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Pancreatic endocrine tumours: mutational and immunohistochemical survey of protein kinases reveals alterations in targetable kinases in cancer cell lines and rare primaries

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Background: Kinases represent potential therapeutic targets in pancreatic endocrine tumours (PETs).
Patients and methods: Thirty-five kinase genes were sequenced in 36 primary PETs and three PET cell lines: (i) 4 receptor tyrosine kinases (RTK), epithelial growth factor receptor (*EGFR*), human epidermal growth factor receptor 2 (*HER2*), tyrosine-protein kinase KIT (*KIT*), platelet-derived growth factor receptor alpha (*PDGFRalpha)*; (ii) 6 belonging to the Akt/mTOR pathway; and (iii) 25 frequently mutated in cancers. The immunohistochemical expression of the four RTKs and the copy number of *EGFR* and *HER2* were assessed in 140 PETs.
Results: Somatic mutations were found in *KIT* in one and *ATM* in two primary neoplasms. Among 140 PETs, EGFR was immunopositive in 18 (13%), HER2 in 3 (2%), KIT in 16 (11%), and PDGFRalpha in 135 (96%). *HER2* amplification was found in 2/130 (1.5%) PETs. KIT membrane immunostaining was significantly associated with tumour aggressiveness and shorter patient survival. PET cell lines QGP1, CM and BON harboured mutations in *FGFR3*, *FLT1/VEGFR1* and *PIK3CA*, respectively.
Conclusions: Only rare PET cases, harbouring either *HER2* amplification or *KIT* mutation, might benefit from targeted drugs. KIT membrane expression deserves further attention as a prognostic marker. *ATM* mutation is involved in a proportion of PET. The finding of specific mutations in PET cell lines renders these models useful for preclinical studies involving pathway-specific therapies.
Key words: carcinoma, endocrine, kinases, pancreas, therapy

introduction

Pancreatic endocrine tumours (PETs) arise sporadically or as part of hereditary cancer syndromes including multiple endocrine neoplasia type 1 (MEN1) [1, 2]. Indeed, MEN1 alterations in the gene sequence and/or protein expression remain the only consistent change also found in the sporadic form of the disease [3]. According to the World Health Organisation classification, PETs are divided into well-differentiated endocrine tumours and carcinomas or poorly differentiated endocrine carcinomas [4]. Surgery should be considered for all patients as first-line treatment offering a chance for cure even in a proportion of malignant PETs [5]. However, only few effective chemotherapic agents are available to date for the treatment of advanced stage disease. Moreover,

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the majority of pancreatic endocrine carcinomas are slowly growing tumours, which show resistance to conventional cytotoxic agents. Therefore, the identification of new therapeutic strategies represents the main challenge for these neoplasms. In this scenario, protein kinases stand out as potential therapeutic targets to be investigated in PETs [6–8].

Protein kinases are key components of signalling pathways involved in the regulation of different and complex cellular processes such as cell cycle progression, differentiation, apoptosis and invasion [9, 10]. The protein kinase complement (defined as 'kinome') represents a significant fraction of the human genome, and recently Manning et al. [11] organised it into a dendogram containing nine broad groups of genes. Alterations in a kinase gene, such as point mutations and deletions in conserved domains, can lead to a constitutively activated kinase, that is a potential target for cancer treatment or to its inactivation, as for genes involved in the maintenance of genome stability [12]. Regarding PETs, the importance of Akt-mTOR pathway and its therapeutic relevance has been largely investigated but no definite data about the mutational profile of the individual kinase components of this pathway in PETs is available to date [13–17].

Lately, extensive sequence analysis of kinase tumour genomes has been conducted in different epithelial tumours [18–21]. These works point out a subset of kinases with known or potential relationship with solid tumour development as they display a relatively high frequency of somatic mutations.

The rationale for the targeting of kinases resides not only in the identification of potentially activating mutations at the gene level but also in the determination of the expression of the corresponding mutant proteins in tumour tissues. This is particularly important when targeting receptor tyrosine kinases (RTKs) with the use of monoclonal antibodies or inhibitors of their tyrosine kinase activity [22–26]. About the presence and role of protein kinases, particularly of RTKs, in PETs, inconsistent results have been reported [27–35].

In the present study, we explored the kinome searching a panel of 36 primary PETs and 3 PET cell lines for mutations in 35 kinase genes including: 25 genes frequently mutated in human cancers other than pancreatic [18–21], 6 genes related to the Akt-mTOR pathway that has been shown to be activated in PET [17], and 4 genes encoding for RTKs targeted by available anticancer drugs. For these latter, the protein expression by immunohistochemistry and gene copy number status by fluorescence *in situ* hybridisation (FISH) was assessed in a large series of 140 primary PETs.

materials and methods

mutational analysis

samples. The panel of 36 primary PETs was collected according to the ethical requirements of the review board of the University of Verona (supplemental Table S1 is available at *Annals of Oncology* online). No patient underwent neoadjuvant therapy. Three PET cell lines, QGP1, BON and CM, were included in the study [36, 37]. Samples containing >80% tumour cells were used. Genomic DNA was isolated using DNAesy Blood and Tissue Kit (Qiagen, Milan, Italy). Matched normal DNA served to determine the somatic or germline nature of mutations.

sequencing and data analysis. The panel of 35 kinase genes selected for mutational analysis is listed in supplemental Table S2 (available at *Annals of Oncology* online). Primers for amplification and sequencing were designed using Primer3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and refer to National Centre for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov). PCR primers were designed to amplify the selected exons and the flanking intronic sequences, including splicing donor and acceptor regions. PCR products were ~400 bp in length, with multiple overlapping amplimers for larger exons. PCR and direct sequencing conditions were described [38]. Sequence differences to the NCBI reference sequence were identified via manual inspection of aligned electropherograms assisted by the Mutation Surveyor software package (SoftGenetics, State College, PA). The genetic alterations identified were cross-referenced to variant information from international databases (NCBI SNP database, The Swiss-Prot and GenBank databases, and the COSMIC database) and literature. In addition to nonsynonymous genetic alterations, we detected numerous silent sequence variations that are not presented and further analysed here.

immunohistochemistry and fluorescence *in situ* hybridisation

tissue microarrays. Paraffin-embedded tissue microarrays (TMAs) contained 140 primary PETs, 38 matched metastasis (22 nodal and 16 liver) and 12 normal pancreas. No patient underwent neoadjuvant therapy. The construction of the TMAs was carried out using a tissue arrayer (Beecher Instruments, Silver Spring, MD) as previously described [39]. For most cases, at least three cores of 1 mm diameter per sample were analysed. Clinicopathological characteristics of PETs are reported in supplemental Table S3 (available at *Annals of Oncology* online).

immunohistochemistry. TMAs were immunostained using the antibodies listed in Table 1. Primary antibodies were omitted in negative controls. Detection was carried out using Dako EnVision Plus-HRP kit (Dako, Carpinteria, CA). Slides were scanned with ScanScope® GL System (Aperio Technologies, Vista, CA) and visualised using ImageScope™ Software (Aperio Technologies). To be considered positive, >10% of tumour or stromal cells had to show positive staining. Three independent observers scored protein expression. The staining intensity was classified into four grades: (0, absent; 1, weak; 2, moderate; 3, strong).

fluorescence in situ hybridisation. FISH was carried out as described [40] using epithelial growth factor receptor (*EGFR*) and human epidermal growth factor receptor 2 (*HER2*) locus-specific and chromosomes 7 and 17 centromeric probes (Vysis, Downers Grove, IL) diluted 1 : 100 in tDenHyb1 buffer (Insitus, Albuquerque, MN). The slides were examined using a Zeiss Axioplan 2 microscope (Zeiss, Gottingen, Germany) and the appropriate filters.

Table 1. Antibodies used in the immunohistochemical analysis

Antibody	Clone/code number	Manufacturer
EGFR	Clone 2-18C9	Dako (Carpinteria, CA)[a]
HER2	Code K5207	Dako
KIT	Code A4502	Dako
PDGFRalpha	Code 3164	Cell Signaling (Danvers, MA)

EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; KIT, tyrosine-protein kinase KIT; PDGFRalpha, platelet-derived growth factor alpha.

[a]Anti-EGFR antibody is part of the FDA approved DakoCytomation EGFR pharmDx™ kit.

statistical analysis

Association of mutational, immunohistochemical and FISH results with clinicopathological variables was evaluated using Pearson's chi-square tests or Fisher's test when appropriate for categorical variables; Kruskal–Wallis test or Wilcoxon test for continuous variables and log-rank tests for time to progression and survival. Time to progression was considered as the time between radical surgery and the examination of the patient in which progression could be detected. In survival analysis, patients dead of causes other than disease were censored at the time of death. All tests were considered significant when $P > 0.05$. For all the calculation the *R* statistical software package was used (http://www.r-project.org).

results

mutational analysis of 35 kinase genes

All exons of the 35 selected genes were analysed in 36 primary PETs and 3 PET cell lines, with 6240 PCR products, spanning over 2.5 Mb of tumour genomic DNA, generated and subjected to direct sequencing. Changes previously described as single nucleotide polymorphisms (SNPs) were excluded from further analysis. To ensure that the observed mutations were not PCR or sequencing artefacts, amplicons were independently re-amplified and resequenced. All verified changes were resequenced in parallel with matched normal DNA to distinguish between somatic mutations and SNPs not previously described. This approach led to the identification of six different nonsynonymous mutations (Table 2). Three were in primary PETs: two in *ATM* and one in tyrosine-protein kinase KIT (KIT). One each in *FGF3*, *FLT1/VEGFR1* and *PIK3CA* were identified in endocrine tumour cell lines.

Two different mutations occurred in *ATM* (p.R823C and p.S2017I), a cancer recessive genes encoding for a serine/threonine kinase involved in DNA damage response [41]. Germline mutations of *ATM* predispose to ataxia telangiectasia and breast cancer [41, 42]. The mutations found in our two PETs were somatic as assessed by sequencing of matched normal DNA. The most interesting mutation is the p.S2017I that occurs in FAT domain of unknown functional significance but highly conserved between the members of the PIKK (phosphatidylinositol 3-kinase-related kinase) family, which includes *ATR* and *DNA-PK* [41]. Inactivating mutations of *ATM* have not been previously implicated in the development of PETs.

Among the four genes encoding for RTKs targeted by available anticancer drugs, we report for the first time a nonsense somatic mutation in the kinase domain of KIT (p.R796stop) in a primary PET, while no mutation was found in *EGFR*, *HER2* and platelet-derived growth factor receptor alpha (*PDGFRalpha*).

No mutation was detected in the six genes belonging to the Akt/mTOR pathway: *AKT2, PIK3CA, RPS6K1, STK11, PDPK1, FRAP1-mTOR*.

immunohistochemical expression of RTKs

Immunohistochemical staining for EGFR, HER2, KIT and PDGFRalpha was evaluated in TMAs containing 140 primary PETs and 38 matched metastasis (22 nodal and 16 liver) (Figure 1). The results are summarised in Table 3. No differences in the staining patterns were observed between primary tumours and matched metastases for all antibodies.

The results of immunohistochemical staining for EGFR, HER2, KIT and PDGFRalpha, of TMAs containing 140 primary PETs and 38 matched metastasis (22 nodal and 16 liver) are summarised in Table 3. Figure 1 shows examples of staining patterns. No differences in staining patterns were observed between primaries and matched metastases for all antibodies.

EGFR expression. Of 140 PETs 18 (13%) stained for EGFR with sharp membranous pattern and signals ranging from very strong to weak (Figure 1B). Specifically, 10 (7%) tumours had strong EGFR signals and 8 (6%) had weak immunoreactivity.

HER2 expression. Only 3 of 140 PETs (2%) stained for HER2 (Figure 1C). Among these, one tumour had a strong membranous signal and two displayed weak and focal immunoreactivity.

KIT expression. Sixteen of 140 PETs (11%) showed KIT immunostaining. A prevalently membranous staining was detected in seven tumours (5%) with variable intensity (six strong and one weak) (Figure 1A). Nine cases showed cytoplasmic staining alone, of these one had strong intensity and five showed a dot-like positivity. Among the cases displaying dot-like positive staining, there was the tumour bearing the mutated *KIT* gene (Table 2). This is somewhat expected since the dot-like *KIT* immunostaining pattern is

Table 2. Mutations indentified in protein kinase genes

Gene	Nucleotide change	Amino acid change	Mutation type	Zygosity	Sample	Cross-reference annotation[b]
ATM	c.2879 C>T	p.R823C	Missense	Heterozygous	528	n.f.
ATM	c.6435 G>T	p.S2017I	Missense	Heterozygous.	502	n.f.
FGFR3	c.1003 G>A	p.E322K	Missense	Heterozygous	QGP[a]	Variant in cancer
FLT1	c.2594 G>A	p.R781Q	Missense	Heterozygous	CM[a]	Variant in cancer
KIT	c.2386 A>T	p.R796stop	Nonsense	Heterozygous	365	n.f.
PI3KCA	c.1790 A>C	p.E545A	Missense	Heterozygous	BON[a]	Variant in cancer

The mutations are listed by gene alongside the samples in which they were found. The nucleotide numbering uses the A of the ATG translation initiation start site as nucleotide + 1, based on reference sequences provided in supplemental Table S2 (available at *Annals of Oncology* online).

[a]PET cell lines. QGP, established from a non-functioning islet cell tumor; CM, established from ascitic fluid of an insulinoma; BON, established from a metastasis of a pancreatic carcinoid tumor.

[b]The genetic alteration identified were cross-referenced with variant information from databases and literature.

c., cDNA sequence; n.f., alterations not previously found in cancer; p., protein sequence; stop, stop codon.

a feature frequently associated with gene mutations [43]. All PET immunopositive cases were sequenced for *KIT* gene revealing no genetic abnormalities.

PDGFRalpha expression. Cytoplasmic staining was found in 135 of 140 (96%); the remaining 5 cases (4%) were negative. Nuclear immunostaining was present in 129 and absent in 6 cases (5%). Cell membranes were always negative, while the stromal component stained in all 140 cases (Figure 1D).

FISH analysis

FISH analysis for EGFR and HER2 was carried out on the same TMAs used for immunohistochemical analysis. Ten of the 140 PETs (7%) were not informative for both EGFR and HER2 analysis. Figure 2 shows examples of fluorescent hybridisation.

No differences were observed between primary tumours and matched metastases for both EGFR and HER2.

EGFR. No case had gene amplification; 86 of 130 PETs (66%) were disomic, 27 (21%) were polysomic–trisomic and 17 (13%) were monosomic (Figure 2A).

HER2. Gene amplification was observed in 2 of 130 (1.5%) cases (Figure 2B): one in a tumour showing strong protein immunostaining and the second in a tumour displaying negative immunoreactivity. Of the 130 informative PETs, 108 (83%) were disomic, 15 (11%) polysomic–trisomic and 7 (5%) monosomic.

correlation of RTK gene status and protein expression with clinical pathological information

Immunohistochemical and FISH results were correlated with clinicopathological features. At univariate analysis, an

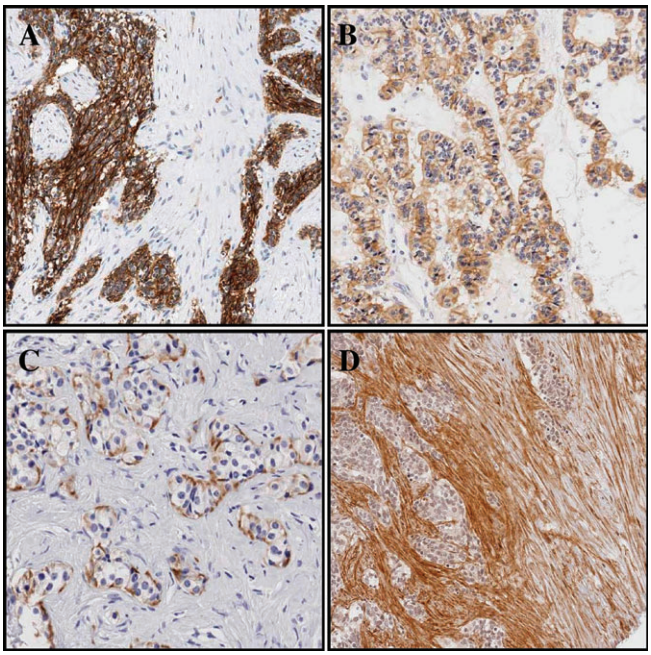

Figure 1. Immunohistochemical staining for receptor tyrosine kinases in pancreatic endocrine tumours. Shown are positive staining for KIT (A), EGFR (B) and HER2 (C) in tumour cells; positive staining in tumour cells and in the stroma is shown for PDGFRalpha (D). Original magnification, x20.

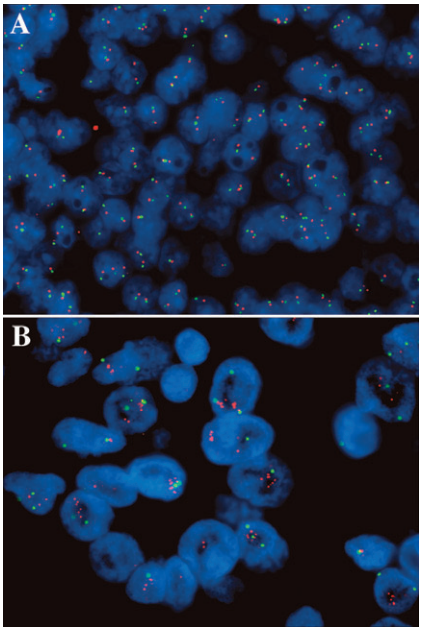

Figure 2. Fluorescent *in situ* hybridisation (FISH) analysis for *EGFR* and *HER2* in pancreatic endocrine tumours. FISH analysis showing monosomy for *EGFR* (A), and gene amplification for *HER2* (B). Original magnification, ×100. EGFR and HER2 signal red, centromeric probes signal green.

Table 3. Expression of receptor tyrosine kinases in pancreatic endocrine tumours (PETs)

	EGFR	HER2	KIT[a]	PDGFRalpha[b]	
				Tumour	Stroma
Total number of PETs	18/140 (13%)	3/140 (2%)	16/140 (11%)	135/135 (100%)	135/135 (100%)
Nonfunctioning	17/106 (16%)	3/106 (3%)	12/106 (11%)	103/103 (100%)	103/103 (100%)
Functioning	1/34 (3%)	0/34 (–)	4/34 (12%)	32/32 (100%)	32/32 (100%)

EGFR, epidermal growth factor receptors; HER2, human epidermal growth factor receptor 2; KIT, tyrosine-protein kinase KIT; PDGFRalpha, platelet-derived growth factor alpha.

[a]Positive immunostaining refers to the detection of both membranous and cytoplasmic signals.

[b]Five cases were not evaluable; positive immunostaining refers to the detection of cytoplasmic signals.

association was found between immunohistochemical KIT membrane positivity and tumour aggressiveness (Table 4). In fact, a positive staining significantly correlated with a diagnosis of carcinoma either well or poorly differentiated ($P < 0.001$), with liver and lymph node metastasis at diagnosis ($P < 0.001$), vascular ($P = 0.004$) and neural ($P = 0.005$) invasion and a Ki67 index >5% ($P = 0.015$). Moreover, a significant association was found between HER2 disomic status at FISH and benign tumours ($P = 0.004$). Conversely, no association was found between mutational data and either clinicopathological data or protein expression and FISH data.

By Mann–Whitney test, no difference was found between primary tumours and metastases for both receptor expression and FISH data.

By Kaplan– Meier analysis, a significant association was found between KIT membrane immunostaining and survival ($P < 0.001$) (Figure 3).

discussion

The need for effective systemic treatment options for patients with PET led us to explore the kinome searching for candidate targets of anticancer drugs.

We first operated a mutational survey of 35 kinase genes in 36 primary PETs and 3 PET cell lines that showed (i) no mutations in *AKT2*, *PIK3CA*, *RPS6K1*, *STK11*, *PDPK1*, and *FRAP1-mTOR*, which are six key genes of the Akt/mTOR pathway; (ii) among the four genes encoding for RTKs targeted by exiting drugs, no mutation was found in *EGFR*, *HER2*, and *PDGFRalpha*, while *KIT* was mutated in one case; (iii) mutations in *ATM* in two different cases were the only alterations affecting the remaining 25 screened genes; (iv) the cell lines QGP1, CM and BON, which represent the most extensively used cellular models for functional and preclinical studies concerning PETs, displayed mutations of kinase genes that are amenable of therapeutic targeting that are *FGFR3*, *FLT1/VEGFR1* and *PIK3CA*, respectively. We then carried out an immunohistochemical survey of the four targetable RTKs in 140 primary PETs showing that (i) EGFR was expressed in 13% of cases, (ii) HER2 in 2% and (iii) KIT in 11%, whereas (iv) PDGFRalpha immunostaining was found in all cases. FISH analysis on 130 PETs showed no gene amplification for *EGFR*, whereas two PETs harboured amplified *HER2* genes.

This study involved the largest panel of primary PETs that has been ever screened for somatic mutations of kinase genes and led to the identification of a total of six different nonsynonymous mutations affecting five kinase genes (*FGFR3*, *FLT1/VEGFR1*, *PIK3CA*, *ATM* and *KIT*).

The mutations found in *FGFR3* (p.E322K), *FLT1/VEGFR1* (p.R781Q) and in *PIK3CA* (p.E545A) were identified in established cell lines and all have been previously related to human cancers [44–50].

The remaining three mutations were found in three different primary tumours and were somatic in origin as assessed by sequencing of their matched normal DNA. None of these mutations were previously described in cancers. Two neoplasms that had previously been shown to lack *MEN1*

Table 4. Correlation of KIT membrane expression with clinicopathological parameters

Parameter	*n*	KIT-positive tumours	*P*[a]
WHO classification	140		
WDET	76	0	
WDEC	59	4	
PDEC	5	3	<0.001
Functional status	140		
F-PET	34	0	
NF-PET	106	7	0.19
Proliferation index	138		
Ki67 ≤5%	101	2	
Ki67 >5%	37	5	0.015
Liver metastases	140		
Absent	110	1	
Present	30	6	< 0.001
Lymph node metastases	140		
Absent	98	0	
Present	42	7	< 0.001
Vascular invasion	136		
Absent	73	0	
Present	63	7	0.004
Neural invasion	136		
Absent	91	1	
Present	45	6	< 0.005

[a]Fisher's exact test.

WDET, well-differentiated endocrine tumours; WDEC, well-differentiated endocrine carcinoma; PDEC, poorly differentiated endocrine carcinoma; F, functioning; NF, nonfunctioning.

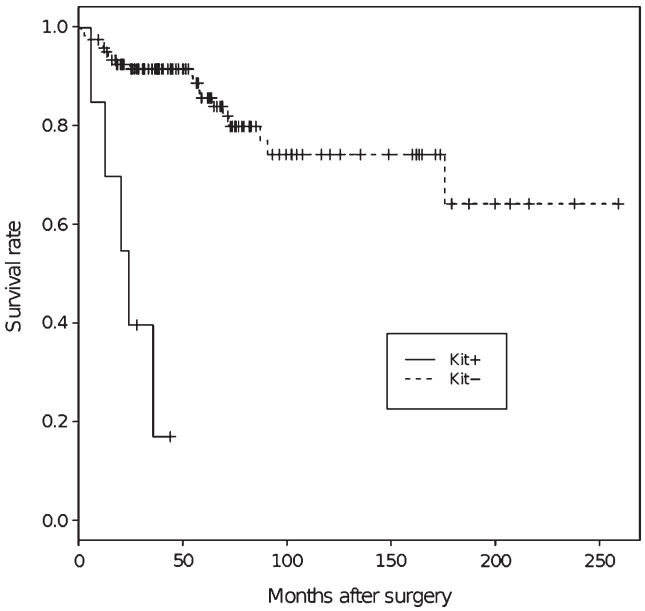

Figure 3. Correlation between KIT membrane immunostaining and patients' survival. Kaplan–Meier estimates of survival with regard to KIT membrane immunostaining ($P < 0.001$). Follow-up, months; KIT+, membrane-positive immunostaining; KIT−, membrane-negative immunostaining.

mutations [3, 51] harboured mutations in *ATM*. Noteworthy, both *ATM* and *MEN1* map to chromosomal arm 11q, which is frequently lost in PET [52]. The finding of *ATM* mutations could partially address the observed discrepancy between the rate of 11q deletion and the lower frequency of *MEN1* mutations in PET [51, 52]. Our mutational screening also revealed a nonsense mutation affecting the catalytic domain of *KIT* that requires further experimental evaluation to assess its functional significance. *KIT* mutational activation is a feature of gastrointestinal stromal tumours [53, 54] and represents a therapeutic target for this malignancy [55]. The role of KIT in PET is still unknown although imatinib mesylate showed a cytotoxic effect on BON cell line [56].

Increasing evidence suggests that the development of efficient therapeutic strategies for cancer treatment implies the recognition of altered pathways rather than their individual components [57]. Regarding PETs, especially the significance of Akt-mTOR pathway and its therapeutic relevance have been addressed by different groups, including ours [13–17]. In this study, we analysed the mutational profiles of six kinases related to the Akt-mTOR pathway: *AKT2*, *PIK3CA*, *RPS6K1*, *STK11*, *PDPK1* and *FRAP1/mTOR*. No primary tumour harboured mutations in these genes, whereas BON cell line displayed an activating mutation of *PIK3CA*. Established from a metastatic pancreatic 'carcinoid' tumour, BON represents the most extensively used model system for the study of PETs and was previously demonstrated to exhibit a constitutive Akt/mTOR activation supposedly due to an autocrine IGF-I loop [58]. QGP1 cell line, derived from a primary pancreatic functioning tumour, contains a mutation in *VEGFR1*. Therefore, our characterisation of established cell lines with the regard to alterations in kinase genes finally provides suitable model systems for preclinical study, particularly in the light of the recent interest for the development of new drugs targeting mTOR/PIK3CA as well as VEGFR activity [59].

The rationale for targeting RTKs resides not only in the identification of gene mutations but also in the determination of their status at both the protein and gene level [22–26]. In line with this, we assessed the protein expression of EGFR, HER2, KIT and PDGFRalpha by immunohistochemistry and gene copy number status of *EGFR* and *HER2* in 140 primary PETs, which included the 36 PETs screened for kinase genes mutations.

The most relevant finding of this analysis regards the expression of KIT. Indeed, 16 of 140 (11%) cases displayed staining for KIT, 7 (5%) of which showed a prevalent membranous signal. This latter feature showed a significant association with tumour aggressiveness and patients' shorter survival at univariate analysis ($P < 0.001$), which was not retained at multivariate analysis. This result differs from that by Zhang et al. [33] who reported that KIT expression was an independent prognostic factor. Concerning EGFR and HER2, neoplastic cells stained for EGFR in 13% (18/140) and for HER2 in 2% (3/140) of samples. No gene amplification was observed for EGFR, whereas two cases showed a high-level *HER2* gene amplification; of these only one expressing the protein. Immunostaining for PDGFRalpha was found in both stroma and neoplastic cells of all cases.

The expression of RTKs had already been investigated in PETs (Table 5), with overlapping results for HER2 and PDGFRalpha in our series [28, 30, 34, 35]. For KIT immunostaining, our data are not dissimilar from those obtained by Zhang et al. [33] using the same antibody, while our figure for EGFR immunostaining is definitely lower than that reported by others [27, 29–32]. However, our EGFR

Table 5. Expression of receptor tyrosine kinases in published series of pancreatic endocrine tumours

Antigen and reference	PET cases,[a] positive/total (%)		Antibody information[b]
EGFR			
Fjällskog et al. [30]	21/38 (55)		Santa Cruz (San Francisco, CA)
Papouchado et al. [29]	12/48 (25)		Santa Cruz
Bergmann et al. [31]	30/65 (46)		Zymed-Invitrogen (Carlsband, CA)
Peghini et al. [27]	6/15 (40)[c]		Santa Cruz
Srivastava et al. [32]	23/35 (65)[d]		Zymed and Oncogene (San Diego, CA)
HER2			
Proca et al. [28]	0/27		Dako (Carpinteria, CA)
Goebel et al. [34]	0/10[c]		Dako
KIT			
Fjällskog et al. [30]	35/38 (92)		Santa Cruz
Zhang et al. [33]	21/97 (22)		Dako
PDGFRalpha	Tumour	Stroma	
Fjällskog et al. [30]	38/38	21/37 (57%)	Santa Cruz
Chaudhry et al. [35]	4/5	5/5	In-house[e]

[a]Except for PDGFRalpha, only the staining of neoplastic cells from primary tumours was considered.
[b]Manufacturers.
[c]These tumours were all gastrinomas.
[d]The overall frequency of EGFR expression refers to the positive immunostaining with either antibodies as reported in this study
[e]For information about PDGFRalpha antibody refers to Eriksson et al. [60]

immunostaining was carried out using FDA approved DakoCytomation EGFR pharmDx™.

In conclusion, in this study we report six different mutations affecting five kinase genes in PET. The finding of specific mutations in the few available PET cell lines renders these models useful for preclinical studies involving pathway-specific therapies. None of the alterations identified in primary tumours were previously related to cancer. Those affecting *ATM* were found among cases lacking MEN1 mutations, thus possibly explaining the observed differences between the rate of chromosome 11q allelic losses and that of *MEN1* mutations. KIT membrane expression seems to be a prognostic marker deserving further attention. Although PET lacked activating mutations in most of the screened genes, we showed that rare cases, namely those harbouring either *HER2* amplification or *KIT* mutation, might benefit from available targeted drugs.

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disclosure

The authors have declared no conflicts of interest.

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