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***Ex vivo* activity of cytotoxic drugs and targeted agents in small intestinal neuroendocrine tumors**

Kosmas Daskalakis¹, Olov Norlén¹, Andreas Karakatsanis¹, Per Hellman¹, Rolf Larsson², Peter Nygren³ and Peter Stålberg¹

¹Department of Surgical Sciences, Uppsala University, Uppsala, Sweden

²Department of Medical Sciences, Uppsala University, Uppsala, Sweden

³Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

Correspondence should be addressed to K Daskalakis: kosmas.daskalakis@surgsci.uu.se

Abstract

Small intestinal neuroendocrine tumors (SI-NETs) are generally considered resistant to systemic treatment. To date, predictive markers for drug activity are lacking. Tumor samples from 27 patients with SI-NETs were analyzed *ex vivo* for sensitivity to a panel of cytotoxic drugs and targeted agents using a short-term total cell kill assay. Samples of renal cancer, colorectal cancer (CRC), ovarian cancer and chronic lymphocytic leukemia (CLL) were included for comparison. For the SI-NET subset, drug sensitivity was analyzed in relation to clinicopathological variables and pre-treatment biomarkers. For cytotoxic drugs, SI-NETs demonstrated similar or higher sensitivity to 5-FU, platinum, gemcitabine and doxorubicin compared with CRC. For several of the targeted kinase inhibitors, SI-NET was among the most sensitive solid tumor types. CLL and ovarian cancer were generally the most sensitive tumor types to both cytotoxic drugs and protein kinase inhibitors. SI-NET was more sensitive to the mTOR inhibitor sirolimus than the other solid tumor types tested. Individual SI-NET samples demonstrated great variability in *ex vivo* sensitivity for most drugs. Cross-resistance between different drugs also varied considerably, being higher among protein kinase inhibitors. Age, stage, grade, peritoneal carcinomatosis and extra-abdominal metastases as well as serum chromogranin A and urine 5-HIAA concentrations at diagnosis did not correlate to drug sensitivity *ex vivo*. SI-NETs exhibit intermediate sensitivity *ex vivo* to cytotoxic and targeted drugs. Clinicopathological factors and currently used biomarkers are not clearly associated to *ex vivo* sensitivity, challenging these criteria for treatment decisions in SI-NET. The great variability in drug sensitivity calls for individualized selection of therapy.

Key Words

- ▶ *ex vivo* activity
- ▶ cytotoxic drugs
- ▶ targeted agents
- ▶ small intestinal neuroendocrine tumor

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Introduction

Small intestinal neuroendocrine tumors (SI-NETs) are indolent, low-proliferative and well-differentiated neoplasms. They are often diagnosed at a late stage and have then a 5-year survival rate of 58% (Norlen *et al.*

2012). However, variable outcomes are hidden within this relative favorable prognosis.

While locoregional surgery followed by active surveillance remains the mainstay approach for the

treatment of localized SI-NETs, the management of patients with metastatic disease involves a multimodality treatment approach, combining targeted and systemic approaches with the aims to control hormone production and tumor progression (Niederle *et al.* 2016).

In contrast to pancreatic NETs and neuroendocrine carcinomas (NECs), SI-NETs are generally considered resistant to systemic cytotoxic drugs with objective response rates in the range of 10–30% (Sun *et al.* 2005, Faure *et al.* 2017). Low proliferation in SI-NETs as well as overexpression of the DNA repair enzyme methylguanine-methyltransferase (MGMT) may contribute to chemotherapy resistance (Kulke *et al.* 2009).

Total cell kill assays that measure early cell damage in the tumor cell population correlate well with clinical treatment outcomes and are technically robust with high evaluability rates (Bosanquet 1991, Blom *et al.* 2017). Sensitivity of approximately 90% and specificity of 70% for predicting response and resistance to treatment has been reported (Larsson & Nygren 1993, Blom *et al.* 2016), indicating that such assays can be used to investigate clinically relevant tumor cell drug sensitivity.

Overall, the experimental and clinical experience on sensitivity of SI-NET to various cancer drugs is limited, leaving a poor basis for treatment decision making in the clinic for advanced SI-NET. The aims of this study were, therefore, to assess sensitivity to a broad panel of cytotoxic drugs and more recently introduced targeted drugs in SI-NETs and to investigate whether established prognostic factors for survival and currently used biomarkers for this tumor type are associated with drug sensitivity.

Materials and methods

Tumor sampling and cell preparation

Tumor tissue sampling in different patients with SI-NET, colorectal cancer (CRC), ovarian cancer and renal cancer was performed intra-operatively during resective surgery for primary or metastatic disease. Sampling sites for SI-NETs were primary tumors as well as regional mesenteric lymph node metastases. Chronic lymphocytic leukemia (CLL) sampling was performed by vein puncture at routine blood sampling. Sampling and data collection were based on patient informed consent as approved by the regional ethical committee in Uppsala (Dnr 2007/237 and Dnr 2011/375/1). Due to shortage of cells, all samples were not tested for all drugs.

Tumor cells from solid tumors were prepared by collagenase digestion (Csoka *et al.* 1997), whereas CLL cells

were collected by Ficoll-Hypaque gradient centrifugation (Pharmacia) (Frost *et al.* 2003). Cells obtained from solid tumors were mostly single cells or small cell clusters with $\geq 90\%$ viability and less than 30% contamination from non-malignant cells, as classified after morphological examinations of May-Grünwald-Giemsa-stained cytocentrifuge preparations.

For subgroup analysis of samples from SI-NETs, data on the presence or absence of peritoneal carcinomatosis and extra-abdominal metastasis, tumor grade, disease stage, age as well as pre-treatment s-CgA and u-5HIAA concentrations were collected from the patient charts. For grading and staging of the SI-NETs included, the WHO 2017 classification system was used (Lloyd *et al.* 2017).

Drug selection and measurement of drug sensitivity *ex vivo*

A panel of cytotoxic drugs, commonly used in solid tumors and also tried in NETs, as well as a broad panel of established and more recently introduced tyrosine kinase inhibitors were tested in the SI-NET samples. For comparison and to obtain clinical references, samples from CRC, ovarian cancer, renal cancer and CLL were tested for the same drugs. Temozolomide, an alkylating pro-drug agent used in NETs (Fine *et al.* 2013, Crespo *et al.* 2017), produced very little cytotoxicity *ex vivo* and is, therefore, not included in the data presentation. Additionally, the mTOR inhibitor sirolimus, precursor to the rapalogue everolimus (Yao *et al.* 2011, 2016), the multi-kinase inhibitor sunitinib, approved in pancreatic NETs (Raymond *et al.* 2011), and the somatostatin analogue (SSA) octreotide, extensively used as first-line treatment in metastatic NETs (Cives & Strosberg 2015), as well as some other tyrosine kinase inhibitors recently approved for other tumor types were tested.

Specifically, the drug panel tested included the cytotoxic drugs 5-fluorouracil (5-FU), cisplatin, docetaxel, doxorubicin, gemcitabine, irinotecan, mitomycin C and oxaliplatin as well as the targeted agents crizotinib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nintedanib, regorafenib, ruxolitinib, sirolimus, sorafenib, sunitinib, and vemurafenib were tested as well as the SSA octreotide.

The drugs were from commercially available clinical preparations or obtained from Selleck Chemicals LLC. The signal transmission pathways targeted by these drugs are indicated in [Supplementary Table 1](#) (see section on [supplementary data](#) given at the end of this article). All the drugs were tested at four 10-fold dilutions from the

maximal concentration (μM) of 30 for cisplatin, 45 for doxorubicin, ruxolitinib and sirolimus, 90 for crizotinib, dasatinib, docetaxel, erlotinib, gefitinib, gemcitabine, imatinib, lapatinib, mitomycin C, nintedanib, oxaliplatin, regorafenib, sorafenib, sunitinib and vemurafenib and 180 for 5-FU, irinotecan and octreotide.

Irinotecan shows relevant activity *ex vivo* under conditions of the fluorometric microculture cytotoxicity assay (FMCA) despite being considered as a pro-drug (Jonsson *et al.* 2000).

384-well microplates (Nunc) were prepared with 5 μL drug solution at 10 times the final drug concentration using the pipetting robot BioMek 2000 (Beckman Coulter). Tumor cells from patient samples (5000 cells/well for the solid tumor samples and 40,000 cells per well for CLL) in 45 μL were seeded in the drug-prepared 384-well plates using the pipetting robot Precision 2000 (Bio-Tek Instruments) or a Biomek 4000 (Beckman-Coulter). Alternatively, and for most samples, drug was added immediately after cell seeding using the liquid handling system ECHO 550 (Labcyte Inc., Sunnyvale, CA, USA). This allows for fast transfer of volumes ≥ 2.5 nL from source plates into destination wells. In ECHO experiments, source plates were prepared with appropriate concentrations of drugs in DMSO and stored in the oxygen and moisture free MiniPod system (Roylean Developments Ltd, Surrey, UK) until further use. The method for drug addition does not affect assay results. Three columns without drugs served as controls and one column with medium only served as blank.

The semi-automated FMCA was used to determine drug sensitivity (Lindhagen *et al.* 2008). FMCA is based on the measurement of fluorescence generated from hydrolysis of fluorescein diacetate (FDA) to fluorescein by cells with intact plasma membranes. The culture plates were incubated at 37°C in humidified atmosphere. After 72-h incubation, the culture medium was washed away and 50 μL /well of a physiological buffer containing 10 $\mu\text{g}/\text{mL}$ of the vital dye fluorescein diacetate (FDA) were added to control, experimental and blank wells. After incubation for 30–45 min at 37°C, the fluorescence from each well was read in a Fluostar Omega (BMG Laboratories).

Quality control and quantification of results

Quality criteria for a successful assay were $\geq 70\%$ tumor cells in the cell preparation prior to incubation and on the assay day in unexposed control wells, as assessed with trypan blue or toluidine blue-stained cytospin preparations, a fluorescence signal in control cultures of

≥ 5 times mean blank values, and a coefficient of variation of cell survival in control cultures of $\leq 30\%$. The results obtained by the viability indicator FDA are presented as survival index (SI), defined as the fluorescence of the test expressed as a percentage of control cultures, with blank values subtracted.

Concentration–response SI data formed the basis for calculation of the 50% inhibitory concentrations (IC_{50}), using a non-linear regression to a standard sigmoidal dose–response model.

For comparisons of *ex vivo* sensitivity between different cancer diagnoses, statistical inference was calculated with 1-way analysis of variance (ANOVA) with Dunnet's post-test and the SI-NET samples as reference. For comparisons of *ex vivo* sensitivity between different clinicopathological factors as well as biomarker concentrations within the subset of SI-NETs, statistical significance was calculated with the Mann–Whitney test due to small subgroups with distributions deviating from normal. Data are presented as mean values $\pm$ standard error or medians with range as appropriate for the number of experiments/samples indicated. The level of significance was set to two-sided $P < 0.05$. Holm–Bonferroni adjustment was used to counteract the problem of multiple comparisons within the SI-NET subset. The statistics software used throughout was GraphPad Prism, version 7 for Macintosh (GraphPad Software).

Results

Approximately 85% of all samples fulfilled the criteria for a successful assay and were subsequently included in this study. Sixteen SI-NET patients (59%) had not received any systemic treatment prior to tumor sampling, while 11 SI-NET patients (41%) were on somatostatin analogues (SSA) prior to surgery. For CRC and ovarian cancer, 76% and 43% of the samples, respectively, were from patients previously treated with chemotherapy, whereas all samples from renal cancer and CLL were from treatment-naïve patients. Demographic and clinical characteristics of the SI-NET samples are detailed in Table 1, whereas Table 2 summarizes number and treatment status of all tumor samples investigated.

Drug sensitivity *ex vivo*

Samples from SI-NET had lower IC_{50} values, i.e. were more sensitive, to cytotoxic drugs compared to renal cancer with the exception of mitomycin C and irinotecan (Fig. 1). Additionally, for half of the protein kinase inhibitors

Table 1 Patient characteristics of the SI-NET group of 27 patients.

Patient characteristics	Number (n, except for age)
Age – mean $\pm$ s.d.	62.4 $\pm$ 8.1
Sex	
Male	16
Female	11
WHO grade	
G1	13
G2	14
TNM stage	
III	13
IV	14
PC	11
EAM	4

EAM, extra-abdominal metastases; PC, peritoneal carcinomatosis.

studied, IC₅₀ values in SI-NETs were lower than in renal cancer (Fig. 2).

SI-NET samples compared to CRC demonstrated lower IC₅₀ values for half of the cytotoxic drugs studied (Fig. 1), and for the majority of protein kinase inhibitors studied (7/12; Fig. 2). The CLL and ovarian cancer samples demonstrated IC₅₀ values similar to or lower than the SI-NETs to both cytotoxic drugs and protein kinase inhibitors (Figs 1 and 2). Of notice, SI-NET samples were relatively sensitive to 5-FU, gemcitabine, gefitinib, nintedanib, ruxolitinib and sirolimus whereas irinotecan and docetaxel are not expected to be active in SI-NET. Of particular interest in SI-NET, sirolimus was relatively active, with marginal statistical significance ($P=0.054$) in comparison with CRC (Fig. 2).

SI-NET samples demonstrated great variability in drug sensitivity with some samples essentially unaffected by the highest drug concentrations tested, whereas other samples exhibited decreased viability even at the lowest concentration (Supplementary Figs 1 and 2). Octreotide demonstrated very low activity (Supplementary Fig. 2).

Cross-resistance between different drugs investigated, varied greatly, being high between protein kinase inhibitors mechanistically related and considerably lower

Table 2 Number of patient samples included in the analyses.

Cell type	Patients (n)	% previously treated
SI-NET	27	41 (SSA only)
CRC	154	76
Ovarian cancer	90	43
Renal cancer	54	0
CLL	16	0

CLL, chronic lymphocytic leukemia; CRC, colorectal cancer; SSA, somatostatin analogue.

for cytotoxic drugs, except for 5-FU and gemcitabine (Supplementary Fig. 3).

Drug sensitivity in subset of SI-NETs

The median IC₅₀ values for each drug according to different clinicopathological categories and biomarker concentrations in the SI-NET subgroup are presented in Table 3. Age, stage and grade at diagnosis as well as the presence of peritoneal carcinomatosis and extra-abdominal metastasis were not related to sensitivity to cytotoxic drugs or the protein kinase inhibitors tested. Likewise s-CgA and u-5HIAA concentrations at diagnosis did not consistently correlate to drug sensitivity.

Discussion

Treatment of metastatic SI-NETs does not routinely involve cytotoxic drugs and/or systemic targeted agents as a first line of therapy, as such treatment is generally considered to have limited activity in this tumor type (Sun *et al.* 2005, Kunz *et al.* 2013). Additionally, symptoms from the secretion of vasoactive mediators from the tumor are often well controlled with SSA and the tumor itself may have an indolent course. However, some well-differentiated SI-NETs may differ in their proliferative activity between the primary tumor and metastasis, thus making start of tumor controlling therapy a higher priority and choice of therapy complicated (Shi *et al.* 2015). With the exception of peptide receptor radionuclide therapy (PRRT) as a standard second line of treatment for metastatic SI-NETs, the mTOR inhibitor everolimus has emerged as the treatment of choice to achieve tumor control if local treatment strategies fail (Pavel *et al.* 2011, Yao *et al.* 2016, Strosberg *et al.* 2017). Activity of cytotoxic drugs such as 5-FU/capecitabine with or without oxaliplatin, dacarbazine and temozolomide, as well as of kinase inhibitors like sunitinib and sorafenib has also been observed (Bajetta *et al.* 2007, Hobday *et al.* 2007, Kulke *et al.* 2008, Walter *et al.* 2010, Fine *et al.* 2013, Crespo *et al.* 2017). However, the roles of these drugs in the treatment strategy for metastatic SI-NETs are still not well defined.

In this study, SI-NETs were found generally more sensitive to cytotoxic drugs and targeted agents *ex vivo* compared to renal cancer, but less sensitive compared to ovarian cancer and CLL. Compared to CRC, SI-NETs were found to be more sensitive to protein kinase inhibitors and some cytotoxic drugs, e.g. 5-FU, cisplatin and doxorubicin. This pattern of drug activity would arbitrarily

Figure 1

IC₅₀ values for cytotoxic drugs commonly used in solid tumors. Results are presented as means values + s.e. Statistical inference was calculated with 1-way ANOVA with Dunnett's post-test and with the SI-NET samples as reference. *, ** and *** denotes $P < 0.05$, 0.001 and 0.0001 vs SI-NET samples, respectively. Absence of asterisks means that no statistical differences compared to SI-NET samples were observed.

label SI-NETs intermediately drug sensitive, similar to e.g. CRC, a tumor type in which several cytotoxic and targeted drugs are now well established to provide benefit in the advanced setting.

Drug activity observed *ex vivo* using the FMCA might not necessarily transmit to drug activity *in vivo*, although the FMCA has been shown to adequately reflect the clinical activity of cytotoxic drugs with considerable inter-individual variability in a broad spectrum of other cancer diagnoses (Cashin *et al.* 2013, Hultman *et al.* 2014,

von Heideman *et al.* 2014, Bjersand *et al.* 2015, Blom *et al.* 2017). However, the tumor microenvironment of SI-NETs might be different than in other cancer types with yet unknown interactions between tumor cells, tumor stroma and/or immune cells that preclude drugs to exert their effects in the patient.

There is to date very scarce or no clinical experience for many of the drugs indicated in the FMCA to be potentially active in SI-NET, notably gemcitabine, gefitinib, nintedanib and ruxolitinib, making it too

Figure 2

IC₅₀ values for mechanistically differentiated targeted drugs. Results are presented as means values + s.e. Statistical inference was calculated with 1-way ANOVA with Dunnet's post-test and with the SI-NET samples as reference. *, ** and *** denotes $P < 0.05$, 0.001 and 0.0001 vs SI-NET samples, respectively. Absence of asterisks means that no statistical differences compared to SI-NET samples were observed.

early to make conclusions on the *ex vivo* – clinical drug activity relationship. In this context, it could be noted that drugs that have demonstrated some effect in the

clinic, i.e. 5-FU, oxaliplatin and sunitinib were all among drugs being relatively active in the FMCA. Based on this *ex vivo* assessment of drug activity in SI-NET, especially

Table 3 Median IC₅₀ values of cytotoxic drugs and protein kinase inhibitors grouped according to clinicopathological categories and biomarker concentrations in SI-NETs.

	Age		Grade		Stage		PC		EAM		S-CgA		U-5HIAA	
	<60 years (n=12)	>60 years (n=15)	1 (n=13)	2 (n=14)	III (n=13)	IV (n=14)	Neg (n=15)	Pos (n=12)	Neg (n=23)	Pos (n=4)	Low (n=11)	High (n=13)	Low (n=14)	High (n=12)
Critzo	8.1	4.78 (0.929)	62.48	5.22 (0.187)	14.95	4.77 (0.359)	14.95	4.77 (0.443)	14.95	4.57 (0.177)	5.55	7.61 (0.731)	14.95	4.78 (0.443)
Dasa	20.74	20.18 (0.961)	20.79	20.43 (0.849)	25.83	20.18 (0.958)	25.83	20.18 (0.929)	25.89	17.2 (0.409)	20.68	43.31 (0.280)	25.83	18.69 (0.853)
Dox	5.41	3.45 (0.612)	5.14	3.58 (0.976)	2.96	19.81 (0.161)	3.14	7.27 (0.482)	3.59	5.58 (0.640)	1.62	32.63 (0.023)	3.14	7.27 (0.482)
Iri	180	77.5 (0.381)	89.4	180 (0.483)	95.16	154.5 (0.972)	83.63	167.3 (0.449)	70.47	180 (0.111)	180	35.85 (0.019)	83.63	90 (0.387)
Lapa	13.41	5.69 (0.132)	5.44	13.41 (0.065)	7.85	9.38 (0.343)	7.85	9.38 (0.343)	7.85	9.34 (0.343)	6.86	7.45 (0.610)	7.85	9.38 (0.343)
Mit C	30.73	18.65 (0.366)	21.23	28.25 (0.687)	20.62	28.21 (0.375)	25.68	25.76 (0.989)	29.47	21.73 (0.785)	20.62	23.75 (0.596)	25.68	25.76 (0.989)
Ninte	7.6	3.98 (0.792)	3.98	7.6 (0.429)	7.6	3.33 (0.527)	7.6	3.33 (0.527)	7.6	3.33 (0.527)	6.18	2.99 (0.476)	7.56	4.08 (0.460)
Oxa	59.53	34.2 (0.600)	40.33	56.77 (0.922)	42.26	56.67 (0.743)	52.57	47.58 (0.930)	56.77	34.2 (0.965)	38.39	43.39 (0.698)	42.26	47.58 (0.722)
Rego	5.79	5.80 (0.118)	12.17	6.54 (0.217)	10.01	6.45 (0.649)	10.01	6.45 (0.689)	10.01	6.2 (0.259)	9.9	6.03 (0.672)	10.01	6.45 (0.689)
Ruxo	35.09	45 (0.307)	45	41.38 (0.707)	27.25	45 (0.015)	32.14	45 (0.052)	35.96	45 (0.078)	27.46	45 (0.017)	32.14	45 (0.052)
Sora	8.63	6.98 (0.811)	6.55	9.73 (0.482)	6.86	6.97 (0.709)	6.86	8.27 (0.586)	6.73	8.27 (0.977)	6.73	9.9 (0.383)	6.86	8.27 (0.856)
Sun	4.52	6.08 (0.890)	5.29	4.64 (0.767)	4.27	7.01 (0.316)	4.26	6.32 (0.366)	4.27	6.32 (0.486)	3.75	8.55 (0.089)	4.27	6.32 (0.366)

Numbers in parentheses represent *P* values from Mann-Whitney test with Holm-Bonferroni correction for multiple testing. Low and high S-CgA and U-5-HIAA are determined by dichotomous division of pre-treatment concentrations of these biomarkers, using median values as cut-off. Drugs with very low *ex vivo* activity and/or fewer than four samples tested per column were not included in this analysis.

Critzo, crizotinib; Dasa, dasatinib; Dox, doxorubicin; EAM, extra-abdominal metastases; Iri, irinotecan; Lapa, lapatinib; Mit C, mitomycin C; neg, negative; Ninte, nintedanib; Oxa, oxaliplatin; PC, peritoneal carcinomatosis; pos, positive; Rego, regorafenib; Ruxo, ruxolitinib; S-CgA, serum chromogranin A; Sora, sorafenib; Sun, sunitinib; u-5-HIAA, urinary 5 hydroxy-indoleacetic acid.

promising drugs in this tumor type are suggested to be nintedanib and ruxolitinib.

Sirolimus (rapamycin) was only tested in five samples of SI-NET and the IC₅₀ values indicated from these was relatively low compared with the other tumor types, which is also in line with the observed clinical benefit from everolimus in SI-NET (Yao *et al.* 2016). Importantly, sirolimus is considered to reflect the activity of everolimus (rapamycin analogue).

Apart from anti-secretory activity, somatostatin analogues have demonstrated a stabilizing effect in gastroenteropancreatic-NETs (Rinke *et al.* 2009, Caplin *et al.* 2014). This is consistent with the very low *ex vivo* cytotoxicity of octreotide in this study and in agreement with the direct and indirect mechanisms of somatostatin analogues anti-proliferative action (Cives & Strosberg 2015).

In the absence of definitive pre-treatment markers as predictive factors for drug response in SI-NET, therapeutic decisions rely currently mostly on clinicopathological criteria. Importantly, the development of novel targeted agents has indeed necessitated the implementation of predictive markers. Mutational status in the PIK3CA/Akt/mTOR pathway has failed to predict response to everolimus treatment in a clinical setting, probably due to errors related to epigenetic changes (Pavel *et al.* 2011, Cros *et al.* 2016, Yao *et al.* 2016). As protein/mRNA markers in this pathway are lacking, *ex vivo* drug sensitivity testing may be utilized in order to select patients for treatment with mTOR inhibitors. Regarding other targeted novel therapies, interleukin-8, sVEGFR-3 and SDF-1α were recently identified as predictors of response to the multiple tyrosine kinase inhibitor sunitinib in a phase II study (Zurita *et al.* 2015). However, protein kinase inhibitors induce a wide spectrum of changes in the cell, which are not all tested for by available markers, and thus, *ex vivo* drug sensitivity testing may have a place in individualizing treatment also with protein kinase inhibitors.

Interestingly, in the SI-NETs of this study, clinicopathological factors linked to worse prognosis such as advanced age and stage at diagnosis, tumor grade and the presence of peritoneal carcinomatosis and extra-abdominal metastasis, as well as the currently used biomarkers s-CgA and u-5HIAA, were not clearly associated to *ex vivo* sensitivity to any kind of drug. These findings indicate that the traditionally used clinicopathological criteria as well as clinically available biomarkers do not reflect tumor cell sensitivity to cytotoxic drugs and targeted agents and are, thus, not immediately predictive for the clinical effect of systemic treatment.

The cross-resistance between the different cytotoxic drugs and targeted agents investigated varied greatly, with reasonably high correlations between kinase inhibitors known to at least partly share their mechanisms of action (Supplementary Fig. 3 and Supplementary Table 1). In clinical practice, this means that resistance to one of these kinase inhibitors drug would also imply resistance to the other. Nevertheless, individual SI-NET samples could be clearly resistant to one but sensitive to another drug, thus supporting the combined or sequential use of drugs, preferably based on a predictive test like the FMCA. Furthermore, the very conspicuous inter-individual variability between the SI-NET samples in sensitivity to most drugs emphasizes the SI-NET heterogeneity as well as the need for a predictive test.

The strength of this study is the assessment of sensitivity to a broad panel of anti-cancer drugs in a rare tumor type using an assay reported to reflect clinical drug activity. An important limitation of *ex vivo* drug sensitivity assay used, however, is that only drugs that act directly on the tumor cells can be assayed. Drugs that mainly target stroma or modulate the immune response will require development of novel assays that can account for complex interactions between different cell types (Blom *et al.* 2017). Furthermore, a considerable fraction of CRC and ovarian cancer samples were from patients previously exposed to chemotherapy. Such samples tend to be somewhat more resistant to drugs *ex vivo* (von Heideman *et al.* 2014, Bjersand *et al.* 2015), implying a certain bias in the comparison between tumor types in this study. Another obvious limitation is the rather low number of tumor samples available for analysis for the SI-NET subset within a reasonably study period, and, thus, few observations on the effect of, e.g. sirolimus and gefitinib. Additionally, FMCA reflects only the pharmacodynamics and not pharmacokinetics of cancer drugs, which explains why this assay is better at predicting *in vivo* resistance than *in vivo* activity.

In conclusion, SI-NETs exhibit intermediate, yet variable sensitivity *ex vivo* to cytotoxic and newly introduced targeted drugs, calling for an individualized choice of therapy in this tumor type. Clinicopathological factors and currently used biomarkers were not associated to *ex vivo* sensitivity, challenging these criteria in clinical practice. Taken together, in the efforts toward personalized cancer medicine, *ex vivo* sensitivity testing might have a place for the identification of appropriate cytotoxic drugs and targeted agents for a subset of SI-NETs patients who might benefit from them.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/ERC-17-0404>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Drs Nygren and Ståhlberg contributed equally to this study. Drs Daskalakis, Nygren and Ståhlberg had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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