

The prognostic and predictive value of sstr₂-immunohistochemistry and sstr₂-targeted imaging in neuroendocrine tumors

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Abstract

Purpose Our aim was to assess the prognostic and predictive value of somatostatin receptor 2 (sstr₂) in neuroendocrine tumors (NETs).

Methods We established a tissue microarray and imaging database from NET patients that received sstr₂-targeted radiopeptide therapy with yttrium-90-DOTATOC, lutetium-177-DOTATOC or alternative treatment. We used univariate and multivariate analyses to identify prognostic and predictive markers for overall survival, including sstr₂-imaging and sstr₂-immunohistochemistry.

Results We included a total of 279 patients. In these patients, sstr₂-immunohistochemistry was an independent prognostic marker for overall survival (HR: 0.82, 95 % CI: 0.67 – 0.99,

$n = 279, p = 0.037$). In DOTATOC patients, sstr₂-expression on immunohistochemistry correlated with tumor uptake on sstr₂-imaging ($n = 170, p < 0.001$); however, sstr₂-imaging showed a higher prognostic accuracy (positive predictive value: +27 %, 95 % CI: 3 – 56 %, $p = 0.025$). Sstr₂-expression did not predict a benefit of DOTATOC over alternative treatment ($p = 0.93$).

Conclusions Our results suggest sstr₂ as an independent prognostic marker in NETs. Sstr₂-immunohistochemistry correlates with sstr₂-imaging; however, sstr₂-imaging is more accurate for determining the individual prognosis.

Keywords PRRT · Targeted therapy · Metabolic therapy · Tailored therapy · Individualized therapy

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Introduction

Prognostic markers offer insight into the biology and natural course of a disease. They provide useful information about the aggressiveness of the disease and the risk of recurrence or death. Predictive markers, on the other hand, indicate a benefit of a certain therapy over alternative treatments, and allow physicians to tailor therapeutic interventions to achieve the most favorable outcome.

Such favorable outcomes have been found after somatostatin receptor 2 (sstr₂)-targeted radiopeptide therapy in patients with neuroendocrine tumors (NETs) that express high levels of sstr₂ [1]. However, it is unclear whether these outcomes reflect treatment effects or favorable prognosis. In other words, is high sstr₂-expression a predictive marker for successful sstr₂-targeted therapy or a prognostic marker for a benign course of disease?

To answer this question, and to provide a framework for improved patient management and meaningful interpretation of treatment effects in NETs, we investigated the prognostic and predictive value of *sstr*₂-imaging and *sstr*₂-immunohistochemistry.

Methods

Patients

We included patients with histologically confirmed NETs who had been enrolled in a prospective trial investigating the benefit of treatment with radiolabeled DOTATOC (tetraazacyclododecane-tetraacetic acid modified somatostatin analog Tyr³-octreotide). This study was designed and carried out according to good clinical practice, Swiss drug laws, and the Declaration of Helsinki. Its protocol was registered (ClinicalTrials.gov identifier: NCT00978211) and approved by the Basel ethics committee for human studies (#120/1997). Written informed consent was obtained from all participants or their legal representatives. Patients were enrolled and treated with yttrium-90-DOTATOC and lutetium-177-DOTATOC at the University Hospital Basel, Switzerland as previously described [2–4].

To assess the prognostic value of *sstr*₂ in a broad patient spectrum, we also included patients with histologically confirmed pancreatic NETs who had not been enrolled and had been systematically investigated at the University Hospital Bern, Switzerland as previously described [5]. Tumor samples were collected at the study center and the main referring centers. Tissue collection and analyses were approved by the Basel ethics committee for human studies (#17/2010) and the Bern ethics committee for human studies (#200/2014).

Follow-up data were obtained from referring physicians, the patients' primary practitioners, or directly from the patient. All follow-up data were centrally collected and each case was reviewed and approved for completeness at the study center. Data were collected until the patient's death, including survival and long-term toxicities.

*Sstr*₂-imaging

*Sstr*₂-imaging was performed in DOTATOC patients as previously described [6, 7]. Specifically, intra-therapeutic DOTA TOC accumulation in the lesion with the highest uptake among all visible tumor lesions was scored by three nuclear medicine physicians, blinded to the patient's baseline and follow-up data, and based on the following four-point scale: no uptake (score 0), or uptake lower (score 1), equal to (score 2), or higher than liver uptake (score 3). Cases of disagreement were re-assessed and scored by consensus.

*Sstr*₂-immunohistochemistry

Two to three NET tissue cores per patient were formalin-fixed and paraffin-embedded in a tissue microarray format. Subsequently, *sstr*₂-staining was performed using the UMB-1 antibody (Biotrend, Cologne, Germany) [8].

*Sstr*₂-expression was scored by two pathologists blinded to patient data using a four-point scale: absent staining (score 0), faint staining at 100x magnification (score 1), strong staining at 100x magnification not involving the entire circumference at 400x magnification (score 2), and strong staining at 100x magnification involving the entire circumference at 400x magnification (score 3). Only membranous *sstr*₂-staining was evaluated, and the highest score of the tissue cores for each patient was used. Cases of disagreement were re-assessed and scored by consensus.

Ki-67-staining was performed using the MIB1-clone antibody (Dako, Carpinteria, CA, USA) [8]. Subsequently, tumors were graded using Ki-67-expression according to the 2010 World Health Organization (WHO) guidelines [9].

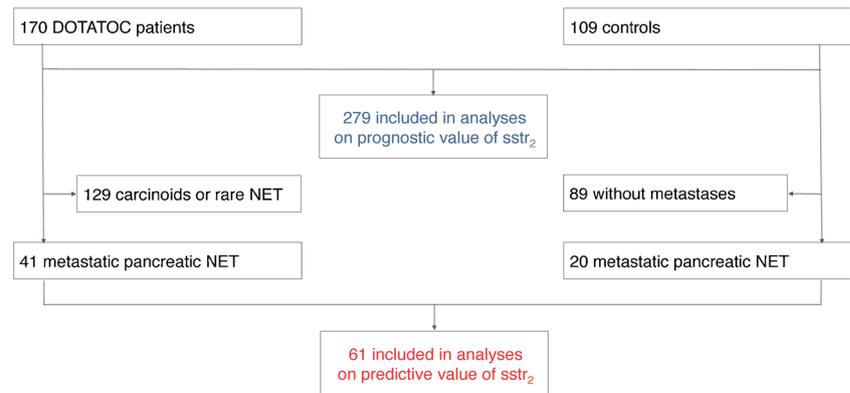
Statistical Analyses

Our *primary hypothesis* was an independent prognostic value of *sstr*₂ in NETs. The association of *sstr*₂-immunohistochemistry (score 0 vs. 1 vs. 2 vs. 3) and survival was examined by a univariate generalized Wilcoxon test and multivariate Cox regression. Cox regression was performed with survival from time of diagnosis as a dependent variable. Independent covariables were age (per year), histology (carcinoid vs. pancreatic NET vs. rare NET), disease extent (metastases vs. no metastases), and Ki-67 expression or tumor grade (*G*₁ vs. *G*₂ vs. *G*₃).

Associations of *sstr*₂-immunohistochemistry with *sstr*₂-imaging and tumor grade were analyzed using Pearson's χ^2 test. The prognostic value of positive *sstr*₂-imaging (score > 2) and positive *sstr*₂-immunohistochemistry (score \geq 2) were compared as described before [10].

Our *secondary hypothesis* was an independent predictive value of *sstr*₂ for a benefit of DOTATOC over alternative treatment. The predictive value was tested using Cox regression with an interaction test for *sstr*₂-immunohistochemistry and DOTATOC treatment. As all DOTATOC patients had metastasized disease and all controls had pancreatic NETs, the predictive value of *sstr*₂ was tested in metastasized pancreatic NETs receiving DOTATOC or alternative treatment.

We had previously found a benefit of yttrium-90-DOTATOC plus lutetium-177-DOTATOC over yttrium-90-DOTATOC alone, without adjusting for tumor grade and *sstr*₂-expression [3]. Thus, we repeated this analysis additionally adjusting for both factors, and assessed the frequency of renal toxicity after DOTATOC taking into account the competing risk of death.

Fig. 1 Patient flow

Effect estimates were expressed as hazard ratios (HRs) with 95 % confidence intervals (CIs). Two-sided p values of < 0.05 were considered statistically significant. Results were reported according to the *reporting recommendations for tumor marker prognostic studies* (REMARK) [11].

Results

Patients

Between October 1997 and February 2010, 170 patients with available tumor samples were enrolled for DOTATOC

(Fig. 1). Treatment prior to DOTATOC included surgery in 140 patients (82.4 %), chemotherapy in 27 patients (15.9 %), and radiation therapy in 16 patients (9.4 %). In addition, 109 controls were included. All of them received surgery; those with metastasized disease additionally received somatostatin analogs (33.9 %), chemotherapy (21.1 %), selective arterial chemo-embolization (0.9 %), and radiation therapy (0.9 %). Patients' characteristics are shown in Table 1.

The 279 NETs comprised 150 (53.8 %) pancreatic NETs, 84 (30.1 %) carcinoids, and 45 (16.1 %) rare NETs. The 84 carcinoids comprised 60 (71.4 %) from the small intestine, 7 (8.3 %) from the large intestine, 1 from the stomach (1.2 %), 9 from the bronchus (10.7 %), 3 (3.6 %) from the thymus, and 4 (4.8 %)

Table 1 Patient Characteristics

Characteristic		All patients ($n = 279$)	DOTATOC patients ($n = 170$)	Controls ($n = 109$)
Gender	<i>Females</i>	118 (42.3 %)	68 (40.0 %)	50 (45.9 %)
	<i>Males</i>	161 (57.7 %)	102 (60.0 %)	59 (54.1 %)
Age (y)	<i>Median</i>	59.6	59.5	59.7
	<i>Range</i>	16.9 - 95.1	16.9 - 81.4	26.4 - 95.1
Histology	<i>Pancreatic NET</i>	150 (53.8 %)	41 (24.1 %)	109 (100 %)
	<i>Carcinoid</i>	84 (30.1 %)	84 (49.4 %)	0 (0 %)
	<i>Rare NET</i>	45 (16.1 %)	45 (26.5 %)	0 (0 %)
Extent	<i>No metastases</i>	57 (20.4 %)	0 (0 %)	57 (52.3 %)
	<i>Metastases</i>	222 (79.6 %)	170 (100 %)	52 (47.7 %)
Grade	<i>G₁</i>	175 (62.7 %)	109 (64.1 %)	66 (60.6 %)
	<i>G₂</i>	74 (26.5 %)	43 (25.3 %)	31 (28.4 %)
	<i>G₃</i>	26 (9.3 %)	15 (8.8 %)	11 (10.1 %)
	<i>Unknown</i>	4 (1.4 %)	3 (1.8 %)	1 (0.9 %)
Ki ₆₇ expression	<i>Median</i>	1.4 %	1.2 %	1.8 %
	<i>Range</i>	0 - 95.8 %	0 % - 85.0 %	0 - 95.8 %
Sstr ₂ expression	<i>Score 0</i>	72 (25.8 %)	45 (26.5 %)	27 (24.8 %)
	<i>Score 1</i>	49 (17.6 %)	38 (22.4 %)	11 (10.1 %)
	<i>Score 2</i>	57 (20.4 %)	39 (22.9 %)	18 (16.5 %)
	<i>Score 3</i>	63 (22.6 %)	47 (27.6 %)	16 (14.7 %)
	<i>Unknown</i>	38 (13.6 %)	1 (0.8 %)	37 (33.9 %)

from an unknown primary. The 45 rare NETs comprised 9 (20.0 %) medullary thyroid cancers, 7 (15.6 %) Merkel cell cancers, 6 (13.3 %) paraganglioma, 1 (2.2 %) pheochromocytoma, 1 (2.2 %) NET of the kidney, 1 (2.2 %) NET of the prostate, and 20 (44.4 %) NETs of unknown primary.

The 279 tumor tissues had a median Ki-67-expression of 1.4 (range: 0–95.8, Table 1). Sstr₂-immunohistochemistry was feasible in 241 cases (86.4 %). Staining was homogenous through all tissue cores in 202 cases (83.8 %) and heterogeneous in 39 cases (16.2 %). The assessment of sstr₂ expression by two pathologists showed a high concordance. Scoring slightly diverged in 8 of 241 cases (3.3 %), with one point difference in scoring, and the final score was assigned by consensus. Low tumor grades were correlated with high scores on sstr₂-immunohistochemistry ($n = 279, p = 0.012$, Pearson's X^2 test).

Sstr₂-immunohistochemistry as prognostic marker in NETs

Patients with score 3 tumor sstr₂-immunohistochemistry had a longer survival from diagnosis than patients with score 2, 1, or 0 (164.2 vs. 140.2 vs. 107.6 vs. 127.8 months; HR: 0.82, 95 % CI: 0.67–0.99; $n = 240, p = 0.037$, multivariate Cox regression, Fig. 2a, Table 2). Similar results were found when adjusting for the actual Ki-67 expression instead of the tumor grade, or when using univariate analysis ($n = 240; p = 0.024$, generalized Wilcoxon test).

Additionally, carcinoid patients survived longer than patients with rare NET (184.9 vs. 82.6 months; HR: 0.37, 95 % CI: 0.21–0.63; $n = 129; p < 0.001$, Cox regression, Fig. 2b), as did patients with pancreatic NETs (164.0 vs. 82.6 months; HR: 0.40, 95 % CI: 0.23–0.69; $n = 195; p = 0.001$, Cox

regression, Fig. 2b). Patients with G₁ tumors survived longer than patients with G₂ or G₃ tumors (185.6 vs. 99.8 vs. 19.1 months; HR: 0.32, 95 % CI: 0.23–0.4; $n = 240; p < 0.001$, Cox regression, Fig. 2c).

Sstr₂-imaging vs. sstr₂-immunohistochemistry

Tumor uptake on sstr₂-imaging correlated with sstr₂-expression on immunohistochemistry ($n = 170, p < 0.001$, Pearson's X^2 test, Fig. 3a).

Sstr₂-imaging had higher accuracy than sstr₂-immunohistochemistry as prognostic marker for survival after DOTATOC (PPV (t): 27 % higher, 95 % CI: 3–56 %; $n = 170, p = 0.025$; NPV (t): 32 % higher, 95 % CI: –21–121 %; $n = 170, p = 0.28$). The positive and negative prognostic values of sstr₂-imaging were higher alone, compared with combining the results from sstr₂-imaging and sstr₂-immunohistochemistry (Fig. 3b).

Sstr₂ as predictive marker for sstr₂-targeted radiopeptide therapy

A total of 61 patients with metastatic pancreatic NETs were treated with DOTATOC ($n = 41$) or alternative treatment ($n = 20$), and had a median survival of 129.9 months from diagnosis.

There was a non-significant trend towards longer survival after DOTATOC vs. alternative treatment (142.5 vs. 95.5 months; HR: 0.70, 95 % CI: 0.29–1.71; $n = 61, p = 0.43$, Cox regression, Fig. 4a). In tumors with sstr₂-expression (scores 1, 2 and 3), this trend was pronounced (139.2 vs. 73.9 months; HR: 0.61, 95 % CI: 0.22–

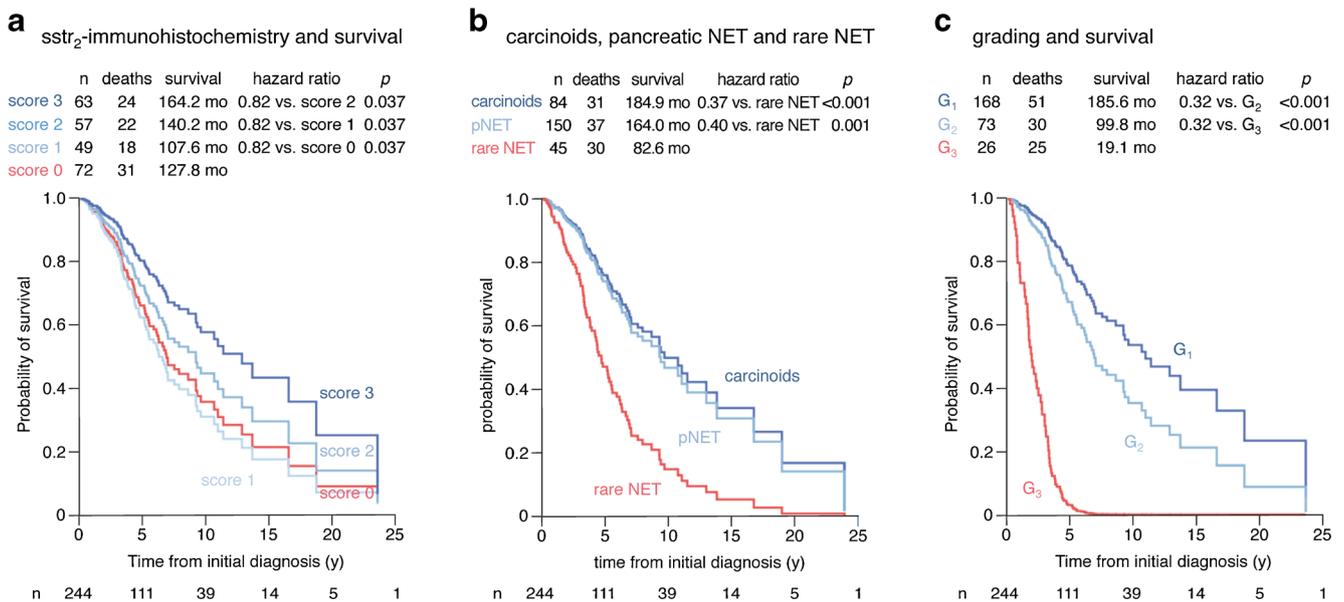


Fig. 2 Sstr₂-immunohistochemistry as prognostic marker in NETs. Cox regression plots demonstrating the survival of NET patients by tumor sstr₂-immunohistochemistry (a), tumor type (b) and tumor grade (c)

Table 2 Prognostic Factors in NETs

Survival from diagnosis (n = 279)			Hazard ratio (95%CI) *	p
Carcinoid	(vs. rare NET)		0.37 (0.21 - 0.63)	< 0.001
Pancreatic NET	(vs. rare NET)		0.40 (0.23 - 0.69)	0.001
Sstr ₂ expression	(per score)		0.82 (0.67 - 0.99)	0.037
Age	(per year)		1.03 (1.01 - 1.05)	0.005
Metastases	(vs. no metastases)		1.90 (0.93 - 3.88)	0.078
Tumor grade	(per grade)		3.15 (2.24 - 4.44)	< 0.001
Survival from DOTATOC (n = 170)			Hazard ratio (95%CI) *	p
Y- plus Lu-DOTATOC	(vs. Y-DOTATOC)		0.43 (0.25 - 0.74)	0.02
Y- plus Lu-DOTATOC	(vs. Lu-DOTATOC)		0.71 (0.28 - 1.85)	0.48
Sstr ₂ expression	(per score)		0.80 (0.65 - 0.98)	0.031
Age	(per year)		1.01 (0.99 - 1.02)	0.42
Tumor grade	(per grade)		2.70 (1.91 - 3.82)	< 0.001

1.67; $n = 50$, $p = 0.33$, Cox regression, Fig. 4b). Conversely, in tumors without sstr₂-expression (score 0), no difference between survivals in the two groups was observed (103.3 vs. 113.7; HR: 1.01, 95 % CI: 0.10–10.5, $n = 11$, $p = 1.0$, Cox regression, Fig. 4c).

The interaction test did not indicate a predictive value of sstr₂; the benefit of DOTATOC over alternative treatment was not significantly different in sstr₂-positive vs. sstr₂-negative cases ($p = 0.93$).

Discussion

Our results suggest sstr₂-immunohistochemistry as a prognostic marker in NETs that is independent from tumor histology, grade, stage, and patient age. While

sstr₂-immunohistochemistry correlates with sstr₂-imaging, sstr₂-imaging has a higher prognostic accuracy. The superior prognostic accuracy might be due to the fact that sstr₂-imaging provides a whole-body read-out, while sstr₂-immunohistochemistry can be associated with a significant sampling error. The results did not confirm sstr₂-expression as a predictive marker for sstr₂-targeted radioreptide therapy, and suggest a trend towards longer survival with sstr₂-targeted radioreptide therapy in sstr₂-positive cases.

Strengths and limitations

Strengths of the present study include the combination of tissue markers, molecular imaging, and long-term follow-up in a wide spectrum of NET patients. The tissue microarray format

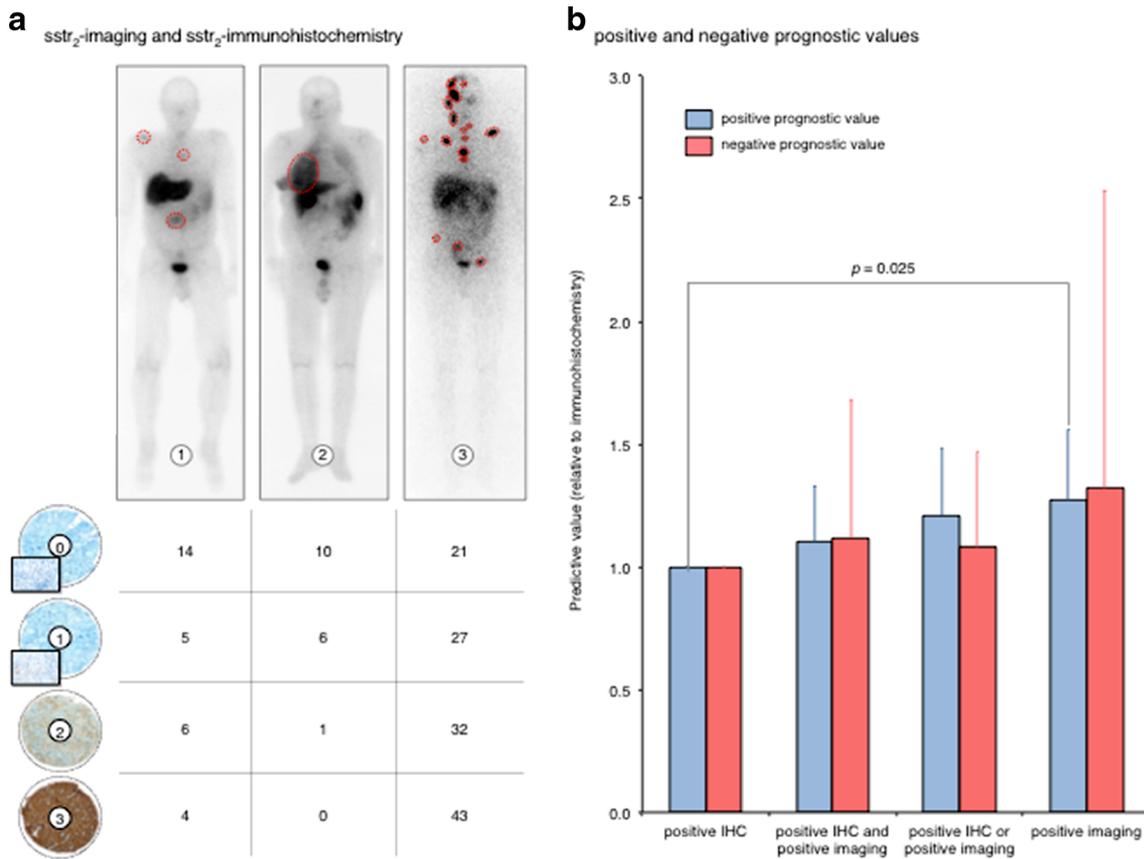


Fig. 3 $Sstr_2$ -imaging vs. $sstr_2$ -immunohistochemistry. Distribution of $sstr_2$ -immunohistochemistry results (score 0, 1, 2, and 3) and $sstr_2$ -imaging results (score 1, 2, and 3; **a**). Positive and negative prognostic

values for survival after DOTATOC of positive $sstr_2$ -immunohistochemistry (score ≥ 2), positive $sstr_2$ -imaging (score > 2) and their combination (**b**, IHC = immunohistochemistry)

facilitated standardized marker analyses, the large number of patients and the completeness of follow-up facilitated comprehensive survival analyses, and the spectrum of tumor

histologies, grades, and stages ensures general applicability of the present results. However, the present study was not a randomized trial, and has the known limitations of a non-

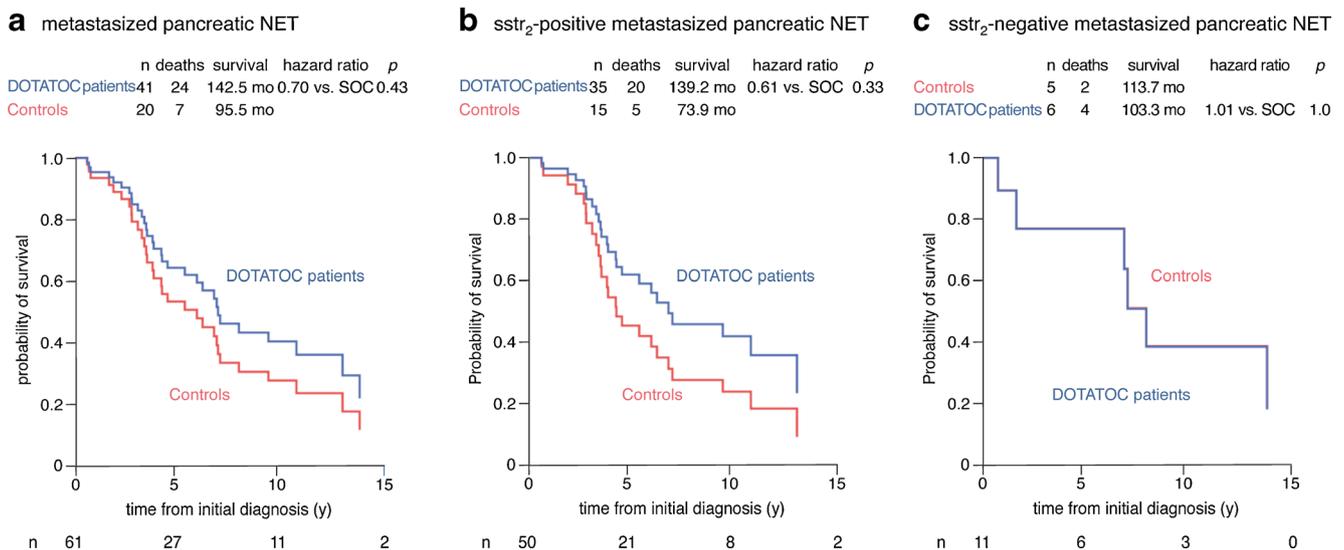


Fig. 4 $Sstr_2$ as predictive marker for $sstr_2$ -targeted radiopeptide therapy. Cox regression plots demonstrating the survival of DOTATOC patients and controls, for all patients (**a**), patients with positive $sstr_2$ -

immunohistochemistry (**b**), and patients with negative $sstr_2$ -

randomized study. To assess the prognostic value of $sstr_2$ in a spectrum of NET patients, we included those that did or did not qualify for DOTATOC treatment. To assess the predictive value of $sstr_2$, we subsequently matched patients from these groups by tumor type (pancreatic NET) and tumor extent (metastasized disease). However, the analysis on the predictive value of $sstr_2$ was limited by the low number of patients with metastasized pancreatic NETs, and the comparison of imaging and immunohistochemistry was limited to DOTATOC patients.

Comparison to other studies

The increasing availability of targeted therapies is generating an increasing demand for modalities that reliably assess target expression, its predictive value, and individual prognosis. Among such modalities, immunohistochemistry allows detailed assessment of specific tissue samples, while imaging allows a less detailed overview over the entire body. Studies that compare the accuracy of both modalities for clinically relevant molecular targets will be increasingly warranted in the future.

To the best of our knowledge, this is the first study to assess the prognostic value of $sstr_2$ -immunohistochemistry in a general NET cohort adjusted for tumor grade and type. Our results concur with other studies, in which $sstr_2$ -immunohistochemistry was found to be prognostic in patients with carcinoids [12] and $sstr_2$ -imaging was shown to be prognostic in NET patients [13], although analyses were not adjusted for important prognostic factors like tumor grade in the latter study. Furthermore, the present study is the first to compare the prognostic value of $sstr_2$ -imaging vs. $sstr_2$ -immunohistochemistry. Its results support the current clinical practice of favoring $sstr_2$ -imaging.

Our results also show a trend towards longer overall survival after $sstr_2$ -targeted radiopeptide therapy over alternative treatment, which is in line with the overall survival benefit of $sstr_2$ -targeted radiopeptide therapy over somatostatin analogue treatment found in the preliminary analysis of the ongoing NETTER-1 trial [14].

Finally, previous studies of NETs with unknown $sstr_2$ -expression and tumor grade indicated superiority of $sstr_2$ -targeted radiopeptide therapy with combined radioisotopes over single radioisotopes [3, 15]. This superiority might be due to lutetium-177 being more effective in irradiating small tumor lesions and yttrium-90 being more effective in irradiating large tumor lesions. The present study confirmed these results, when adjusting for $sstr_2$ -expression and tumor grade.

Implications

Our results have implications for patient management and further research. Prognostic markers have particular value

for tumors with a wide spectrum of courses, such as NETs [11]. Based on our results, $sstr_2$ can serve as prognostic marker and decision aid in determining whether a NET patient should receive treatment and how aggressive the treatment should be.

Furthermore, immunohistochemistry and imaging can be used to assess tumor $sstr_2$ -expression. However, our results suggest that, in cases of discrepancies, imaging is more accurate for determining the patient's individual prognosis.

Finally, studies that stratify patients according to $sstr_2$ -expression, e.g. surgical resection of NETs visible in $sstr_2$ -imaging, are prone to bias favoring interventions in $sstr_2$ -positive cases, and need to be interpreted cautiously. Moreover, upcoming studies to evaluate prognostic factors in NETs should take $sstr_2$ -expression into account for all analyses.

Conclusions

Our results suggest $sstr_2$ as an independent prognostic marker in NETs. $sstr_2$ -immunohistochemistry correlates with $sstr_2$ -imaging; however, $sstr_2$ -imaging is more accurate for determining the individual prognosis. Studies exploring prognostic factors in NETs should include $sstr_2$ -expression in all analyses.

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Compliance with Ethical Standards The authors declare no potential conflicts of interest. All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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