwekkeboom et al. 2008), although in NETTER-1 no evidence of renal

adverse effects was observed in patients treated with ^{177}Lu (Strosberg et al. 2017). We observed renal toxicity both in patients treated with ^{90}Y and ^{177}Lu. Furthermore, we found similar frequency of toxicity for patients receiving PRRT as first line vs. later line of treatment.

Limitations

High-grade GEP NEN patients treated with PRRT are probably highly selected on factors as being positive on SRI imaging and having a rather low median Ki-67 compared to the NEN G3 group as a whole. RR, PFS and OS should be interpreted carefully in light of the retrospective design of the study. However most of our patients were classified as having radiological progression of disease at the start of PRRT, and approximately half were based on RECIST. The rate of side-effects of PRRT in our analysis was in line with that previously reported for PRRT, but toxicity reports in a retrospective study must be interpreted cautiously. Pathologist reports were mainly from NET expert centers and reclassification was done in reports with missing data when sections were available. Though, a general problem is that the distinction between well and poor differentiation is not standardized (Tang, et al. 2016). Addition of molecular data on DAXX, ATRX (loss of expression in well-differentiated tumors) and Rb1, KRAS and p53 (expressed in poorly-differentiated tumors, could aid further in classification of differentiation (Sorbye et al. 2018).

Conclusion

This large retrospective multicenter study is at present the most comprehensive report on which to base treatment decisions regarding the use of PRRT in high-grade GEP NEN. It shows promising RR, DCR, PFS and OS and acceptable toxicity after PRRT in patients with mainly progressive

disease. This suggests that PRRT is active and potentially effective in patients with GEP NEN G3. Awaiting further data, PRRT may therefore be a treatment option for GEP NEN G3 patients.

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Table 1. Baseline characteristics of 149 patients with GEP NEN G3 receiving PRRT.

Characteristics	Value
Age (years), median (range)	57 (24-85)
Time since diagnosis (m), median (range)	8 (0-174)
Gender, n (%): Male	76 (51.0)
Female	73 (49.0)
PS, n (%): 0	74 (49.7)
1	41 (27.5)
2	11 (7.4)
Missing	23 (15.4)
Primary tumor site, n (%): Pancreas	89 (59.7)
Gastrointestinal	34 (22.8)
Unknown primary	26 (17.4)
Metastatic disease, n (%)	147 (98.7)
Liver metastases	141 (94.6)
Tumor differentiation, n (%): Well	60 (40.3)
Intermediate	9 (6.0)
Poor	62 (41.6)
Not specified	18 (12.1)
Ki-67 (%), median (range)	30 (21-100)
21-54%	125 (83.9)
≥ 55%	23 (15.4)
Not specified	1 (0.7)
Ki-67 and differentiation: NET G3	58 (38.9)
NEC; Ki-67 21-54%	44 (29.5)
NEC; Ki-67 ≥ 55%	17 (11.4)
Not specified	30 (20.1)
CgA staining of tumor: Strongly positive	90 (60.4)
Partly positive	19 (12.8)
Negative	9 (6.0)
Not specified	31 (20.8) *
Synaptophysin staining of tumor: Strongly positive	105 (70.5)
Partly positive	11 (7.4)
Not specified	33 (22.1)
SRI available	146 (98.0%)
Uptake: None	0
< liver	5 (3.4%)
= liver	10 (6.7%)
> liver	131 (87.9%)
^{18}F-FDG PET/CT available	39 (26.2%)
Tumor positive	34 (87.2%)
Plasma-CgA (n, %): Normal	15 (10.1)
Elevated	83 (55.7)
Missing	51 (34.2)
Plasma-LDH, n(%): Normal	76 (51.0)
Elevated	35 (23.5)
Missing	38 (25.5)
Plasma-ALP, n (%): Normal	54 (36.2)
Elevated	67 (45.0)
Missing	28 (18.8)
Number of prior lines of medical treatment: 0	30 (20.1)
1	62 (41.6)
2	31 (20.8)
> 2	26 (17.5
Prior treatment, n (%)	

Primary tumor resected	58 (38.9)
Somatostatin analog	74 (49.7)**
Chemotherapy/targeted therapy	88 (59.1)
Cisplatin	31 (20.8)
Carboplatin	26 (17.4)
Etoposide	46 (30.9)
Capecitabine or 5-fluorouracil	38 (25.5)
Temozolomide	19 (12.8)
Streptozotocin	13 (8.7)
Everolimus	9 (6.0)
Doxorubicin	5 (3.4)
Sunitinib	4 (2.7)
Oxaliplatin	4 (2.7)
Interferon	2 (1.3)

PS: Performance status. CgA: chromogranin A. SRI: Somatostatin receptor imaging. LDH: lactate dehydrogenase. ALP: alkaline phosphatase.

* In 29 patients, CgA and synaptophysin staining results were not available; hereof 28 patients had SRI available that showed tumor uptake.

**Missing values for seven patients.

Table 2. Treatment details and toxicity of PRRT for 149 patients with GEP NEN G3.

	Value
Radiologically progressive disease at start of PRRT, n (%)	104 (69.8)
No	35 (23.5)
Unknown	10 (6.7)
Indication for PRRT, n (%)	
Progression of disease	97 (65.1)
First line	30 (20.1)
Side effects to other therapies	6 (4.0)
Other	16 (10.7)
Radioisotope, n (%)	
^{177}Lutetium	101 (67.8)
^{90}Yttrium	34 (22.8)
^{177}Lutetium + ^{90}Yttrium	12 (8.1)
^{111}Indium	1 (0.7)
Not specified	1 (0.7)
Cumulative activity (GBq), median (range)	18.0 (4-85)
Number of PRRT cycles, median (range)	4.0 (1-15)
Fulfilled planned number of cycles	98 (65.8)
Discontinuation of PRRT:	
Disease progression	19 (12.8)
Clinical deterioration	6 (4.0)
Hematological side effects	5 (3.4)
Renal side effects	1 (0.7)
Lack of compliance	1 (0.7)
Other	17 (11.4)
Not specified	2 (1.3)
WHO performance status after treatment, n (%)	
0	74 (49.7)
1	34 (22.8)
2	11 (7.4)
3	5 (3.4)
Not specified	25 (16.8)
Absence of acute toxicity (grade 3-4), n (%)	121 (81.2)
Acute toxicity	19 (12.8)
Hematological, grade 3/grade 4, n *	8/1
Renal	2/1
Diarrhea	0/2
Nausea	0/2
Other, not specified	14/1
Unknown	9 (6.0)
Absence of long-term toxicity (grade 3-4), n (%)	101 (67.8)
Long-term toxicity	19 (12.8)
Hematological, grade 3/grade 4, n *	13/2
Renal	3/0
Other, not specified	3/3
Unknown	29 (19.5)

*More than one may be reported for a patient.

Table 3. PRRT response rates (n=114) and outcomes (n=149) in GEP NEN G3.

	CR (%)	PR (%)	SD (%)	PD (%)	PFS (m), (95% CI)	OS (m), (95% CI)	
All patients	1 (1)	47 (41)	43 (38)	23 (20)	14.0 (10.4-17.6)	29.0 (23.3-34.7)	
PS							
• 0	1 (2)	21 (36)	26 (45)	10 (17)	**16.0 (11.0-21.0)**	**39.0 (28.1-49.9)**	*
• 1	0	17 (53)	8 (25)	7 (22)	**14.0 (8.2-19.8)**	**23.0 (16.2-29.8)**	
• 2	0	3 (38)	2 (25)	3 (38)	**3.0 (0-6.2)**	**4.0 (0-12.6)**	
SRI tumor uptake							
• ≤liver	1 (9)	3 (27)	4 (36)	3 (27)	16.0 (7.9-24.1)	25.0 (8.6-41.4)	
• > liver	0	44 (43)	38 (37)	20 (20)	14.0 (10.0-18.0)	29.0 (21.6-36.4)	
Primary tumor site							
• Pancreas	0	32 (48)	23 (34)	12 (18)	14.0 (10.4-17.6)	29.0 (21.7-36.3)	
• Gastrointestinal	0	11 (42)	9 (35)	6 (23)	10.0 (0-21.2)	31.0 (7.5-54.5)	
• Unknown	1 (5)	4 (19)	11 (52)	5 (24)	16.0 (8.4-23.6)	29.0 (11.4-46.6)	
Differentiation							
• Well	0	19 (42)	23 (51)	3 (7)	**19.0 (13.9-24.1)**	**44.0 (25.2-62.8)**	*
• Poor	1 (2)	21 (41)	13 (25)	16 (31)	**8.0 (3.3-12.7)**	**19.0 (11.7-26.3)**	
Proliferation							
• Ki-67 21-54%	1 (1)	41 (41)	41 (41)	16 (16)	**16.0 (12.7-19.3)**	**31.0 (24.2-37.8)**	*
• Ki-67≥ 55%	0	6 (43)	2 (14)	6 (43)	**6.0 (3.0-9.0)**	**9.0 (4.5-13.5)**	
Differentiation and proliferation							*
• NET G3	0	18 (42)	22 (51)	3 (7)	**19.0 (14.4-23.6)**	**44 (25.3-62.7)**	
• NEC; Ki-67 21-54%	1 (3)	16 (41)	12 (31)	10 (26)	**11 (5.4-16-6)**	**22.0 (16.0-28.0)**	
• NEC; Ki-67 ≥ 55%	0	5 (45)	1 (9)	5 (45)	**4 (0.8-7.2)**	**9.0 (1.6-16.4)**	
Line of treatment							
• First line	0	10 (42)	9 (38)	5 (21)	13 (6.3-19.7)	29 (12.5-45.5)	
• Second line	0	20 (45)	16 (36)	8 (18)	12.0 (6.5-17.5)	29 (16.8-41.2)	
• Later line	1 (2)	17 (37)	18 (39)	10 (22)	19.0 (13.6-24.4)	29.0 (18.0-40.0)	
plasma-LDH							
• Normal	1 (2)	30 (48)	24 (38)	8 (13)	**18.0 (14.3-21.7)**	**39.0 (30.8-47.2)**	*
• Elevated	0	8 (30)	8 (30)	11 (41)	**4.0 (0.5-7.5)**	**13.0 (7.4-18.6)**	
plasma-ALP							
• Normal	1 (2)	21 (47)	18 (40)	5 (11)	**18.0 (9.9-26.1)**	**39.0 (30.8-47.2)**	*
• Elevated	0	22 (40)	19 (35)	14 (25)	**14.0 (9.2-18.8)**	**21.0 (16.8-25.2)**	

*Denotes statistically significant difference in PFS and OS. P-values as shown in Figures 1-2.

CR: complete response. PR: partial response. SD: stable disease. PD: progressive disease per Response Evaluation Criteria In Solid Tumours. PFS: progression-free survival. OS: overall survival. CI: confidence interval. SRI: somatostatin receptor imaging. LDH: lactate dehydrogenase. ALP: alkaline phosphatase.

Table 4. Multiple Cox regression analysis of predictors for PFS and OS in 75 GEP NEN G3 patients treated with PRRT. (74 missing)

Model 1	**PFS**		**OS**	
Covariate	**Hazard ratio (95% CI)**	**P-value**	**Hazard ratio (95% CI)**	**P-value**
Age	**0.98 (0.95-1.00)**	**0.045**	0.99 (0.96-1.02)	0.42
Male	1.53 (0.85-2.74)	0.16	1.05 (0.53-2.10)	0.89
PS 0	1		1	
1	**2.57 (1.33-4.93)**	**0.005**	**2.35 (1.13-4.89)**	**0.02**
2	3.42 (0.90-13.06)	0.07	**4.20 (0.98-18.01)**	**0.05**
SRI ≤ liver	0.72 (0.13-4.03)	0.70	0.43 (0.04-4.33)	0.47
Primary tumor site (unknown primary)	1		1	
Gastrointestinal	0.80 (0.32-2.02)	0.64	0.78 (0.26-2.37)	0.66
Pancreas	0.66 (0.28-1.57)	0.35	0.46 (0.18-1.22)	0.12
Poorly differentiated	1.69 (0.88-3.23)	0.11	**2.92 (1.31-6.50)**	**0.009**
Ki-67 ≥ 55%	1.11(0.51-2.42)	0.80	1.97 (0.83-4.66)	0.13
Line of treatment (first line)	1		1	
Second line	0.76 (0.38-1.54)	0.46	1.55 (0.70-3.43)	0.28
Later line	1.04 (0.48-2.27)	0.91	1.77 (0.67-4.65)	0.25
Elevated plasma-LDH	**2.66 (1.29-5.49)**	**0.008**	**2.61 (1.16-5.90)**	**0.02**
Elevated plasma-ALP	**2.24 (1.22-4.09)**	**0.009**	**2.79 (1.42-5.49)**	**0.003**

Supplementary Table 1. Univariate analyses of predictors for PFS and OS in 149 GEP NEN G3 patients treated with PRRT.

	PFS		OS	
Covariate	**Hazard ratio (95% CI)**	**P-value**	**Hazard ratio (95% CI)**	**P-value**
Age	1.00 (0.99-1.02)	0.84	1.01 (0.99-1.03)	0.20
Male	0.96 (0.68-1.34)	0.79	0.78 (0.53-1.1)	0.19
PS 0	1		1	
1	1.36 (0.91-2.04)	0.14	**1.65 (1.04-2.63)**	**0.04**
2	**3.53 (1.83-6.83)**	**< 0.001**	**6.84 (3.40-13.76)**	**< 0.001**
SRI ≤ liver	1.17 (0.67-2.04)	0.59	0.79 (0.40-1.57)	0.50
Primary tumor site (unknown primary)	1		1	
Gastrointestinal	1.13 (0.65-1.95)	0.67	0.75 (0.41-1.39)	0.36
Pancreas	1.29 (0.80-2.07)	0.30	0.83 (0.50-1.37)	0.46
Poorly differentiated	**1.62 (1.11-2.36)**	**0.01**	**2.55 (1.62-4.02)**	**< 0.001**
Ki-67 ≥ 55%	**2.15 (1.34-3.47)**	**0.002**	**2.48 (1.51-4.06)**	**< 0.001**
Differentiation and proliferation (NET G3)	1		1	
NEC; Ki-67 21-54%	1.38 (0.91-2.07)	0.13	**2.06 (1.26-3.39)**	**0.004**
NEC; Ki-67 ≥ 55%	**2.81 (1.55-5.11)**	**0.001**	**4.77 (2.51-9.06)**	**< 0.001**
Line of treatment (first line)	1		1	
Second line	1.08 (0.69-1.69)	0.73	1.04 (0.63-1.71)	0.87
Later line	0.79 (0.50-1.24)	0.31	0.86 (0.52-1.42)	0.55
Elevated plasma-LDH	**2.35 (1.54-3.59)**	**< 0.001**	**3.14 (1.96-5.02)**	**< 0.001**
Elevated plasma-ALP	**1.53 (1.04-2.24)**	**0.03**	**2.21 (1.42-3.45)**	**< 0.001**

CI: Confidence interval. PS: performance status. SRI: Somatostatin receptor imaging. GI: gastrointestinal. LDH: lactate dehydrogenase. ALP: alkaline phosphatase.

Supplementary table 2. Multiple Cox regression analysis of predictors for PFS and OS in 106 GEP NEN G3 patients treated with PRRT. (43 missing)

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Model 2	**PFS**		**OS**	
Covariate	**Hazard ratio (95% CI)**	**P-value**	**Hazard ratio (95% CI)**	**P-value**
Age	1.00 (0.99-1.02)	0.66	1.02 (0.99-1.04)	0.16
Male	1.13 (0.71-1.80)	0.60	0.86 (0.48-1.54)	0.62
PS 0	1		1	
1	**1.74 (1.07-2.86)**	**0.03**	**1.86 (1.07-3.24)**	**0.03**
2	**4.10 (1.82-9.24)**	**< 0.001**	**5.39 (2.15-13.52)**	**< 0.001**
SRI ≤ liver	0.86 (0.33-2.21)	0.75	0.16 (0.02-1.20)	0.07
Primary tumor site (unknown primary)	1		1	
Gastrointestinal	1.01 (0.50-2.01)	0.98	0.51 (0.23-1.11)	0.09
Pancreas	1.16 (0.63-2.12)	0.63	0.62 (0.32-1.19)	0.15
Poorly differentiated	**1.84 (1.16-2.94)**	**0.01**	**3.16 (1.73-5.76)**	**< 0.001**
Ki-67 ≥ 55%	1.30 (0.68-2.48)	0.43	1.69 (0.81-3.52)	0.16
Line of treatment (first line)	1		1	0.19
Second line	1.15 (0.64-2.07)	0.63	1.55 (0.78-3.10)	0.21
Later line	0.75 (0.40-1.43)	0.39	0.98 (0.45-2.13)	0.95

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Figure 1. Kaplan-Meier curves of PFS for 149 patients with GEP NEN G3 treated with PRRT. Stratification by Ki-67 index (n=148), differentiation (n=122), performance status (n=126), combined Ki-67 index and differentiation (n=119), LDH (n=111) and ALP (n=121), respectively. P-values shown in figures for statistically significant differences.

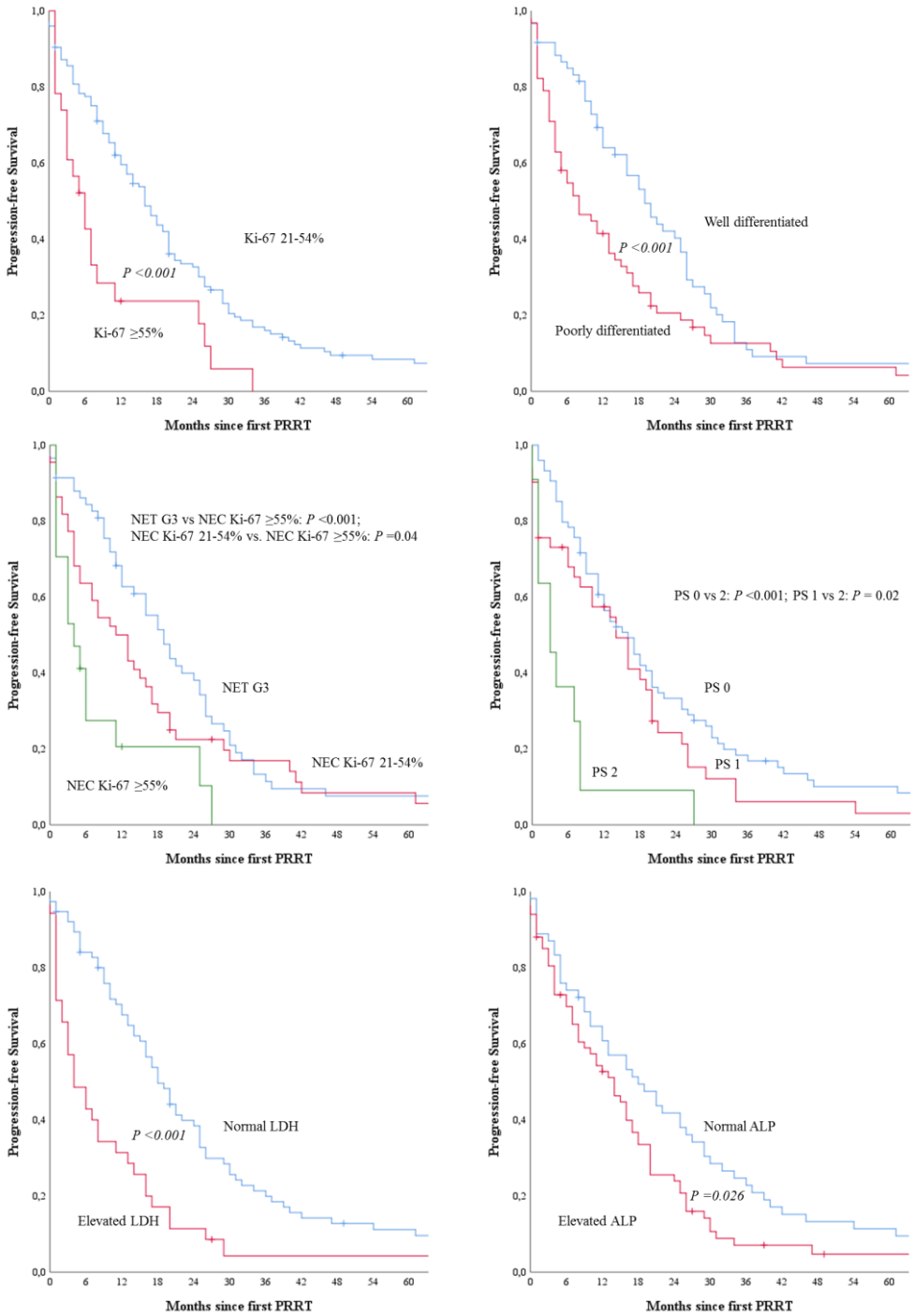

Figure 2. Kaplan-Meier analysis of OS for 149 patients with GEP NEN G3 treated with PRRT. Stratification by Ki-67 index (n=148), differentiation (n=122), performance status (n=126), combined Ki-67 index and differentiation (n=119), LDH (n=111) and ALP (n=121), respectively. P-values shown in figures for statistically significant differences.