

## MANAGEMENT OF ENDOCRINE DISEASE

**Precision medicine in neuroendocrine neoplasms: an update on current management and future perspectives****Germano Gaudenzi<sup>1</sup>, Alessandra Dicitore<sup>2</sup>, Silvia Carra<sup>3</sup>, Davide Saronni<sup>2</sup>, Carlotta Pozza<sup>4</sup>, Elisa Giannetta<sup>4</sup>, Luca Persani<sup>2,3</sup> and Giovanni Vitale<sup>1,2</sup>**

<sup>1</sup>Istituto Auxologico Italiano, IRCCS, Laboratorio Sperimentale di Ricerche di Neuroendocrinologia Geriatrica ed Oncologica, Milan, Italy, <sup>2</sup>Department of Clinical Sciences and Community Health (DISCCO), University of Milan, Milan, Italy, <sup>3</sup>Laboratory of Endocrine and Metabolic Research, Istituto Auxologico Italiano IRCCS, Milan, Italy, and <sup>4</sup>Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy

Correspondence should be addressed to G Vitale  
**Email**  
[giovanni.vitale@unimi.it](mailto:giovanni.vitale@unimi.it)

**Abstract**

Neuroendocrine neoplasms (NENS) are traditionally considered as a single group of rare malignancies that originate from the highly spread neuroendocrine system. The clinical management is complex due to the high heterogeneity of these neoplasms in terms of clinical aggressiveness and response to the therapy. Indeed, a multidisciplinary approach is required to reach a personalization of the therapy, including cancer rehabilitation. In this review, we discuss the possibility to adopt a precision medicine (PM) approach in the management of NENS. To this purpose, we summarize current knowledge and future perspectives about biomarkers and preclinical *in vitro* and *in vivo* platforms, potentially useful to inform clinicians about the prognosis and for tailoring therapy in patients with NENS. This approach may represent a breakthrough in the therapy and tertiary prevention of these tumors.

*European Journal of Endocrinology*  
(2019) **180**, R1–R10

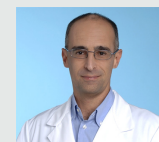
**Introduction**

Neuroendocrine neoplasms (NENS) are a group of neoplasms derived from the neuroendocrine system, expressing markers of neuroendocrine differentiation, such as chromogranin A (CgA), synaptophysin and neuron-specific enolase (NSE), as well as several hormones

(1). Although surgery remains the cornerstone of treatment for localized tumors, most patients with NENS are diagnosed once metastases have occurred. These patients require a chronic medical management defined through a multidisciplinary approach. The main factors

**Invited Author's profile**

**Giovanni Vitale** is an associate professor in endocrinology at the University of Milan – Italy – and director of the Laboratory of Geriatric and Oncologic Neuroendocrinology Research at Istituto Auxologico Italiano IRCCS. His research interests involve the development of new therapeutic strategies in the field of neuroendocrine tumors with a translational approach from bench to bedside. In recent years, he has developed an innovative preclinical model for neuroendocrine tumors based on xenograft in zebrafish embryos.



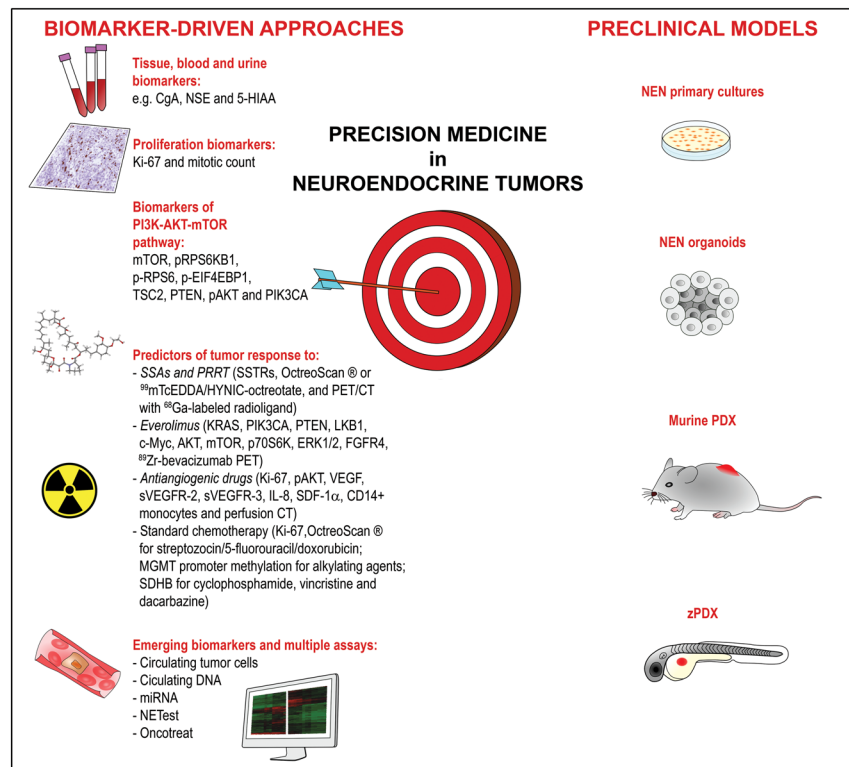
that currently play an important role in establishing the treatment are substantially the grade and the stage of the tumor, the anatomic site of origin and the presence of a functioning syndrome. However, clinical efficacy of current treatment strategies is limited by the high biological heterogeneity of these neoplasms in the clinical aggressiveness and response to the therapy (2). In this context, a precision medicine (PM)-based strategy, through the biomarker-driven approach and preclinical models, could be helpful for the management of NENs (Fig. 1).

## Biomarkers in PM for NENs

Tissue biomarkers, routinely used in the clinical practice, have a diagnostic role in verifying the neuroendocrine phenotype (CgA, synaptophysin and NSE), determining the grade (Ki-67 and mitotic count) and discriminating between functioning (secreting serotonin, insulin, gastrin, glucagon, VIP, somatostatin, catecholamines, PTHrp, ACTH, GH, ADH, calcitonin, GNRH, CRH, etc.) or non-functioning tumors (3). However, currently available neuroendocrine phenotype markers have some limitations in the diagnostic phase when dosed in the blood. Despite being long identified as the most relevant NEN-related

serum marker, the utility of circulating CgA is limited for the diagnosis of NEN. CgA assays still lack standardization, thus limiting not only clinical management but also the comparison between different analyses. Moreover, the test specificity is hampered by non-oncological causes, such as benign diseases and iatrogenic conditions (proton pump inhibitors and histamine type-2 receptor antagonists), and by the fact that a variety of non-NEN malignancies are characterized by increased CgA levels (4, 5, 6). Another limitation of CgA assays is the sensitivity that ranges between 32 and 92% and is dependent on the type of NEN, the functional status and the size of the tumor (3). Circulating NSE is also not relevant for the diagnosis of NEN, being not actively secreted but released by tumor cells with an intense cytolysis in poorly differentiated NEN (4). Conversely, the 24h urinary 5-hydroxyindoloacetic acid (5-HIAA) has a potential diagnostic utility as all markers of functioning endocrine syndromes. 5-HIAA is increased in typical carcinoid syndrome, and therefore, represents a crucial marker, particularly in ileal NENs associated with carcinoid syndrome where the sensitivity and specificity can reach 85 and 100%, respectively (4).

In addition to diagnostic biomarkers, another area of interest for NENs includes the research of biomarkers with a prognostic value. Ki-67 index and mitotic count are routinely used to determine the tumor grading and



**Figure 1**

Overview of available biomarker-driven approaches and preclinical models for the development of PM in NENs. CgA, chromogranin A; NEN, neuroendocrine neoplasm; NSE, neuron-specific enolase; PDX, patient-derived xenograft; PET-CT, positron emission tomography/computed tomography; PRRT, peptide receptor radionuclide therapy; SSAs, somatostatin analogs; sVEGFR-2/3, soluble VEGF receptor-2/3; zPDX, zebrafish PDX.

cell proliferation rate. While Ki-67 index has a prognostic role in gastroenteropancreatic (GEP)-NENs, relatively less data support the same use for bronchial NENs (7, 8). The mitotic count has been shown to be prognostic in most of the NENs (9, 10). Elevated CgA and NSE levels are associated with poor progression-free survival (PFS) and overall survival (OS) in NENs. In addition, NSE expression is usually elevated in poorly differentiated NENs (11).

Other studies have been focused on the research of prognostic biomarkers between members of PI3K-AKT-mTOR pathway (12). In this context, the overexpression of mTOR protein has been suggested as a negative prognostic factor (13). In pancreatic NENs mutations in PI3K-AKT-mTOR pathway genes have been reported in 15% of patients. These genetic alterations seem to confer worse prognosis than other mutations linked to NENs (14). Genetic alterations of *TSC2* have been reported in 8% of pancreatic NENs and resulted to be associated with a reduced OS (15). On the other hand, although loss of 10q, the region containing *PTEN*, was present in about 30% of pancreatic NENs (16) and mutations in *PIK3CA/PTEN* have been found in 22% of poorly differentiated NENs, no prognostic value has been reported for these genetic modifications (17, 18). Indeed, given the complexity of this pathway, increased by the cross-talk with other molecular signaling, further studies are needed to get new insights into the prognostic role of its genetic and molecular alterations.

A great interest in the research of NEN biomarkers is represented by the identification of predictors of tumor response to the medical therapy. Although Ki-67 and mitotic count are currently used in the decision making of NENs treatment, technical issues about measurement of these parameters and tumor heterogeneity may weaken their predictive role (19). In addition, none of conventional circulating biomarkers have shown a high predictive accuracy (20). However, recent studies have investigated the potential predictive role of therapeutic response to current anti-cancer therapy for several biomarkers in NENs. These biomarkers are grouped on the basis of therapeutic interventions as follows:

- *Somatostatin analogs (SSAs) and peptide receptor radionuclide therapy (PRRT)*: Although most clinicians agree that the presence of somatostatin receptors (SSTRs) should be verified before treatment with SSAs is initiated (21), only few studies showed that SSTR expression can predict the response to the therapy with SSAs in NENs (22, 23, 24). However, in CLARINET and PROMID trials, showing a significant

delay in disease progression after treatment with SSA, SSTR scintigraphy was positive in 100 and 86% of enrolled patients, respectively (25, 26). One of the most clinically relevant therapeutic innovation in NEN has been the development of PRRT through the use of SSAs labeled with beta-emitting radionuclides, in patients with unresectable grade 1 or 2 NENs and high SSTR expression (27). In these cases, nuclear medicine imaging, such as scintigraphy with <sup>111</sup>Indium pentetreotide (OctreoScan®) or <sup>99m</sup>TcEDDA/HYNIC-octreotate and positron emission tomography (PET) with <sup>68</sup>Ga-labeled radioligands, has some predictive ability in determining the functional response.

- *mTOR inhibitors*: Although in several clinical studies, no valid biomarker has been identified so far to predict response to mTOR inhibitors, such as everolimus, few preclinical studies have recently explored mechanisms involved in the resistance to mTOR inhibitors. These data would be useful in future for the identification of predictive biomarkers. Besides KRAS and PIK3CA mutations, that conferred resistance to everolimus therapy (28), it has been showed that loss of PTEN and LKB1 with activation of c-Myc decreased sensitivity to treatment with mTOR inhibitors in pancreatic NEN cell lines (29). Evidence collected on human bronchial NEN primary cultures suggested that lower expression of mTOR, p70S6K, AKT and ERK1/2 could be predictive markers of resistance to mTOR inhibitors (30). Other genetic alterations correlated with resistance to everolimus therapy, such as the FGFR4-G388R single nucleotide polymorphism (31).

Given that mTOR inhibition reduced VEGF-A secretion in three murine GEP-NEN cell lines (32), it has been proposed that levels of this cytokine could measure the response to everolimus (33). However, circulating VEGF-A in NEN patients treated with everolimus has not shown a clear predictive value yet. The tumor uptake of <sup>89</sup>Zr-bevacizumab, a radioactive-labeled VEGF-A antibody, diminished during everolimus treatment in patients with well-differentiated NENs. Therefore, serial <sup>89</sup>Zr-bevacizumab PET might be useful as an early predictive imaging-based biomarker for the treatment with everolimus or other drugs targeting VEGF system in NEN patients (33).

- *Antiangiogenic therapies*: Sunitinib is a multi-targeted tyrosine kinase inhibitor. Among tissue biomarkers, low Ki-67 and pAKT expression correlated with a better response to sunitinib in NENs (34). The predictive role of circulating levels of VEGF, soluble VEGF receptors (sVEGFR-2 and sVEGFR-3), IL-8 and stromal

cell-derived factor (SDF)-1 $\alpha$ , have been analyzed in patients with pancreatic NEN and carcinoid tumors treated with sunitinib. Baseline level of sVEGFR-2 resulted more elevated in patients with pancreatic NEN and longer OS (35), as previously reported in NEN patients treated with pazopanib, another tyrosine kinase inhibitor (36). In carcinoid patients low pre-treatment IL-8 levels were associated with longer PFS and OS. In addition, low baseline concentrations of sVEGFR-3 and SDF-1 $\alpha$  were associated with longer PFS and OS in both pancreatic NENs and carcinoid tumors patients (35). A significant increase from baseline of VEGF, IL-8 and SDF-1 $\alpha$ , and a decrease in sVEGFR-2 and sVEGFR-3 were observed in patients with NENs at the end of the first cycle of treatment with sunitinib (35).

Bevacizumab, a VEGF monoclonal antibody, is another targeted therapy used in advanced NENs. In patients with metastatic or unresectable NEN, the decrease in blood flow and blood volume observed through perfusion CT during treatment with bevacizumab and everolimus correlated with RECIST response (37). However, larger prospective studies are needed for validation of these potential predictive biomarkers.

- *Standard chemotherapy:* Ki-67 has been proposed for selecting patients for chemotherapy in NENs due to the direct association between tumor grade and response (38). However, clinically useful threshold for Ki-67 has not well defined (38). Other predictive biomarkers have been currently identified for cytotoxic drugs. In pancreatic NENs, a positive OctreoScan® was predictive of an objective response to streptozocin/5-fluorouracil/doxorubicin (39). Moreover, it has been reported that MGMT promoter methylation status and protein deficiency were associated with a better response rate to alkylating agents (40, 41). In pheochromocytomas and paraganglioma patients with SDHB mutations showed a higher risk for developing metastatic disease, but they responded better than non-mutation carriers to the therapy with cyclophosphamide, vincristine and dacarbazine (42).

In the last years, due to a better understanding of molecular mechanisms involved in the development of NENs (43) and advances in the technologies, a new generation of biomarkers and multiple assays have been recently developed. Preliminary data are available in NENs:

- *Circulating tumor cells (CTCs)* could provide prognostic information in real time and in terms of tumor

progression and OS in NENs (44, 45). Patients with a negative CTC count showed a better prognosis in terms of PFS and OS as compared to patients with  $\geq 1$  CTC. In addition, a >50% reduction of CTCs count after the treatment was associated with a better outcome (45). Therefore, CTC detection could be also an attractive method to monitor disease progression and response during the treatment. In addition, the molecular characterization of isolated CTCs might have clinical relevance for therapeutic decision making through the identification of specific molecular targets (45). SSTRs have been recently measured in CTCs isolated from patients with GEP-NENs (46). This could be useful in future for the selection of patients to treat with SSAs or PPRT. A recent study showed that CTC copy number alteration may represent a new predictor of response to chemotherapy in patients with small-cell lung cancer (47).

- *Circulating tumor DNA (ctDNA)* consists of short nuclear fragments (~166bp) released in the blood from apoptotic or necrotic tumor cells. ctDNA analysis can be potentially useful for NEN management. It has been observed that the ctDNA levels rise during tumor progression, whereas decline after therapy. In this way, ctDNA levels surveillance could guide drug treatment and provide a more comprehensive representation of the mutational landscape of the NEN, as recently reported in few anecdotal reports (48, 49). In addition, changes in allele frequencies over time could reflect subclonal evolution, supplying the opportunity to adjust the therapy in order to overcome newly developing resistance. Finally, it has been noted an upward trend in ctDNA concentration earlier than current biomarkers, useful for an earlier prediction of disease recurrence (50).
- *miRNAs* are endogenous small non-coding RNAs that control post-transcriptional eukaryotic gene expression. Their tissue and blood levels have been associated to prognosis and prediction of therapeutic outcome in several cancers. As reported by Zatelli *et al.*, few evidences are currently available about the prognostic potential of miRNAs in NENs and they are limited to their tissue expression in lung NENs, pancreatic NENs, medullary thyroid cancer and pheochromocytoma (51).
- *NETest* is a multianalyte liquid biopsy procedure that measures the circulating expression level of 51 genes involved in cancerogenesis, cell proliferation, signaling, secretion and metastasis formation through a peripheral blood real-time polymerase chain reaction.

This procedure has been tested in GEP and pulmonary NENs. The NETest provides with high sensitivity (85–98%) and specificity (93–97%) information about the diagnosis, completeness of surgical resection and the presence of residual disease in patients with NENs. This test can also predict the therapeutic efficacy of SSAs and PRRT (52). Moreover, NETest is standardized, reproducible and is not influenced by age, gender, ethnicity, fasting or proton pump inhibitors (53).

- *OncoTreat* is an innovative platform based on the systematic prioritization of anti-cancer drugs. The rationale of *OncoTreat* starts from the ability of drugs to invert the expression profile of master regulator proteins, whose coordinated activity is necessary for the modulation of tumor check points (54). *OncoTreat* was set up in a cohort of 212 patients with GEP-NEN. In the first phase, a transcriptome analysis identified several master regulator proteins that include key regulators of neuroendocrine lineage progenitor state and immunoevasion. In the second phase, a prioritization of small molecules was performed by a transcriptome analysis aimed at identifying which molecules were able to invert the activity of GEP-NEN master regulator proteins in H-STS cells, a cell line derived from GEP-NEN patient (55). Interestingly, results of this study lead FDA to approve the Investigational New Drug Application for entinostat in GEP-NENs (54). Therefore, *OncoTreat* appears to be a promising strategy for the development of PM applications, ideally complemented by preclinical models to predict which drugs a patient will respond to.

### The promising role of preclinical models in PM

Although the biomarker-driven approach is contributing to the evolution of tailored therapies for some tumors, the characterization of the genetic and molecular profiles of tumor cells does not often translate into a successful clinical outcome, due to the spatial and temporal heterogeneity of these cells (56). Several preclinical models have been indicated as promising platform for the development of PM applications, able to capture the heterogeneous nature of human cancers. For instance, short-term primary culture cells derived from solid tumors, also known as *ex vivo*, have gained significant importance in personalized cancer therapy (57). Recently, a human platform technology called CANscript™ has been developed to predict the response to anti-cancer

drugs in patients with head and neck squamous cell carcinoma and colorectal cancer. Thin tumor sections were cultured *ex vivo*, on grade-matched tumor matrix support in a medium addicted with autologous patient serum and treated with selected drugs. Then, the clinical response was predicted from several parameters detected on *ex vivo* cultures, by a sophisticated machine-learning trained algorithm, showing a 100% sensitivity and 92% specificity (58).

Nowadays, patient-derived xenografts (PDXs) in mice represent the most robust and investigated experimental platform for the development of PM applications (59). To generate PDXs, solid tumors, collected after surgery or biopsy procedures, are inoculated as pieces or single-cell suspensions subcutaneously into the flank or in the same organ, as the original tumor of the animal. Several mouse strains, having different degrees of immunosuppression are currently available for these studies (60). Although an engraftment-associated selection has been reported, PDXs preserve the histological organization, the genetic and epigenetic mutational profile and the gene expression patterns, as in the patient counterpart. PDXs have showed also a high potential in predicting drug response to pharmacological treatments, as demonstrated in colorectal cancer (61). PDXs have been recently used to perform co-clinical studies, in which patient-derived tumor cells, isolated from a patient enrolled in a clinical trial, are implanted into immunocompromised mice that are subsequently treated with the same drugs of the patient to emulate clinical response (62). Compared to conventional phase I/II clinical trials, PDX-based co-clinical studies have the advantage of analyzing and integrating preclinical and clinical data in a real-time manner. This aspect is crucial to study mechanisms of drug resistance and to explore the therapies that can be administered to the patient (60).

### Preclinical models for PM in NENs

Several preclinical models have been recently developed in NENs (Fig. 1) with relevant advantages and potential application in the clinical management of this tumor (Table 1).

#### *In vitro* models

Although human immortalized NEN cell lines have significantly contributed to the study of pathways involved in carcinogenesis and the screening of

**Table 1** Advantages and limitations of NEN preclinical models.

	Advantages	Limitations
Immortalized cell lines	<ul style="list-style-type: none"> <li>• Unlimited lifespan</li> <li>• Easy to handle and manipulate</li> <li>• Large number of cells</li> <li>• Study of pathways involved in carcinogenesis and preliminary drug screening</li> </ul>	<ul style="list-style-type: none"> <li>• Lack of cellular heterogeneity</li> <li>• Accumulation of genetic changes over time</li> <li>• Loss of well-differentiated neuroendocrine phenotype</li> </ul>
Primary cultures (2D and 3D organoids)	<ul style="list-style-type: none"> <li>• Fast procedure</li> <li>• Identification of novel molecular targets and drug screening</li> </ul>	<ul style="list-style-type: none"> <li>• Possible loss of tumor heterogeneity</li> <li>• Difficulties in the culture establishment due to the low proliferation rate of NENs</li> </ul>
Murine patient-derived xenografts	<ul style="list-style-type: none"> <li>• Realistic heterogeneity of tumor cells</li> <li>• Preservation of genetic and epigenetic characteristics of primary tumor</li> <li>• Preclinical drug screening and co-clinical trials</li> <li>• High prognostic and predictive potential</li> </ul>	<ul style="list-style-type: none"> <li>• Large number of tumor cells</li> <li>• Long time to establish</li> <li>• Immunosuppressed animals limit a realistic tumor microenvironment</li> <li>• Difficulties to generate mouse xenograft models able to metastasize</li> <li>• Possibility of engraftment-associated selection</li> <li>• Low engraftment rate for NENs</li> </ul>
Zebrafish patient-derived xenografts	<ul style="list-style-type: none"> <li>• Small number of tumor cells for the implant</li> <li>• Possibility to implant high number of embryos</li> <li>• Real-time visualization</li> <li>• Fast model for the analysis of tumor-induced angiogenesis and migration</li> <li>• Lack of an acquired immune system in embryos and larvae</li> <li>• High engraftment rate</li> <li>• Preclinical drug screening and co-clinical trials</li> <li>• High predictive potential</li> </ul>	<ul style="list-style-type: none"> <li>• Difficulties in orthotopic implantation</li> <li>• Difficulties in long-term analyses</li> <li>• Several organs or systems are still developing in embryos</li> <li>• Little knowledge about the maintenance of tumor microenvironment in zebrafish</li> </ul>

compounds with antitumor activity and related drug resistance mechanisms (63), they have some limitations, such as the accumulation of genetic changes over time in culture and the lack of cellular heterogeneity. In addition, some NEN cell lines do not display well-differentiated neuroendocrine phenotype or have a very low expression of some key receptors for drug treatment (such as SSTRs). In this context, experimental data obtained from NEN cell lines should be carefully validated with primary cell cultures derived from NEN patients (64), even if establishment of these cells could be difficult, due to the low proliferation rate of NEN cells. In the context of PM, the main advantage of primary tumor cell culture is the possibility to evaluate the potential efficacy of several antitumor compounds in a short time, through a system where intratumor heterogeneity and the original genetic signature of the tumor are preserved (57). NEN primary cell cultures could also be used to perform preliminary preclinical studies for the identification of novel druggable molecular targets.

Powerful *in vitro* platforms, which can facilitate the development of PM strategies, have been recently set up using 3D patient-derived organoid cultures also in

NENs (65). Organoids can be cultured from a small sample size, derived from needle biopsy and generated from different areas of the tumor in order to better mimic genetic and phenotypic heterogeneity of the tumor (66). These models could serve as a platform to combine high-throughput drug screening and genomic analysis on patient-derived tumor samples, thus offering a unique opportunity to stratify and identify efficacious therapies for individual patients.

### ***In vivo* models**

NEN-PDX murine models of MTC (67) and high-grade pulmonary NENs (68) have been used in preclinical research to investigate the efficacy of experimental anti-cancer drugs. NEN-PDX murine models recapitulate some peculiarity of tumors in patients. For instance, the gastric neuroendocrine carcinoma PDX model GA0087 has showed a metastatic behavior supported by the high expression of VEGF-A as in patients with gastric NEC (69). A genomewide analysis on a PDX model of neuroendocrine prostate cancer in mice has showed that CBX2 and EZH2, members of the polycomb group family

of transcriptional repressors, are upregulated, as in patients with this disease (70). PDXs of pancreatic NEN can develop resistance to everolimus. In this study, the inhibitor of the mTOR pathway sapanisertib showed a potent antitumor effect also on everolimus-resistant PDXs, leading to the suggestion of a new alternative pharmacological strategy for everolimus-resistant NEN (71). However, the use of murine NEN-PDX models is very limited in the research of PM strategies probably due to the rarity of NENs, the limited size of post-surgical samples for most of these tumors and the low rate of successful tumor engraftment (72).

Recently, zebrafish PDX (zPDX) has been suggested as promising platform for the development of PM applications (73, 74). Fior and collaborators have demonstrated that zPDX has a strong predictive potential in patients with colorectal cancer treated with chemotherapy and biological therapy (74). In this respect, we have recently set up a NEN-zPDX platform, based on the injection of red fluorescent labeled NEN cells into the subperidermal cavity of *Tg(fli1a:EGFP)<sup>v1</sup>* zebrafish embryos (73, 75). This transgenic line, expressing the enhanced green fluorescent protein (EGFP) in the endothelial cells of the entire vascular tree, offers the possibility to estimate the proangiogenic potential and the metastatic behavior of injected tumor cells derived from each patient tissue. In addition to the advantages due to the intrinsic features of the zebrafish model, as the high fecundity, the outer fertilization and the optical transparency, our PDX platform can overcome some general drawbacks of murine engraftment procedure. Although mouse is considered the gold standard for PDXs, several limitations have been reported, such as the large number of tumor cells to be implanted (about 1 million for each animal) and the long time required for the implantation (from several weeks to months), the need of immunosuppressed animals to avoid transplant rejection and the difficulties to generate mouse xenotransplant models able to metastasize (76). We have demonstrated that NEN PDXs can stimulate angiogenesis in zebrafish embryos within few days and without the need of immunosuppression, because the adaptive immune response is not completely developed during the early development of zebrafish (73). Compared to mouse tumor models, in which the spread of tumor cells cannot be analyzed in real time after the transplantation, the transparency of zebrafish embryos allows to follow in real time the invasive behavior of fluorescent-labeled tumor cells (73). Besides, in zebrafish model, the possibility to study the effects of small tumor implants (100 cells/embryo) resulted particularly suitable for

NENs, where post-surgical availability of tumor cells is often limited. Interestingly, the success of NEN cells transplantation in zebrafish embryos resulted to be extraordinary higher compared than that reported for murine PDXs (72).

In the near future, additional studies will be fundamental to clarify if NEN zPDXs and NEN patients might have similar response to the available therapeutic options, as recently reported for colorectal cancer (74). These studies could be supported by the versatility of zebrafish embryos in drug screening. Indeed, because of the permeability of zebrafish embryos to small molecules, a number of compounds can be added directly to the embryo water, whereas larger or not water-soluble molecules need to be injected into the body of the embryo to ensure drug uptake. The effects of antitumor compounds on tumor-induced angiogenesis, invasiveness, metastatic dissemination and tumor cell proliferation can be easily evaluated by epifluorescence microscopy and confocal microscopy within 3 days after implantation.

## Conclusions

Several biomarkers are routinely used for the classification of NENs and are currently relevant for the treatment selection. Although several prognostic and predictive biomarkers, which could support tailoring therapies have been recently identified (Fig. 1), most of them are far from being routinely adopted in clinical practice and further insights are needed.

Few available preclinical *in vitro* and *in vivo* models, derived from NEN patient cells, have provided first evidences of preserving molecular and behavioral features of the original NEN. The most promising preclinical platforms for PM in NENs are PDXs in mice and zebrafish embryos (Fig. 1). However, additional studies are needed to analyze the predictive potential of these innovative tools, as well as their translatability into the clinical practice, in order to improve the survival and quality of life in patients with advanced NENs.

---

### Declaration of interest

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

---

### Funding

This work was supported by the ministerial research project: PRIN 2017Z3N3YC.

## References

- Rindi G & Wiedenmann B. Neuroendocrine neoplasms of the gut and pancreas: new insights. *Nature Reviews Endocrinology* 2012 **8** 54–64. (<https://doi.org/10.1038/nrendo.2011.120>)
- Uri I & Grozinsky-Glasberg S. Current treatment strategies for patients with advanced gastroenteropancreatic neuroendocrine tumors (GEP-NETs). *Clinical Diabetes and Endocrinology* 2018 **4** 16. (<https://doi.org/10.1186/s40842-018-0066-3>)
- Hofland J, Zandee WT & de Herder WW. Role of biomarker tests for diagnosis of neuroendocrine tumours. *Nature Reviews: Endocrinology* 2018 **14** 656–669. (<https://doi.org/10.1038/s41574-018-0082-5>)
- Ferolla P, Faggiano A, Mansueto G, Avenia N, Cantelmi MG, Giovenali P, Del Basso De Caro ML, Milone F, Scarpelli G, Masone S *et al.* The biological characterization of neuroendocrine tumors: the role of neuroendocrine markers. *Journal of Endocrinological Investigation* 2008 **31** 277–286. (<https://doi.org/10.1007/BF03345602>)
- Marotta V, Nuzzo V, Ferrara T, Zuccoli A, Masone M, Nocerino L, Del Prete M, Marciello F, Ramundo V, Lombardi G *et al.* Limitations of chromogranin A in clinical practice. *Biomarkers* 2012 **17** 186–191. (<https://doi.org/10.3109/1354750X.2012.654511>)
- Marotta V, Zatelli MC, Sciammarella C, Ambrosio MR, Bondanelli M, Colao A & Faggiano A. Chromogranin A as circulating marker for diagnosis and management of neuroendocrine neoplasms: more flaws than fame. *Endocrine-Related Cancer* 2018 **25** R11–R29. (<https://doi.org/10.1530/ERC-17-0269>)
- Strosberg J, Nasir A, Coppola D, Wick M & Kvols L. Correlation between grade and prognosis in metastatic gastroenteropancreatic neuroendocrine tumors. *Human Pathology* 2009 **40** 1262–1268. (<https://doi.org/10.1016/j.humpath.2009.01.010>)
- Pelosi G, Rindi G, Travis WD & Papotti M. Ki-67 antigen in lung neuroendocrine tumors: unraveling a role in clinical practice. *Journal of Thoracic Oncology* 2014 **9** 273–284. (<https://doi.org/10.1097/JTO.0000000000000092>)
- Pape UF, Berndt U, Muller-Nordhorn J, Bohmig M, Roll S, Koch M, Willich SN & Wiedenmann B. Prognostic factors of long-term outcome in gastroenteropancreatic neuroendocrine tumours. *Endocrine-Related Cancer* 2008 **15** 1083–1097. (<https://doi.org/10.1677/ERC-08-0017>)
- Beasley MB, Thunnissen FB, Brambilla E, Hasleton P, Steele R, Hammar SP, Colby TV, Sheppard M, Shimamoto Y, Koss MN *et al.* Pulmonary atypical carcinoid: predictors of survival in 106 cases. *Human Pathology* 2000 **31** 1255–1265. (<https://doi.org/10.1053/hupa.2000.19294>)
- Lawrence B, Gustafsson BI, Kidd M, Pavel M, Svejda B & Modlin IM. The clinical relevance of chromogranin A as a biomarker for gastroenteropancreatic neuroendocrine tumors. *Endocrinology and Metabolism Clinics of North America* 2011 **40** 111–134, viii. (<https://doi.org/10.1016/j.ecl.2010.12.001>)
- Zatelli MC, Fanciulli G, Malandrino P, Ramundo V, Faggiano A, Colao A & NIKE Group. Predictive factors of response to mTOR inhibitors in neuroendocrine tumours. *Endocrine-Related Cancer* 2016 **23** R173–R183. (<https://doi.org/10.1530/ERC-15-0413>)
- Qian ZR, Ter-Minassian M, Chan JA, Imamura Y, Hooshmand SM, Kuchiba A, Morikawa T, Brais LK, Daskalova A, Heafield R *et al.* Prognostic significance of MTOR pathway component expression in neuroendocrine tumors. *Journal of Clinical Oncology* 2013 **31** 3418–3425. (<https://doi.org/10.1200/JCO.2012.46.6946>)
- Jiao Y, Shi C, Edil BH, de Wilde RF, Klimstra DS, Maitra A, Schulick RD, Tang LH, Wolfgang CL, Choti MA *et al.* DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science* 2011 **331** 1199–1203. (<https://doi.org/10.1126/science.1200609>)
- Gleeson FC, Voss JS, Kipp BR, Kerr SE, Van Arnam JS, Mills JR, Marcou CA, Schneider AR, Tu ZJ, Henry MR *et al.* Assessment of pancreatic neuroendocrine tumor cytologic genotype diversity to guide personalized medicine using a custom gastroenteropancreatic next-generation sequencing panel. *Oncotarget* 2017 **8** 93464–93475. (<https://doi.org/10.18632/oncotarget.18750>)
- Perren A, Komminoth P, Saremaslani P, Matter C, Feurer S, Lees JA, Heitz PU & Eng C. Mutation and expression analyses reveal differential subcellular compartmentalization of PTEN in endocrine pancreatic tumors compared to normal islet cells. *American Journal of Pathology* 2000 **157** 1097–1103. ([https://doi.org/10.1016/S0002-9440\(10\)64624-X](https://doi.org/10.1016/S0002-9440(10)64624-X))
- Vijayvergia N, Boland PM, Handorf E, Gustafson KS, Gong Y, Cooper HS, Sheriff F, Astsaturov I, Cohen SJ & Engstrom PF. Molecular profiling of neuroendocrine malignancies to identify prognostic and therapeutic markers: a Fox Chase Cancer Center Pilot Study. *British Journal of Cancer* 2016 **115** 564–570. (<https://doi.org/10.1038/bjc.2016.229>)
- Yuan F, Shi M, Ji J, Shi H, Zhou C, Yu Y, Liu B, Zhu Z & Zhang J. KRAS and DAXX/ATRX gene mutations are correlated with the clinicopathological features, advanced diseases, and poor prognosis in Chinese patients with pancreatic neuroendocrine tumors. *International Journal of Biological Sciences* 2014 **10** 957–965. (<https://doi.org/10.7150/ijbs.9773>)
- Chan DL, Clarke SJ, Diakos CI, Roach PJ, Bailey DL, Singh S & Pavlakis N. Prognostic and predictive biomarkers in neuroendocrine tumours. *Critical Reviews in Oncology/Hematology* 2017 **113** 268–282. (<https://doi.org/10.1016/j.critrevonc.2017.03.017>)
- Falconi M, Eriksson B, Kaltsas G, Bartsch DK, Capdevila J, Caplin M, Kos-Kudla B, Kwekkeboom D, Rindi G, Kloppel G *et al.* Enets consensus guidelines update for the management of patients with functional pancreatic neuroendocrine tumors and non-functional pancreatic neuroendocrine tumors. *Neuroendocrinology* 2016 **103** 153–171. (<https://doi.org/10.1159/000443171>)
- Mazziotti G, Mosca A, Frara S, Vitale G & Giustina A. Somatostatin analogs in the treatment of neuroendocrine tumors: current and emerging aspects. *Expert Opinion on Pharmacotherapy* 2017 **18** 1679–1689. (<https://doi.org/10.1080/14656566.2017.1391217>)
- Janson ET, Westlin JE, Eriksson B, Ahlstrom H, Nilsson S & Oberg K. [111In-DTPA-D-Phe1]octreotide scintigraphy in patients with carcinoid tumours: the predictive value for somatostatin analogue treatment. *European Journal of Endocrinology* 1994 **131** 577–581. (<https://doi.org/10.1530/eje.0.1310577>)
- Cives M, Kunz PL, Morse B, Coppola D, Schell MJ, Campos T, Nguyen PT, Nandoskar P, Khandelwal V & Strosberg JR. Phase II clinical trial of pasireotide long-acting repeatable in patients with metastatic neuroendocrine tumors. *Endocrine-Related Cancer* 2015 **22** 1–9. (<https://doi.org/10.1530/ERC-14-0360>)
- Volante M, Brizzi MP, Faggiano A, La Rosa S, Rapa I, Ferrero A, Mansueto G, Righi L, Garancini S, Capella C *et al.* Somatostatin receptor type 2A immunohistochemistry in neuroendocrine tumors: a proposal of scoring system correlated with somatostatin receptor scintigraphy. *Modern Pathology* 2007 **20** 1172–1182. (<https://doi.org/10.1038/modpathol.3800954>)
- Caplin ME, Pavel M & Ruszniewski P. Lanreotide in metastatic enteropancreatic neuroendocrine tumors. *New England Journal of Medicine* 2014 **371** 1556–1557. (<https://doi.org/10.1056/NEJMc1409757>)
- Rinke A, Muller HH, Schade-Brittinger C, Klose KJ, Barth P, Wied M, Mayer C, Aminossadati B, Pape UF, Blaker M *et al.* Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. *Journal of Clinical Oncology* 2009 **27** 4656–4663. (<https://doi.org/10.1200/JCO.2009.22.8510>)
- Bodei L, Mueller-Brand J, Baum RP, Pavel ME, Horsch D, O'Dorisio MS, O'Dorisio TM, Howe JR, Cremonesi M, Kwekkeboom DJ *et al.* The joint IAEA, EANM, and SNMMI practical guidance on peptide receptor radionuclide therapy (PRRT) in



- neuroendocrine tumours. *European Journal of Nuclear Medicine and Molecular Imaging* 2013 **40** 800–816. (<https://doi.org/10.1007/s00259-012-2330-6>)
- 28 Di Nicolantonio F, Arena S, Tabernero J, Grosso S, Molinari F, Macarulla T, Russo M, Cancelliere C, Zecchin D, Mazzucchelli L *et al.* Deregulation of the PI3K and KRAS signaling pathways in human cancer cells determines their response to everolimus. *Journal of Clinical Investigation* 2010 **120** 2858–2866. (<https://doi.org/10.1172/JCI37539>)
- 29 Chang TM, Shan YS, Chu PY, Jiang SS, Hung WC, Chen YL, Tu HC, Lin HY, Tsai HJ & Chen LT. The regulatory role of aberrant phosphatase and tensin homologue and liver kinase B1 on AKT/mTOR/c-Myc axis in pancreatic neuroendocrine tumors. *Oncotarget* 2017 **8** 98068–98083. (<https://doi.org/10.18632/oncotarget.20956>)
- 30 Gagliano T, Bellio M, Gentilin E, Mole D, Tagliati F, Schiavon M, Cavallesco NG, Andriolo LG, Ambrosio MR, Rea F *et al.* mTOR, p70s6K, AKT, and ERK1/2 levels predict sensitivity to mTOR and PI3K/mTOR inhibitors in human bronchial carcinoids. *Endocrine-Related Cancer* 2013 **20** 463–475. (<https://doi.org/10.1530/ERC-13-0042>)
- 31 Serra S, Zheng L, Hassan M, Phan AT, Woodhouse LJ, Yao JC, Ezzat S & Asa SL. The FGFR4-G388R single-nucleotide polymorphism alters pancreatic neuroendocrine tumor progression and response to mTOR inhibition therapy. *Cancer Research* 2012 **72** 5683–5691. (<https://doi.org/10.1158/0008-5472.CAN-12-2102>)
- 32 Guillaume K, Blanc M, Gouysse G, Walter T, Couderc C, Nejari M, Vercherat C, Cordier-Bussat M, Roche C & Scoazec JY. VEGF secretion by neuroendocrine tumor cells is inhibited by octreotide and by inhibitors of the PI3K/AKT/mTOR pathway. *Neuroendocrinology* 2010 **91** 268–278. (<https://doi.org/10.1159/000289569>)
- 33 van Asselt SJ, Oosting SF, Brouwers AH, Bongaerts AH, de Jong JR, Lub-de Hooge MN, Oude Munnink TH, Fiebrich HB, Sluiter WJ, Links TP *et al.* Everolimus reduces (89)Zr-bevacizumab tumor uptake in patients with neuroendocrine tumors. *Journal of Nuclear Medicine* 2014 **55** 1087–1092. (<https://doi.org/10.2967/jnumed.113.129056>)
- 34 Pellat A, Dreyer C, Couffignal C, Walter T, Lombard-Bohas C, Niccoli P, Seitz JF, Hentic O, Andre T, Coriat R *et al.* Clinical and biomarker evaluations of sunitinib in patients with grade 3 digestive neuroendocrine neoplasms. *Neuroendocrinology* 2018 **107** 24–31. (<https://doi.org/10.1159/000487237>)
- 35 Zurita AJ, Khajavi M, Wu HK, Tye L, Huang X, Kulke MH, Lenz HJ, Meropol NJ, Carley W, DePrimo SE *et al.* Circulating cytokines and monocyte subpopulations as biomarkers of outcome and biological activity in sunitinib-treated patients with advanced neuroendocrine tumours. *British Journal of Cancer* 2015 **112** 1199–1205. (<https://doi.org/10.1038/bjc.2015.73>)
- 36 Grande E, Casanovas O, Earl J, Castellano DE, Garcia-Carbonero R, Teule A, Duran I, Fuster J, Sevilla I, Escudero P *et al.* sVEGFR2 and circulating tumor cells to predict for the efficacy of pazopanib in neuroendocrine tumors (NETs): PAZONET subgroup analysis. *Journal of Clinical Oncology* 2013 **31** 4140–4147.
- 37 Yao JC, Phan AT, Hess K, Fogelman D, Jacobs C, Dagohoy C, Leary C, Xie K & Ng CS. Perfusion computed tomography as functional biomarker in randomized run-in study of bevacizumab and everolimus in well-differentiated neuroendocrine tumors. *Pancreas* 2015 **44** 190–197. (<https://doi.org/10.1097/MPA.0000000000000255>)
- 38 Childs A, Kirkwood A, Edeline J, Luong TV, Watkins J, Lamarca A, Alrifai D, Nsiah-Sarheng P, Gillmore R, Mayer A *et al.* Ki-67 index and response to chemotherapy in patients with neuroendocrine tumours. *Endocrine-Related Cancer* 2016 **23** 563–570. (<https://doi.org/10.1530/ERC-16-0099>)
- 39 Krug S, Boch M, Daniel H, Nimphius W, Muller D, Michl P, Rinke A & Gress TM. Streptozocin-based chemotherapy in patients with advanced neuroendocrine neoplasms – predictive and prognostic markers for treatment stratification. *PLoS ONE* 2015 **10** e0143822. (<https://doi.org/10.1371/journal.pone.0143822>)
- 40 Kulke MH, Hornick JL, Fraumeni C, Hooshmand S, Ryan DP, Enzinger PC, Meyerhardt JA, Clark JW, Stuart K, Fuchs CS *et al.* O6-methylguanine DNA methyltransferase deficiency and response to temozolomide-based therapy in patients with neuroendocrine tumors. *Clinical Cancer Research* 2009 **15** 338–345. (<https://doi.org/10.1158/1078-0432.CCR-08-1476>)
- 41 Campana D, Walter T, Pusceddu S, Gelsomino F, Graillot E, Prinzi N, Spallanzani A, Fiorentino M, Barrिताult M, Dall'Olio F *et al.* Correlation between MGMT promoter methylation and response to temozolomide-based therapy in neuroendocrine neoplasms: an observational retrospective multicenter study. *Endocrine* 2018 **60** 490–498. (<https://doi.org/10.1007/s12020-017-1474-3>)
- 42 Fishbein L, Ben-Maimon S, Keefe S, Cengel K, Pryma DA, Loaiza-Bonilla A, Fraker DL, Nathanson KL & Cohen DL. SDHB mutation carriers with malignant pheochromocytoma respond better to CVD. *Endocrine-Related Cancer* 2017 **24** L51–L55. (<https://doi.org/10.1530/ERC-17-0086>)
- 43 Walenkamp A, Crespo G, Fierro Maya F, Fossmark R, Igaz P, Rinke A, Tamagno G, Vitale G, Oberg K & Meyer T. Hallmarks of gastrointestinal neuroendocrine tumours: implications for treatment. *Endocrine-Related Cancer* 2014 **21** R445–R460. (<https://doi.org/10.1530/ERC-14-0106>)
- 44 Khan MS, Kirkwood A, Tsigani T, Garcia-Hernandez J, Hartley JA, Caplin ME & Meyer T. Circulating tumor cells as prognostic markers in neuroendocrine tumors. *Journal of Clinical Oncology* 2013 **31** 365–372. (<https://doi.org/10.1200/JCO.2012.44.2905>)
- 45 Khan MS, Kirkwood AA, Tsigani T, Lowe H, Goldstein R, Hartley JA, Caplin ME & Meyer T. Early changes in circulating tumor cells are associated with response and survival following treatment of metastatic neuroendocrine neoplasms. *Clinical Cancer Research* 2016 **22** 79–85. (<https://doi.org/10.1158/1078-0432.CCR-15-1008>)
- 46 Childs A, Vesely C, Ensell L, Lowe H, Luong TV, Caplin ME, Toumpanakis C, Thirlwell C, Hartley JA & Meyer T. Expression of somatostatin receptors 2 and 5 in circulating tumour cells from patients with neuroendocrine tumours. *British Journal of Cancer* 2016 **115** 1540–1547. (<https://doi.org/10.1038/bjc.2016.377>)
- 47 Carter L, Rothwell DG, Mesquita B, Smowton C, Leong HS, Fernandez-Gutierrez F, Li Y, Burt DJ, Antonello J, Morrow CJ *et al.* Molecular analysis of circulating tumor cells identifies distinct copy-number profiles in patients with chemosensitive and chemorefractory small-cell lung cancer. *Nature Medicine* 2017 **23** 114–119. (<https://doi.org/10.1038/nm.4239>)
- 48 Wang VE, Young L, Ali S, Miller VA, Urisman A, Wolfe J, Bivona TG, Damato B, Fogh S & Bergsland EK. A case of metastatic atypical neuroendocrine tumor with ALK translocation and diffuse brain metastases. *Oncologist* 2017 **22** 768–773. (<https://doi.org/10.1634/theoncologist.2017-0054>)
- 49 Sharabi A, Kim SS, Kato S, Sanders PD, Patel SP, Sanghvi P, Weihe E & Kurzrock R. Exceptional response to nivolumab and stereotactic body radiation therapy (SBRT) in neuroendocrine cervical carcinoma with high tumor mutational burden: management considerations from the Center for Personalized Cancer Therapy at UC San Diego Moores Cancer Center. *Oncologist* 2017 **22** 631–637. (<https://doi.org/10.1634/theoncologist.2016-0517>)
- 50 Allin DM, Shaikh R, Carter P, Thway K, Sharabiani MTA, Gonzales-de-Castro D, O'Leary B, Garcia-Murillas I, Bhide S, Hubank M *et al.* Circulating tumour DNA is a potential biomarker for disease progression and response to targeted therapy in advanced thyroid cancer. *European Journal of Cancer* 2018 **103** 165–175. (<https://doi.org/10.1016/j.ejca.2018.08.013>)
- 51 Zatelli MC, Grossrubatscher EM, Guadagno E, Sciammarella C, Faggiano A & Colao A. Circulating tumor cells and miRNAs as prognostic markers in neuroendocrine neoplasms. *Endocrine-Related Cancer* 2017 **24** R223–R237. (<https://doi.org/10.1530/ERC-17-0091>)
- 52 Cwikla JB, Bodei L, Kolasinska-Cwikla A, Sankowski A, Modlin IM & Kidd M. Circulating transcript analysis (NETest) in GEP-NETS

- treated With somatostatin analogs defines therapy. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** E1437–E1445. (<https://doi.org/10.1210/jc.2015-2792>)
- 53 Modlin IM, Kidd M, Malczewska A, Drozdov I, Bodei L, Matar S & Chung KM. The NETest: the clinical utility of multigene blood analysis in the diagnosis and management of neuroendocrine tumors. *Endocrinology and Metabolism Clinics of North America* 2018 **47** 485–504. (<https://doi.org/10.1016/j.ecl.2018.05.002>)
- 54 Alvarez MJ, Subramaniam PS, Tang LH, Grunn A, Aburi M, Rieckhof G, Komissarova EV, Hagan EA, Bodei L, Clemons PA *et al.* A precision oncology approach to the pharmacological targeting of mechanistic dependencies in neuroendocrine tumors. *Nature Genetics* 2018 **50** 979–989. (<https://doi.org/10.1038/s41588-018-0138-4>)
- 55 Pfragner R, Behmel A, Hoger H, Beham A, Ingolic E, Stelzer J, Svejda B, Moser VA, Obenaus AC, Siegl V *et al.* Establishment and characterization of three novel cell lines – P-STS, L-STS, H-STS – derived from a human metastatic midgut carcinoid. *Anticancer Research* 2009 **29** 1951–1961.
- 56 McMillin DW, Delmore J, Weisberg E, Negri JM, Geer DC, Klippel S, Mitsiades N, Schlossman RL, Munshi NC, Kung AL *et al.* Tumor cell-specific bioluminescence platform to identify stroma-induced changes to anticancer drug activity. *Nature Medicine* 2010 **16** 483–489. (<https://doi.org/10.1038/nm.2112>)
- 57 Mitra A, Mishra L & Li S. Technologies for deriving primary tumor cells for use in personalized cancer therapy. *Trends in Biotechnology* 2013 **31** 347–354. (<https://doi.org/10.1016/j.tibtech.2013.03.006>)
- 58 Majumder B, Baraneedharan U, Thiyagarajan S, Radhakrishnan P, Narasimhan H, Dhandapani M, Brijwani N, Pinto DD, Prasath A, Shanthappa BU *et al.* Predicting clinical response to anticancer drugs using an ex vivo platform that captures tumour heterogeneity. *Nature Communications* 2015 **6** 6169. (<https://doi.org/10.1038/ncomms7169>)
- 59 Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, Zhang C, Schnell C, Yang G, Zhang Y *et al.* High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nature Medicine* 2015 **21** 1318–1325. (<https://doi.org/10.1038/nm.3954>)
- 60 Hidalgo M, Amant F, Biankin AV, Budinska E, Byrne AT, Caldas C, Clarke RB, de Jong S, Jonkers J, Maeldandsmo GM *et al.* Patient-derived xenograft models: an emerging platform for translational cancer research. *Cancer Discovery* 2014 **4** 998–1013. (<https://doi.org/10.1158/2159-8290.CD-14-0001>)
- 61 Bertotti A, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, Cora D, Di Nicolantonio F, Buscarino M, Petti C *et al.* A molecularly annotated platform of patient-derived xenografts ('xenopatients') identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. *Cancer Discovery* 2011 **1** 508–523. (<https://doi.org/10.1158/2159-8290.CD-11-0109>)
- 62 Byrne AT, Alferez DG, Amant F, Annibaldi D, Arribas J, Biankin AV, Bruna A, Budinska E, Caldas C, Chang DK *et al.* Interrogating open issues in cancer precision medicine with patient-derived xenografts. *Nature Reviews: Cancer* 2017 **17** 254–268. (<https://doi.org/10.1038/nrc.2016.140>)
- 63 Grozinsky-Glasberg S, Shimon I & Rubinfeld H. The role of cell lines in the study of neuroendocrine tumors. *Neuroendocrinology* 2012 **96** 173–187. (<https://doi.org/10.1159/000338793>)
- 64 Hofving T, Arvidsson Y, Almobarak B, Inge L, Pfragner R, Persson M, Stenman G, Kristiansson E, Johanson V & Nilsson O. The neuroendocrine phenotype, genomic profile and therapeutic sensitivity of GEPNET cell lines. *Endocrine-Related Cancer* 2018 **25** 367–380. (<https://doi.org/10.1530/ERC-17-0445>)
- 65 Date S & Sato T. Mini-gut organoids: reconstitution of the stem cell niche. *Annual Review of Cell and Developmental Biology* 2015 **31** 269–289. (<https://doi.org/10.1146/annurev-cellbio-100814-125218>)
- 66 Aboulkheyr Es H, Montazeri L, Aref AR, Vosough M & Baharvand H. Personalized cancer medicine: an organoid approach. *Trends in Biotechnology* 2018 **36** 358–371. (<https://doi.org/10.1016/j.tibtech.2017.12.005>)
- 67 Lin H, Jiang X, Zhu H, Jiang W, Dong X, Qiao H, Sun X & Jiang H. ZME2 inhibits the activated hypoxia-inducible pathways by cabozantinib and enhances its efficacy against medullary thyroid carcinoma. *Tumour Biology* 2016 **37** 381–391. (<https://doi.org/10.1007/s13277-015-3816-1>)
- 68 Saunders LR, Bankovich AJ, Anderson WC, Aujay MA, Bheddah S, Black K, Desai R, Escarpe PA, Hampl J, Laysang A *et al.* A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells in vivo. *Science Translational Medicine* 2015 **7** 302ra136. (<https://doi.org/10.1126/scitranslmed.aac9459>)
- 69 Jiang J, Wang DD, Yang M, Chen D, Pang L, Guo S, Cai J, Wery JP, Li L, Li HQ *et al.* Comprehensive characterization of chemotherapeutic efficacy on metastases in the established gastric neuroendocrine cancer patient derived xenograft model. *Oncotarget* 2015 **6** 15639–15651. (<https://doi.org/10.18632/oncotarget.3712>)
- 70 Clermont PL, Lin D, Crea F, Wu R, Xue H, Wang Y, Thu KL, Lam WL, Collins CC, Wang Y *et al.* Polycomb-mediated silencing in neuroendocrine prostate cancer. *Clinical Epigenetics* 2015 **7** 40. (<https://doi.org/10.1186/s13148-015-0074-4>)
- 71 Chamberlain CE, German MS, Yang K, Wang J, VanBrocklin H, Regan M, Shokat KM, Ducker GS, Kim GE, Hann B *et al.* A patient-derived xenograft model of pancreatic neuroendocrine tumors identifies Sapanisertib as a possible new treatment for everolimus-resistant tumors. *Molecular Cancer Therapeutics* 2018 **17** 2702–2709. (<https://doi.org/10.1158/1535-7163.MCT-17-1204>)
- 72 Morton CL & Houghton PJ. Establishment of human tumor xenografts in immunodeficient mice. *Nature Protocols* 2007 **2** 247–250. (<https://doi.org/10.1038/nprot.2007.25>)
- 73 Gaudenzi G, Albertelli M, Dicitore A, Wurth R, Gatto F, Barbieri F, Cotelli F, Florio T, Ferone D, Persani L *et al.* Patient-derived xenograft in zebrafish embryos: a new platform for translational research in neuroendocrine tumors. *Endocrine* 2017 **57** 214–219. (<https://doi.org/10.1007/s12020-016-1048-9>)
- 74 Fior R, Povoia V, Mendes RV, Carvalho T, Gomes A, Figueiredo N & Ferreira MG. Single-cell functional and chemosensitive profiling of combinatorial colorectal therapy in zebrafish xenografts. *PNAS* 2017 **114** E8234–E8243. (<https://doi.org/10.1073/pnas.1618389114>)
- 75 Lawson ND & Weinstein BM. In vivo imaging of embryonic vascular development using transgenic zebrafish. *Developmental Biology* 2002 **248** 307–318. (<https://doi.org/10.1006/dbio.2002.0711>)
- 76 Vitale G, Gaudenzi G, Dicitore A, Cotelli F, Ferone D & Persani L. Zebrafish as an innovative model for neuroendocrine tumors. *Endocrine-Related Cancer* 2014 **21** R67–R83. (<https://doi.org/10.1530/ERC-13-0388>)

Received 8 January 2019

Revised version received 6 April 2019

Accepted 2 May 2019