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 chemotherapeutic agents are available to date for the treatment of advanced stage disease. Moreover,
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 a panel of 36 primary PETs and 3 PET cell lines for mutations expression by immunohistochemistry and gene copy number available anticancer drugs. For these latter, the protein in PET [17], and 4 genes encoding for RTKs targeted by the Akt-mTOR pathway that has been shown to be activated (supplemental Table S1 is available at 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 DNAeasy Blood and Tissue Kit (Qiagen, Milan, Italy). Matched normal DNA served to determine the somatic or germline nature of mutations.

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).

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 DNAeasy 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 (Soft Genetics, 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.

Table 1. Antibodies used in the immunohistochemical analysis

| Antibody       | Clone/code number | Manufacturer                |
|----------------|-------------------|----------------------------|
| EGFR           | Clone 2-18C9       | Dako (Carpinteria, CA)*    |
| HER2           | Code K5207        | Dako                       |
| KIT            | Code A4S02        | 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.

*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 \( KIT \) (KIT). One each in \( FGF3, FLT1/VEGFR1 \) and \( PIK3CA \) were identified in endocrine tumour cell lines.

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: \( AKT2, PIK3CA, RPS6K1, STK11, PDPK1, FRAP1-mTOR \).

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.                          |
| ATMicro    | c.6435 G>T        | p.S2017I          | Missense      | Heterozygous | 502    | n.f.                          |
| FGF3       | c.1003 G>A        | p.E322K           | Missense      | Heterozygous | QGP*   | Variant in cancer             |
| FLT1       | c.2594 G>A        | p.R781Q           | Missense      | Heterozygous | CM*    | Variant in cancer             |
| KIT        | c.2386 A>T        | p.R796stop        | Nonsense      | Heterozygous | 365    | n.f.                          |
| PIK3CA     | c.1790 A>C        | p.E545A           | Missense      | Heterozygous | BOON*  | 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).

^bThe 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](image1.png)

*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](image2.png)

*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 | PDGFRalpha |
|--------------|-------|------|-----|------------|
| **Total number of PETs** |       |      |     |            | 135/135 (100%) | 135/135 (100%) |
| **Nonfunctioning** | 17/106 (16%) | 3/106 (3%) | 16/106 (11%) | 135/135 (100%) | 103/103 (100%) | 103/103 (100%) |
| **Functioning** | 1/34 (3%) | 0/34 (–) | 4/34 (12%) | 32/32 (100%) | 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.

*aPositive immunostaining refers to the detection of both membranous and cytoplasmic signals.

*bFive 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 \( MEN1 \)

| Parameter                  | n   | KIT-positive tumours | \( P^a \) |
|----------------------------|-----|----------------------|-----------|
| WHO classification         |     |                      |           |
| 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, PDK1 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 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, positive/total (%) | Antibody information |
|-----------------------|------------------------------|----------------------|
| **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)                     | 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**        |                              |                      |
| Fjällskog et al. [30] | 38/38 21/37 (57%)         | Santa Cruz |
| Chaudhry et al. [35] | 4/5                          | In-house^e |

^aExcept for PDGFRalpha, only the staining of neoplastic cells from primary tumours was considered.
^bManufacturers.
^cThese tumours were all gastrinomas.
^dThe overall frequency of EGFR expression refers to the positive immunostaining with either antibodies as reported in this study
^eFor information about PDGFRalpha antibody refers to Eriksson et al. [60]
immunostaining was carried out using FDA approved DakoCytoation 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**

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**references**

1. Antonello D, Gobbo S, Corbo V et al. Update on the molecular pathogenesis of pancreatic tumors other than common ductal adenocarcinoma. Pancreatology 2009; 9: 25–33.
2. Capelli P, Martignoni G, Pedica F et al. Endocrine neoplasms of the pancreas: pathologic and genetic features. Arch Pathol Lab Med 2009; 133: 350–364.
3. Corbo V, Dalai I, Scardoni M et al. MEN1 in pancreatic endocrine tumors: analysis of gene and protein status in 169 sporadic neoplasms reveals alterations in the vast majority of cases. Endocr Relat Cancer 2010; 17: 771–783.
4. Kloppel G, Ferren A, Heitz PU. The gastroenteropancreatic neuroendocrine cell system and its tumors: the WHO classification. Ann N Y Acad Sci 2004; 1014: 13–27.
5. Bettini R, Mantovani W, Boninsegna L et al. Primary tumour resection in metastatic nonfunctioning pancreatic endocrine carcinomas. Dig Liver Dis 2009; 41: 49–55.
6. Steeghs N, Nortier JW, Gelderblom H. Small molecule tyrosine kinase inhibitors in the treatment of solid tumours: an update of recent developments. Ann Surg Oncol 2007; 14: 942–953.
7. Leary A, Johnston SR. Small molecule signal transduction inhibitors for the treatment of solid tumours. Cancer Invest 2007; 25: 347–365.
8. Fabbro D, Ruetz S, Buchdunger E et al. Protein kinases as targets for anticancer agents: from inhibitors to useful drugs. Pharmacol Ther 2002; 93: 79–98.
9. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. Nature 2001; 411: 355–365.
10. Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell 2000; 103: 211–225.
11. Manning G, Whyte DB, Martinez R et al. The protein kinase complement of the human genome. Science 2002; 298: 1912–1934.
12. Boulton J. Ataxia telangiectasia gene mutations in leukemia and lymphoma. J Clin Pathol 2001; 54: 512–516.
13. Duran I, Salazar R, Casanovas O et al. New drug development in digestive neuroendocrine tumors. Ann Oncol 2007; 18: 1307–1313.
14. Grozinsky-Glasberg S, Franchi G, Teng M et al. Octreotide and the mTOR inhibitor RAD001 (everolimus) block proliferation and interact with the Akt-mTOR-p70S6K pathway in a neuro-endocrine tumour cell line. Neuroendocrinology 2008; 87: 168–181.
15. Moreno A, Akcakanat A, Munsell MF et al. Antitumor activity of rapamycin and octreotide as single agents or in combination in neuroendocrine tumors. Endocr Relat Cancer 2008; 15: 257–266.
16. Strosberg JR, Kvisl K. A review of the current clinical trials for gastroenteropancreatic neuroendocrine tumors. Expert Opin Investig Drugs 2007; 16: 219–224.
17. Missiaglia E, Dalai I, Baril S et al. Pancreatic endocrine tumors: expression profiling evidences a role for AKT-mTOR pathway. J Clin Oncol 2010; 28: 245–255.
18. Bardelli A, Parsons DW, Silliman N et al. Mutational analysis of the tyrosine kinase kinome in colorectal cancers. Science 2003; 300: 949.
19. Davies H, Hunter C, Smith R et al. Somatic mutations of the protein kinase gene family in human lung cancer. Cancer Res 2005; 65: 7591–7595.
20. Stephens P, Edkins S, Davies H et al. A screen of the complete protein kinase gene family identifies diverse patterns of somatic mutations in human breast cancer. Nat Genet 2005; 37: 590–592.
21. Thomas RK, Baker AC, DeBiasi RM et al. High-throughput oncogene mutation profiling in human cancer. Nat Genet 2007; 39: 347–351.
22. Tokunaga E, Oki E, Nishida K et al. Trastuzumab and breast cancer: developments and current status. Int J Clin Oncol 2006; 11: 199–208.
23. Vogel CL, Cobleigh MA, Tripathy D et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 2002; 20: 719–726.
24. Hirsch F, Varella-Garcia M, Cappuzzo F et al. Combination of EGFR gene copy number and protein expression predicts outcome for advanced non-small-cell lung cancer patients treated with gefitinib. Ann Oncol 2007; 18: 752–760.
25. John T, Liu G, Tsao MS. Overview of molecular testing in non-small-cell lung cancer: mutational analysis, gene copy number, protein expression and other biomarkers of EGFR for the prediction of response to tyrosine kinase inhibitors. Oncogene 2009; 28 (Suppl 1): S14–S23.
26. Shepherd FA, Tsao MS. Unraveling the mystery of prognostic and predictive factors in epidermal growth factor receptor therapy. J Clin Oncol 2006; 24: 1219–1220; author reply 1220–1211.
27. Peghini PL, Iwamoto M, Raffeld M et al. Overexpression of epidermal growth factor and hepatocyte growth factor receptors in a proportion of gastrinomas correlates with aggressive growth and lower curability. Clin Cancer Res 2002; 8: 2273–2285.
28. Proca DM, Frankel WL. Pancreatic endocrine tumors-c-erb B2 (Her-2/neu), bcl-2, and p53 immunohistochemical testing and their value in assessing prognosis. Appl Immunohistochem Mol Morphol 2008; 16: 44–47.
29. Papachouda B, Erickson LA, Rohlinger AL et al. Epidermal growth factor receptor and activated epidermal growth factor receptor expression in gastrointestinal carcinoids and pancreatic endocrine carcinomas. Mod Pathol 2005; 18: 1329–1335.
30. Fiallos LV, Lejonklou MH, Oberg KE et al. Expression of molecular targets for tyrosine kinase receptor antagonists in malignant endocrine pancreatic tumors. Clin Cancer Res 2003; 9: 1469–1473.
31. Bergmann F, Breining M, Hopfner M et al. Expression pattern and functional relevance of epidermal growth factor receptor and cyclinB1/CCNB1: novel chemotherapeutic targets in pancreatic endocrine tumors? Am J Gastroenterol 2009; 104: 171–181.
Ikenoue T, Kanai F, Hikiba Y et al. Functional analysis of PIK3CA gene mutations in pancreatic endocrine tumors. Hum Pathol 2001; 32: 1184–1189.

Zhang L, Smyrk TC, Oliveira AM et al. KIT is an independent prognostic marker for pancreatic endocrine tumors: a finding derived from analysis of islet cell differentiation markers. Am J Surg Pathol 2009; 33: 1562–1569.

Goebel SU, Iwamoto M, Raffeld M et al. Her-2/neu expression and gene amplification in gastrinomas: correlations with tumor biology, growth, and aggressiveness. Cancer Res 2002; 62: 3702–3710.

Chaudhry A, Papanicolaou V, Oberg K et al. Expression of platelet-derived growth factor receptor in neuroendocrine tumors of the digestive system. Cancer Res 1992; 52: 1006–1012.

Capurso G, Lattimore S, Cnogorac-Jurcevic T et al. Gene expression profiles of progressive pancreatic endocrine tumours and their liver metastases reveal potential novel markers and therapeutic targets. Endocr Relat Cancer 2006; 13: 541–558.

Cecconi D, Donadelli M, Rinalducci S et al. Proteomic analysis of pancreatic endocrine tumor cell lines treated with the histone deacetylase inhibitor trichostatin A. Proteomics 2007; 7: 1644–1653.

Corbo V, Ritelli R, Barbi S et al. Mutational profiling of kinases in human tumours of pancreatic origin identifies candidate cancer genes in ductal and ampulla of vater carcinomas. PLoS One 2010; 5: e12653.

Kononen J, Bubendorf L, Kallioniemi A et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998; 4: 844–847.

Brunelli M, Eble JN, Zhang S et al. Eosinophilic and classic chromophobe renal cell carcinomas have similar frequent losses of multiple chromosomes from among chromosomes 1, 2, 6, 10, and 17, and this pattern of genetic abnormality is not present in renal oncocytoma. Mod Pathol 2005; 18: 161–169.

Shihoh Y. ATM and related protein kinases: safeguarding genome integrity. Nat Rev Cancer 2003; 3: 155–168.

Renwick A, Thompson D, Seal S et al. ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. Nat Genet 2006; 38: 873–875.

Pauls K, Merkellbach-Bruse S, Thal D et al. PDGFRalpha- and c-kit-mutated gastrointestinal stromal tumours (GISTs) are characterized by distinctive histological and immunohistochemical features. Histopathology 2005; 46: 166–175.

Samuels Y, Wang Z, Bardelli A et al. High frequency of mutations of the PIK3CA gene in human cancers. Science 2004; 304: 554.

Ikenoue T, Kanai F, Hikiba Y et al. Functional analysis of PIK3CA gene mutations in human colorectal cancer. Cancer Res 2005; 65: 4562–4567.

Bader AG, Kang S, Vogt PK. Cancer-specific mutations in PIK3CA are oncogenic in vivo. Proc Natl Acad Sci U S A 2006; 103: 1475–1479.

Jang JH, Shin KH, Park JG. Mutations in fibroblast growth factor receptor 2 and fibroblast growth factor receptor 3 genes associated with human gastric and colorectal cancers. Cancer Res 2001; 61: 3541–3543.

Piotnikov AN, Schlessinger J, Hubbard SR, Mohammadi M. Structural basis for FGFR receptor dimerization and activation. Cell 1999; 98: 641–650.

Lee JW, Soung YH, Kim SY et al. PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. Oncogene 2005; 24: 1477–1480.

Samuels Y, Diaz LA Jr., Schmidt-Kittler O et al. Mutant PIK3CA promotes cell growth and invasion of human cancer cells. Cancer Cell 2005; 7: 561–573.

Moore PS, Missiaglia E, Antonello D et al. Role of disease-causing genes in sporadic pancreatic endocrine tumors: MEN1 and VHL. Genes Chromosomes Cancer 2001; 32: 177–181.

Rindi G, Bardi C. Endocrine tumours of the gastrointestinal tract: aetiology, molecular pathogenesis and genetics. Best Pract Res Clin Gastroenterol 2005; 19: 519–534.

Hirota S, Itozaki K, Moriyama Y et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 1998; 279: 577–580.

Zamo A, Bertola A, Franceschetti I et al. Microfluidic deletion/insertion analysis for rapid screening of KIT and PDGFRα mutations in CD117-positive gastrointestinal stromal tumors: diagnostic applications and report of a new KIT mutation. J Mol Diagn 2007; 9: 151–157.

Demetri GD, von Mehren M, Blanke CD et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002; 347: 472–480.

Yao JC, Zhang JX, Rashid A et al. Clinical and in vitro studies of imatinib in advanced carcinoid tumors. Clin Cancer Res 2007; 13: 234–240.

Jones S, Zhang X, Parsons DW et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008; 321: 1801–1806.

von Wichert G, Jehle PM, Hoeflich A et al. Insulin-like growth factor-I is an autocrine regulator of chromogranin A secretion and growth in human neuroendocrine tumor cells. Cancer Res 2000; 60: 4573–4581.

Kulke MH, Lenz HJ, Meropol NJ et al. Activity of sunitinib in patients with advanced neuroendocrine tumors. J Clin Oncol 2008; 26: 3403–3410.

Eriksson A, Siegbahn A, Westermark B et al. PDGF alpha- and beta-receptors activate unique and common signal transduction pathways. Embo J 1992; 11: 543–550.