Research Square

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Can SSTR2 expression in previously resected SI-NETs predict overall survival after PRRT treatment of remaining lesions?

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Abstract

Purpose

Small intestinal neuroendocrine tumours (SI-NET) often present with distant metastases at diagnosis. Peptide receptor radiotherapy (PRRT) with radiolabelled somatostatin analogues is a systemic treatment that increases overall survival (OS) in patients with SI-NET. However, PRRT treatment response is variable and predictive factors have not been established. PRRT predominantly targets somatostatin receptor 2 (SSTR2). This study evaluates if SSTR2 expression in SI-NET tumours predicts OS after PRRT treatment.

Methods

Using a previously constructed Tissue Micro Array (TMA) consisting of 412 SI-NET patients we identified a subgroup consisting of 44 patients (95 tissue samples) that had received PRRT treatment during 2006-2016 at Sahlgrenska University hospital. IHC expression of SSTR2, Ki-67 and neuroendocrine markers were assessed. A retrospective estimation of 177 Lu-DOTATATE uptake in 33 patients was performed. An additional subgroup of 34 patients with paired samples from 3 tumour sites was identified. SSTR2 expression was assessed in corresponding tissue samples (n=102). Data regarding OS and non-surgical treatment were collected for both groups.

Results

SSTR2 expression did not vary between tumour sites but correlated among the patients' lesions. Patients were grouped into Low SSTR2 or High SSTR2 depending on levels of SSTR2 expression. OS based on SSTR2 expression was not significantly different. However, PRRT treated patients with low SSTR2 expression received less additional treatment compared to patients with high SSTR2 expression and had a tendency towards higher 177 Lu-DOTATATE uptake.

Conclusion

The results from the present study suggest that low SSTR2 expression should not exclude patients from PRRT.

Introduction

Neuroendocrine tumours (NETs), originating from enterochromaffin cells (EC-cells) in small intestinal mucosa (SI-NET), is the most common small intestinal neoplasm with a reported incidence of 1–5/100 000 [2, 25].

SI-NET patients are often diagnosed with regional or distant metastases (WHO stage III-IV). The only potentially curative treatment is radical surgical resection. Although a large proportion of SI-NET patients with stage III-IV disease cannot receive curative treatment, 5 year-overall survival (OS) is 91% for stage III and 72% for stage IV possibly reflecting the generally low proliferative rate in SI-NET tumors. As a

consequence, a large number of SI-NET patients will over time require alternative treatment other than surgical resection.

SI-NETs generally express the somatostatin receptor subtype 2 (SSTR2). Treatment with somatostatin analogues (SSA) reduces hormone secretion and has an anti-tumoural effect [4, 18]. SSTR2 expression is also used clinically for molecular imaging, by scintigraphy (^{111}In-octreotide) or the more recently developed SSTR PET/CT (^{68}Ga-DOTATATE-PET). The SSTR2 expression can further be exploited therapeutically by targeting NET tumours with radiolabelled SSA (^{177}Lu-DOTATATE or ^{177}Lu-DOTATOC), i.e. peptide receptor mediated radionuclide therapy (PRRT)[14].

PRRT in combination with SSA has recently been evaluated in a randomized clinical trial, in which PRRT and SSA increased time to progression and overall survival compared to SSA alone [21]. It has therefore been suggested that PRRT could be considered as first line systemic treatment [8]. However, even though patients are selected for PRRT by evaluation of SSA uptake with SSTR imaging, the objective response of PRRT is still highly variable [5] and, especially for SI-NETS, difficult to assess due to the slow progression rate.

A high uptake on ^{68}Ga-DOTATATE PET has been reported to predict treatment response [13]. Some studies suggest a correlation between immunohistochemically (IHC) quantified SSTR2 expression in tissue samples and uptake in SSTR imaging with ^{68}Ga-DOTATATE-PET or ^{111}In-octreotide scintigraphy [3, 10, 15].

Based on these previous observations, a low or negative SSTR2 expression in SI-NETs could theoretically predict an inferior PRRT treatment response, compared to SI-NETS with a high SSTR2 expression. As other treatment options exist and as PRRT is a resource demanding treatment with potential side effects, such as decreased renal function and bone marrow toxicity, one could then argue that patients with low SSTR2 expressing tumours should not be selected for PPRT as first line treatment.

Typically, SI-NET metastases are not biopsied prior to PRRT and therefore SSTR2 expression in PRRT treated lesions is not possible to quantify with IHC. However, several patients undergo tumour reductive surgery prior to PRRT and we hypothesized that SSTR2 expression in previously resected tissue samples would reflect SSTR2 expression in the remaining lesions that are treated with PPRT.

The aims of this study were to investigate if SSTR2 expression could predict outcome after PRRT, and if SSTR2 expression in resected tumours can be representative of lesions remaining after surgical resection.

Material And Methods

Tumour tissue samples

A tissue microarray (TMA) block was assembled as previously described [1]. Briefly, all patients who underwent surgery for SI-NET at Sahlgrenska University Hospital from 1986 to 2013 were included in a tissue microarray. Formalin-fixed and paraffin-embedded tumour tissue from this cohort was retrieved from the Department of Clinical Pathology and Genetics, Sahlgrenska University Hospital, Gothenburg. The diagnosis was confirmed by reviewing hematoxylin and eosin-stained sections and IHC stainings. Sufficient tumour material for construction of the tissue microarray was available from 412 patients. A total of 8 recipient blocks were constructed, derived from 846 tumours. The quality of the constructed tissue microarray was evaluated on hematoxylin and eosin-stained sections and on IHC stainings for chromogranin A, synaptophysin, SSTR2, and Ki-67. Approval was obtained from the Regional Ethical Review Board in Gothenburg, Sweden, for the use of clinical material for research purpose.

Immunohistochemistry and scoring

Sections from the TMA blocks were placed on coated glass slides and were subjected to antigen retrieval using EnVision FLEX Target Retrieval Solution (high pH) in a Dako PT-Link. IHC staining was performed in a Dako Autostainer Link using EnVision FLEX according to the manufacturer's instructions (DakoCytomation).

The following primary antibodies were used: anti-SSTR2a (clone UMB1; cat no. 134152 Abcam), anti-chromogranin A (MAB319; Chemicon), anti-synaptophysin (SY38, M0776; Dako) and anti-Ki67 (MIB1; Dako), positive and negative controls were included in each run. The fraction of Ki67-positive cells was estimated by manually counting 500–2000 tumour cells per sample, using printouts [23].

Stained TMA slides were scanned using Leica SCN 4000 at × 40 magnification. The scoring system was based on the immunoreactive scoring (IRS) method as previously described by Specht et al [20]. In short, a score for staining intensity between 0-3 was determined using all 846 tumours. A scoring (1-4) of percentage stained cells was also performed according to the following: 1 = <10 % positive cells, 2 = 10-50% positive cells, 3 = 51-80 % positive cells, 4 = >80 % positive cells. These two scores are multiplied for a combined score of 0-12 which is then divided into separate groups (score 0-1 = group 0, score 2-3= group 1, score 4-8= group 2 and score 9-12 = group 3.

When we applied this method to our samples, we found a consistently homogenous expression pattern with over 80% stained cells in all our samples with the exception of 4 negative samples (i.e. score 0), therefore staining intensity was the primary determinant for final score (0-3) (Figure 1 A). SSTR2 expression in the entire TMA was initially scored by a board certified pathologist (O.N.) Samples corresponding to patients included in the present study were reassessed by two blinded observers (E.E and A-K.E). Two cases differed in SSTR2 score between the 3 observers and for these cases the score that 2 out of the 3observers agreed upon was chosen. Synaptofysin and CgA were scored by a single observer (E.E).

Patients and clinical characteristics

Among the specimens on the TMA block we identified samples from 44 patients treated with PRRT during the years 2006-2016 at Sahlgrenska University Hospital (cohort A). Clinical characteristics are presented in Table 1. Clinical data regarding overall survival (OS) and other treatments were obtained. Two patients in cohort A died before they could complete the intended PRRT treatment and were therefore excluded from survival analysis.

Patients were paired samples from primary tumour, lymph node metastases and hepatic metastases were present on the TMA were identified and clinical data for these patients were collected (cohort B, n=34) (Table 2). In this cohort the majority (n=28) had not received PRRT. The reasons for not offering these patients PRRT were stable disease (n=7), predominantly hepatic metastatic burdenore suitable for regional treatment (n=6) or due to low performance score not eligible for treatment (n=4). Seven patients died before PRRT was an approved treatment in Sweden. Clinical data regarding overall survival (OS) and other treatments were obtained.

Activity concentration in tumours

In PRRT treated patients (cohort A) an estimation of the uptake of radionuclide was done by measuring the activity concentration in the tumours. In SPECT images acquired 24 h after the first PRRT treatment reconstructions were done with the recently developed Monte Carlo based ordered subset expectation maximization algorithm SARec [19]. Tumours were identified by visual inspection and the three tumours containing the highest maximum voxel values in each patient were chosen for assessment. Activity concentration calculation was done by dividing the maximum voxel value with SPECT sensitivity and mass of the tissue represented by the voxel.

Statistical analysis

For all statistical analysis of data generated from IHC scoring non-parametric tests were used. For comparisons between 2 groups Mann-Whitney U test was used. For comparisons between 3 or more groups Kruskal-Wallis one-way ANOVA was used. For survival curve comparisons Mantel-Cox log-rank test was used. A level of significance was set to $p < 0.05$ in all tests. Prism software (v.7) was used for statistical analysis.

Results

SSTR2 scoring and distribution of SSTR2 expression among samples

Representative images of SSTR2 expression score are presented in Figure 2 A) Amount of Low SSTR2 (SSTR2 score 1 and 2) samples in the entire TMA, cohort A and cohort B were similar (Figure 2 B). In 95 samples from the 44 PRRT treated patients (cohort A), 16 samples (16.8 %) had no or low STTR2 expression (score 0 or 1) (Figure 1 B). The majority of samples (n= 79) had medium or high SSTR2 expression (score 2 or 3). In 102 samples from the 34 patients in cohort B (paired samples from primary

tumour, lymph node and hepatic metastases), 23 samples (23%) had a low STTR2 expression (score 0 or 1).

To investigate if the SSTR2 expression differed depending on tumour site the samples from cohort B (*n*=102), with paired samples from three separate locations, were analysed. SSTR2 expression levels did not vary significantly between primary, lymph node or hepatic metastases (average score Primary tumour 2.18 *vs* average score Lymph node metastases 2.03 *vs* average score Liver metastases 2.24, n=34 for all groups, p=ns).

Further, to determine if SSTR2 expression was consistent among all lesions in a patient, all samples from cohort B were sorted according to SSTR2 expression in the primary tumour. Three groups of primary tumours (score 1-3, no primary tumour had score 0) were established and SSTR2 expression in corresponding metastases was assessed (Figure 2 C). SSTR2 expression was significantly different in metastases when sorted according to SSTR2 expression in the primary tumors (Group 1-3) (Lymph node metastases group 1 *vs* Lymph node metastases group 2, *p=***, Lymph node metastases group 1 *vs* Lymph node metastases group 3, *p=***,* and Liver metastases group 1 *vs* Liver metastases group 3 *p=*** .

SSTR2 expression and Ki-67

To determine the association between SSTR2 and Ki-67%, Ki-67% was assessed in 70 samples from cohort A. Samples were grouped according to SSTR2 expression and Ki-67% in corresponding samples was analysed. There was a non-significant tendency towards higher Ki-67% in tumours with SSTR2 score 2-3 (p=ns) (Figure 1 C)

SSTR2 expression does not correlate with synaptophysin or chromogranin A expression

We also assessed if SSTR2 expression was associated with the IHC expression of established SI-NET markers synaptohysin and chromogranin A (CgA). A subset of samples (n=49) from PRRT treated patients (cohort A) were divided into groups based on SSTR2 expression, and synaptophysin and CgA expression for each sample was assessed. IHC expression of synaptophysin and CgA was consistent among samples, regardless of SSTR2 expression (Figure 1 D)

SSTR2 expression and activity concentration

In PRRT treated patients (cohort A) an estimation of the uptake of radionuclide was done by measuring the activity concentration in 3 tumours per patient (in two patients only 2 tumours were measured). Measurements were possible in 33 patients: 27 with high and 6 with low SSTR2 expressing tumours, respectively. Results showed a large variability between treated patients but consistency among the tumours within patients. There was a tendency towards higher uptake in the Low SSTR2 group but this was not statistically significant *(p=0.06)* (Figure 4 D). There was no correlation between average tumour activity concentration and average Ki-67% in PRRT treated patients (Figure 4 C).

SSTR2 expression and long-term outcome

The patients in cohort A and cohort B were divided into two groups based on SSTR2 expression. Patients who had at least one lesion with a score of 0 or 1 were assigned to the “Low SSTR2” group. The remaining patients, with a score of 2 or 3 in every sample (n=32), were assigned to the “High SSTR2” group. Patient characteristics are presented in table 1 and table 2.

4 patients had lesions with a Ki-67% over 10%, these patients all were assigned to the “High SSTR2” group. When the sample with the highest Ki-67% for each patient was used, patients with low SSTR2 expressing tumours had significantly lower Ki-67% than patients with high SSTR2 expression *(p=0.049).*

Both cohorts A and B were analysed regarding long-term outcome and SSTR2 expression. PRRT treated patients in cohort A included 10 patients in the Low SSTR2 group and 32 patients in the High SSTR2 group. Among non-PRRT treated patients (cohort B) 11 patients had low SSTR2 expressing tumours and 17 had high SSTR2 expressing tumours. Kaplan-Meier curves depicting overall survival (OS) based on SSTR2 expression in these two patient cohorts show that there was no statistically significant difference between the groups *(p = 0.12 for cohort A; p = 0.11 for cohort B)* (Fig 3). Although there was no statistical difference, we observed a trend towards longer OS in the Low SSTR2 group.

SSTR2 expression and treatment patterns

In cohort A all patients received PRRT. After PRRT, patients continued SSA, and were offered additional treatments in case of progression. Eight of the 32 patients (25%) with high SSTR2 expression received additional treatment after initial PRRT. None of the patients (0/10) with low SSTR2 expression received additional treatment during follow-up, Table 3.

Among the non-PRRT treated patients all but two patients, with no evident signs of disease, were treated with SSA after surgery. The main additional treatment was hepatic artery embolization (n=16), followed by interferon-a (n=5) (Table 4).

Discussion

The aims of this study were to investigate if SSTR2 expression could predict outcome after PRRT, and if SSTR2 expression in resected tumours is representative of SSTR2 expression in lesions remaining after surgical resection.

Using a previously constructed TMA consisting of 412 SI-NET patients with grade 1 or 2 we identified a subgroup consisting of 42 patients that had received at least one complete PRRT treatment cycle during 2006–2016 at Sahlgrenska University hospital (cohort A). SSTR2, CgA, Synaptofysin and Ki-67% expression was determined in corresponding tissue samples. Clinical data including overall survival after PRRT and additional treatment after PRRT was collected. A retrospective estimation of ^{177}Lu-DOTATATE uptake in 33 patients was also performed. Our analysis show that 10/42 patients had at least one tissue

sample with a low SSTR2 expression and these patients were grouped into a low SSTR2 expression group. The “Low SSTR2” group had a slightly lower Ki-67%, a tendency towards increased OS and a tendency towards increased SSA uptake. Furthermore, no patients in this group received additional treatment after PRRT during follow up. We therefore conclude that low SSTR2 expression is not suitable as marker to exclude patients from PRRT treatment.

We also identified an additional subgroup consisting of 34 patients were paired samples from primary, lymph node- and liver metastases were present on the TMA (cohort B). SSTR2 expression was determined in corresponding samples. In concordance with other studies we found varying SSTR2 expression in tumour lesions among SI-NET patients [3]. SSTR2 expression did not differ solely based on tumour location. However, when we studied the intra-patient variability, we found that lesions at different locations within a patient had similar SSTR2 expression. We therefore concluded that IHC assessment of SSTR2 expression in resected tumour samples could be representative for the remaining lesions.

There are some methodological considerations to address. Several different methods of scoring for evaluating SSTR2 IHC have been proposed. Körner et al compared SSTR2 expression, using the then newly developed UMB-1 antibody, with SSTR autoradiography and found that immunohistochemical staining of > 10% of tumour cells corresponded to SSTR levels high enough for clinical applications [12]. The use of a scoring system based on both staining intensity and percentage of stained cells have been advocated by some authors [9, 17], while others have emphasized the importance of the subcellular localization [24]. Staining patterns can be influenced by a specific IHC methodology, representing a challenge when comparing results from different studies. We adapted the IRS-score to quantify SSTR2 expression in our samples. In general, our samples had a homogenous staining pattern and all cells within a section exhibited the same staining pattern and we therefore chose to only evaluate staining intensity. A faint SSTR2 staining could be caused by suboptimal tissue fixation with impaired antigen preservation. As the varying expression of synaptophysin and CgA was not associated with SSTR2 expression, we concluded that the variation in SSTR2 expression was not caused by impaired antigen preservation.

All patients had performed a pre-treatment SSTR imaging (^{111}In-octreotide) with only planar scintigraphy images. We therefore chose to assess tumour SSA uptake by SPECT in relation to the first ^{177}Lu-DOTATATE treatment, as this investigation enables direct evaluation of activity concentrations and should correspond well with pre-treatment SSTR imaging [7]. Our data do not show that a low IHC expression of SSTR2 predicts a lesser uptake of SSA, which is concordant with some studies [17] but in contrast to others, where a correlation was found between SSTR2 expression and uptake on SSTR imaging [3, 10, 15]. However, these studies include neuroendocrine tumours from various origins and both low- and high-grade tumours, which might explain this discrepancy.

The present study indicated a trend towards prolonged survival among both PRRT treated and non-PRRT treated patients with low SSTR2 expressing tumours, although there was no statistically significant difference. This is in contrast to other studies, where high SSTR2 expression was associated with

increased OS [11, 16, 17]. In our study the PRRT treated patients in the low SSTR2 group had a significantly lower Ki-67% than the high SSTR2 group due to four patients that had a Ki-67% over 10% Hence, the trend towards a difference in long-term outcome after PRRT could in part be a result of differences in Ki-67%, which is an independent predictor for OS [6, 22]. However, the same trend was seen in cohort B where there was no statistical difference in Ki-67% between groups.

The present study has some limitations. The limited number of patients increases the risk for type 2 errors. Also, the tumour samples that were used where obtained some time before PRRT. In some cases, several years differed between the time of the sample collection and the PRRT treatment. During this time SSTR2 expression could have changed in the remaining lesions. Therefore, data should be interpreted with some caution. However, these long periods of stationary disease are typical of disseminated SI-NET and therefore the results from our study could be applicable in a clinical setting.

The strength of the study lies in the long follow-up, which given the natural course of SI-NETs, is required for evaluating long-term outcome such as survival. Another strength is the homogenous patient cohorts, containing only SI-NET grade 1 or 2, and similar treatments. In the literature, reports evaluating PRRT often include cohorts with tumours of diverse origins and grades, making it more difficult to extrapolate these results [3, 10, 15].

Interestingly, some of the data in the present paper suggest that a low SSTR2 expression could be a positive prognostic marker. Ki-67% was lower in the group of patients with at least one low SSTR2 expressing tumour. This group also had a tendency to increased OS regardless of PRRT treatment and surprisingly a tendency towards higher SSA tumour uptake.

Conclusion

In order to optimise individualized systemic therapy for SI-NETs there is a need for accurate prognostic markers for SI-NET and predictive markers for PRRT. Altogether, the results from present study suggest that low SSTR2 expression should not exclude patients from PRRT and that low SSTR2 expression might be a prognostic marker in low grade SI-NET.

Declarations

Ethics approval: This article does not contain any studies with human participants performed by any of the authors.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Author contributions:

Study conception and design: AKE, VJ, EE, JS, PB

Acquisition of data: AKE, EE, VJ, JS, PB

Analysis and interpretation of data AKE, EE, VJ, AB, IM, ON

Drafting of manuscript AKE, EE, VJ

Critical revision of manuscript AKE, EE, BW, ON, PB, JS

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Tables

Table 1

Clinical characteristics of patients in cohort A

	All Patients	High SSTR2 [a]	Low SSTR2 [b]	
Patients (*n*)	42	32	10	
Age at PRRT treatment, mean (range)	66 (45-78)	68 (48-77)	65 (45-78)	*p=ns*
Male/female (*n*)	19/23	15/17	4/6	*p=ns*
Number of PRRT treatments, mean (range)	3,8 (2-6)	3,8 (2-6)	3,7 (2-6)	*p=ns*
Ki-67, median (%; range)	1.87	2.18 (0.25-15.2)	1.87 (0.62-3.1)	*p=0,049*

[a] SSTR2 score 2 or 3 in all lesions

[b] SSTR2 score 0 or 1 in $\geq$1 lesion

Table 2

Clinical characteristics of non-PRRT treated patients in cohort B

	All Patients	High SSTR2 [a]	Low SSTR2 [b]	
Patients (*n*)	28	17	11	
Age at tissue sampling mean (range)	66 (37-84)	68 (40-84)	63 (37-80)	*p=ns*
Male/female (*n*)	16/12	12/5	4/7	*p=ns*
Ki-67, median (%; range)	1	1 (0.4-19)	1 (0.5-10)	*p=ns*

[a] SSTR2 score 2 or 3 in all lesions

[b] SSTR2 score 0 or 1 in ≥1 lesion

Table 3

Treatments after PRRT in cohort A

	All Patients	High SSTR2 [a]	Low SSTR2 [b]
Number of patients	42	32	10
Additional PRRT and/or HAE and/or external radiation	8	8	0
No additional treatment	34	24	10

[a] SSTR2 score 2 or 3 in all lesions

[b] SSTR2 score 0 or 1 in ≥1 lesion

Table 4

Treatment patterns in cohort B

	All Patients	High SSTR2 [a]	Low SSTR2 [b]
Number of patients	28	17	11
Interferon-	5	3	2
HAE / RF / SIRT	15/2/1	8/0/1	7/2/0
No additional treatment	6	5	1

[a] SSTR2 score 2 or 3 in all lesions

[b] SSTR2 score 0 or 1 in ≥ lesions

Figures

Figure 1

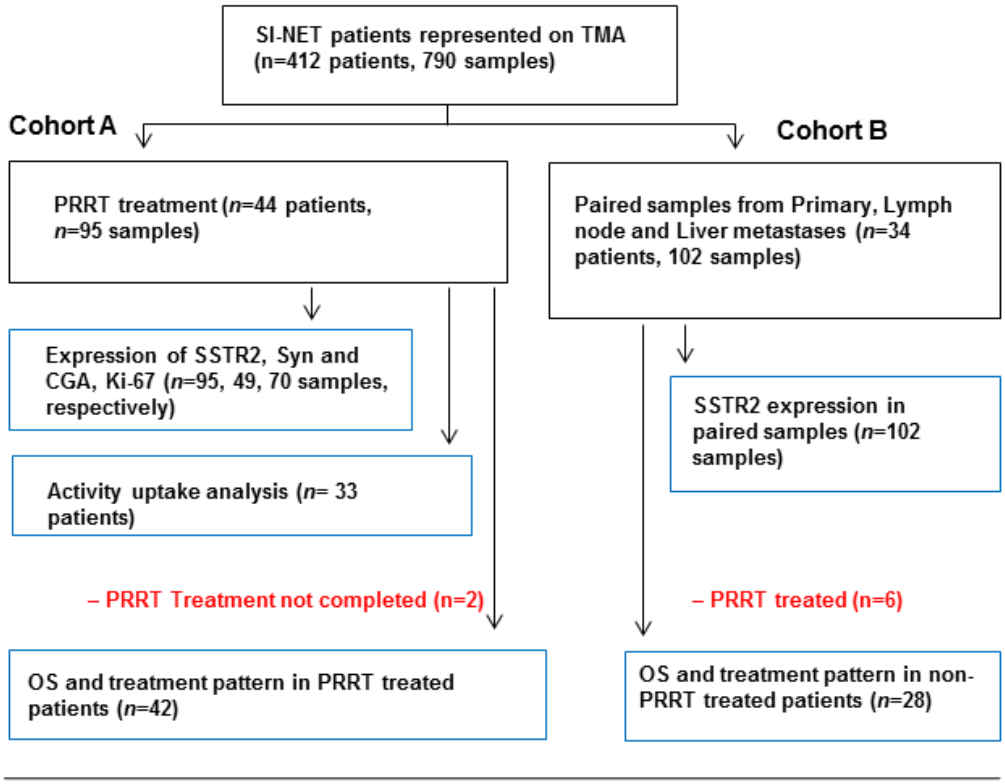

Figure 1

Flowchart describing identification of patient cohorts and corresponding analysis.

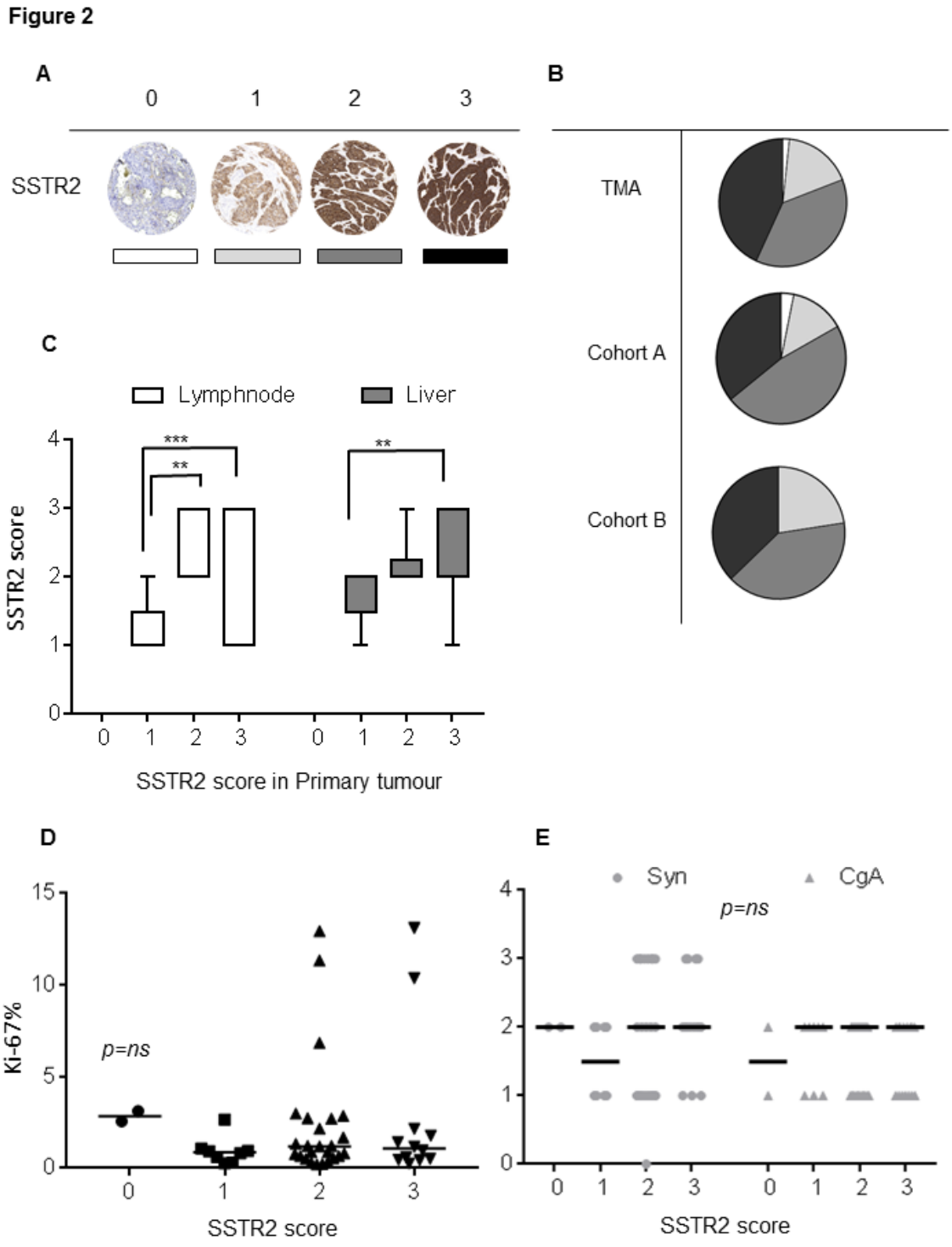

Figure 2

a A representative image of SSTR2 scoring. b SSTR2 score in tumour samples is similarly distributed in TMA (n=790, Cohort A (n=95 and Cohort B (n=102). c Samples from cohort B consisting of primary tumour, lymph node metastases and liver metastases from each patient were analysed (102 samples from 34 patients). When samples from metastases were sorted according to the SSTR2 score in

corresponding primary tumour a consistency between primary tumours and metastases was observed. The figure illustrates a “Box and whiskers” plot, box shows 25th to 75th percentiles, whiskers indicate range and line shows median. SSTR2 expression is significantly different in metastases when sorted according to SSTR2 expression in the primary tumours (*=p<0.05, **=p< 0.01, ***=p<0.001). d Samples (n=70) from patients in cohort A were grouped according to SSTR2 score and Ki-67% was assessed in full section slides corresponding to the TMA core biopsy. There was a tendency towards a higher Ki-67% in samples with a higher SSTR2 score although it was not significant, (p=ns). e A subset of samples (n=49) from cohort A, were immunohistochemically stained for SI-NET tumour markers chromogranin A and synaptophysin. Staining intensity was consistent among samples regardless of SSTR2 expression level. Line show median, (p=ns).

Figure 3

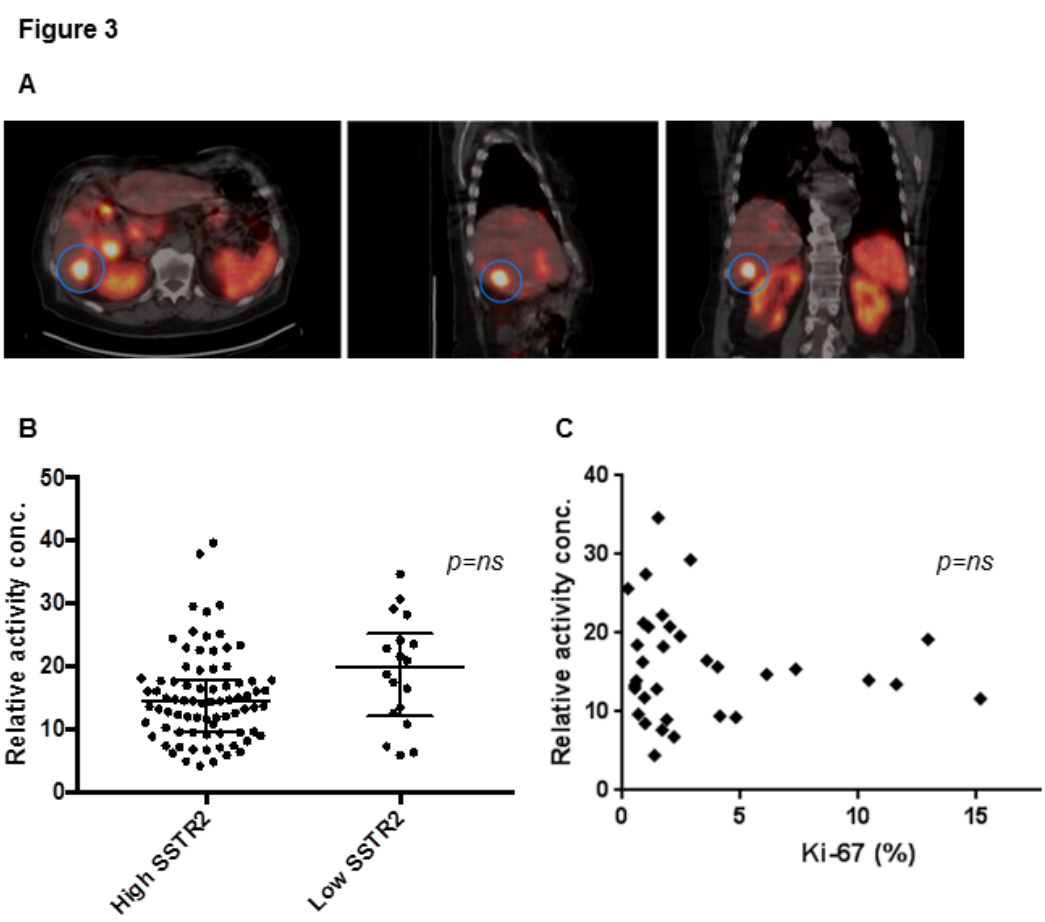

Figure 3

a Representative planary gammakamera image. b Representative image illustrating relative activity concentration at SPECT 24h after PRRT in a single lesion. c Mean relative activity concentration in relation to mean Ki-67% in PRRT treated patients (n=33, p=ns.) d Relative activity concentration at SPECT 24h after PRRT was estimated in tumours in 33 patients in cohort A: 27 with high and 6 with low SSTR2

expressing tumours, respectively. 2-3 tumours were measured in each patient. No significant difference was seen between groups (p=0.06, Mann-Whitney). Bars show median values and 95% CI.

Figure 4

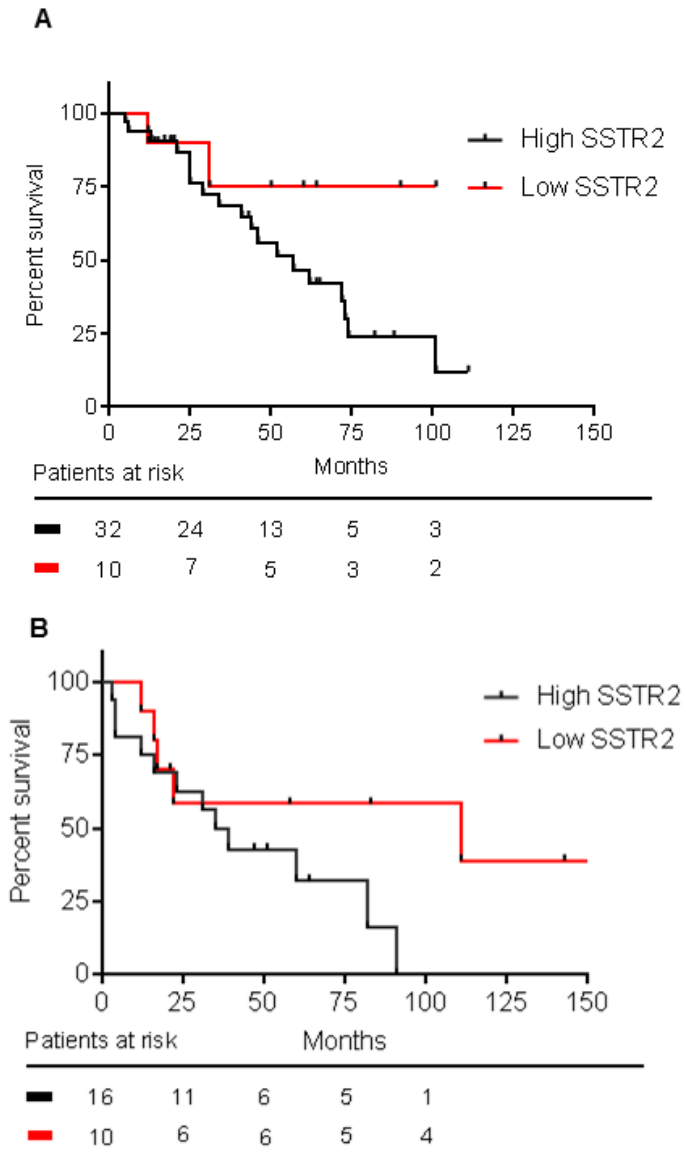

Figure 4

a OS analysis of PRRT- treated patients (Cohort A) grouped according to SSTR2 expression (Low SSTR2=10, High SSTR2 =32. Two patients (one from each group) were excluded due to progressive disease, which precluded them from completing PRRT treatment. No significant difference was seen

between high and low SSTR2, (p=0.12). b OS analysis of non-PRRT treated patients from Cohort B (Low SSTR2=11, High SSTR2=17). No significant difference was seen between high and low SSTR2, (p=0.11).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- IHCstainingsofSSTR2andscoring.pptx