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REVIEW

Translational research in neuroendocrine tumors: pitfalls and opportunities

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Interest in research on neuroendocrine tumors (NETs) has grown in the past 10 years, coinciding with improvements in our understanding of the molecular pathogenesis of NETs. In addition, NETs have become one of the most exciting settings for drug development. Two targeted agents for the management of advanced pancreatic NETs have been approved, but the development of targeted agents for NETs is limited by problems with both patient selection and demonstration of activity. In this review, we analyze these limitations and discuss ways to increase the predictive value of preclinical models for target discovery and drug development. The role of translational research and 'omics' methodologies is emphasized, with the final aim of developing personalized medicine. Because NETs usually grow slowly and metastatic tumors are found at easily accessible locations, and owing to improvements in techniques for liquid biopsies, NETs provide a unique opportunity to obtain tumor samples at all stages of the evolution of the disease and to adapt treatment to changes in tumor biology. Combining clinical and translational research is essential to achieve progress in the NET field. Slow growth and genetic stability limit and challenge both the availability and further development of preclinical models of NETs, one of the most crucial unmet research needs in the field. Finally, we suggest some useful approaches for improving clinical drug development for NETs: moving from classical RECIST-based response end points to survival parameters; searching for different criteria to define response rates (for example, antiangiogenic effects and metabolic responses); implementing randomized phase II studies to avoid single-arm phase II studies that produce limited data on drug efficacy; and using predictive biomarkers for patient selection.

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INTRODUCTION

Although the incidence of neuroendocrine tumors (NETs) has been increasing over the past few decades, such neoplasms are still considered rare tumors with an age-adjusted incidence of fewer than six cases per 100 000 individuals per year.[1] Furthermore, as NETs are likely to represent a variety of tumor types depending on site of the primary tumor, the incidence rate for each type is even lower. However, the incidence rate of these tumors does not match their prevalence rate because the natural history of NETs is usually less aggressive than that of other solid tumors and they are the second most prevalent advanced tumors of the gastrointestinal tract after advanced colorectal cancer.[2]

Interest in research on NETs has grown in the past 10 years, coinciding with improvements in knowledge about the molecular aberrations that characterize NET pathogenesis. Therefore, NETs are no longer considered to be orphan tumors and instead have emerged as one of the most exciting settings for drug development, with more than 100 active clinical trials registered at www.clinicaltrials.gov and currently ongoing worldwide. This increasing interest in NET research has facilitated the discovery of two targeted agents (sunitinib and everolimus) for the management of advanced pancreatic NETs (pNETs) after decades with no substantial advances in the field. However, their small incidence and large tumor heterogeneity along with other preclinical research limitations compromise the specific development of targeted agents.[3]

The limited availability of preclinical models of NETs and the performance of non-selective translational and clinical research have not allowed the optimal development of targeted agents in this setting. Furthermore, new approved drugs have not produced marked responses or have a demonstrated effect on overall survival, although they can control tumor growth in some patients for long periods of time.

We aim to review these limitations in all aspects of NET research, determine how we can increase the predictive value of preclinical models in target discovery and drug development, and propose methods to help to avoid recurrent mistakes and optimize resource investment in future clinical research.

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NEW AVENUES IN TRANSLATIONAL RESEARCH

Many of the impressive advances witnessed over the past two decades in the field of oncology have arisen from the synergic emergence of three approaches: translational research, 'omics' methodologies and the determined pursuit of personalized medicine. Indeed, the basic knowledge gained regarding the cellular and molecular mechanisms underlying tumor biology has inspired novel perspectives and strategies in clinical oncology. These strategies, in turn, constantly raise novel questions and reveal unexpected failures, further providing us with new and increasingly challenging questions whereas requiring more refined answers for the development of successful therapies for patients. A global network for translational research in oncology has enabled new targets, drugs and biomarkers to be developed on the basis of clinical–biological correlative studies and preclinical models, including both specific cell lines and appropriate animal models. These advances have led to changes in the design of phase II trials to include new end points and novel biomarker-driven programs for improved patient selection (Figure 1).

Prominent examples of the fruitful progress of this emerging translational research network can be traced to the development of affordable 'omics' technologies: genomics, transcriptomics, proteomics, metabolomics and so on. High-throughput sequencing methodologies have enabled the identification of specific gene mutations and variations, and alterations in gene products, that are involved in cancer, and these discoveries have been combined with pharmacodynamic strategies to develop new therapies in preclinical models and, subsequently, in clinical settings.[4] A widely recognized and paradigmatic effort that pioneered this combined strategy was the development of trastuzumab for the treatment of patients with breast cancer overexpressing HER2, which involved the parallel development and approval of both the drug and the identification of patients who would be likely to benefit from the treatment.[5] The application of similar strategies to hypothesis-driven translational research in NETs has lagged behind other most prevalent cancers (breast, lung, prostate and colon cancer), partly owing to the rarity and remarkable heterogeneity of NETs. However, as in other cancers, a thorough knowledge of the basic molecular mechanisms underlying tumor initiation and progression is expected to precede and facilitate advances in the development of novel clinical strategies to improve the diagnosis and treatment of NETs. Fortunately, the molecular research landscape of NETs is increasingly better known and better used for clinical and translational research.

FROM GENETICS TO GENOMICS, PROTEOMICS AND EPIGENETICS OF NETS

An overview of recent efforts to gain deeper knowledge of the molecular basis of NETs reveals that, although key pieces of knowledge already available for other cancers are still lacking, major advances have been achieved in identifying relevant mechanisms involved in the pathogenesis of NETs. Early efforts to identify the genetic basis of this complex family of tumors revealed a number of chromosomal alterations related to tumor development, and, subsequently, molecular cytogenetics provided large databases with more precise information concerning these chromosomal changes.[6,7] These findings support the notion that chromosomal instability is a source of tumor progression; for example, in pancreatic pNETs, loss of genetic material is more frequent than gain and genomic alterations seem to accumulate during tumor growth and progression.[6,8] More recent studies have confirmed that these altered regions contain a handful of genes that are potentially involved in NET oncogenesis; however, we are still far from identifying all the oncogenes and tumor suppressor genes that presumably reside in these regions that are likely to underlie NET initiation and progression.

A landmark in this quest to discover 'driver genes' for NETs was recently achieved through the application of high-throughput unbiased genetic methodologies, (exome sequencing) to analyze 10 non-familial pNETs, and subsequent screening for the most commonly mutated genes in an additional set of 58 pNETs.[9] This work led to the identification of somatic inactivating mutations in

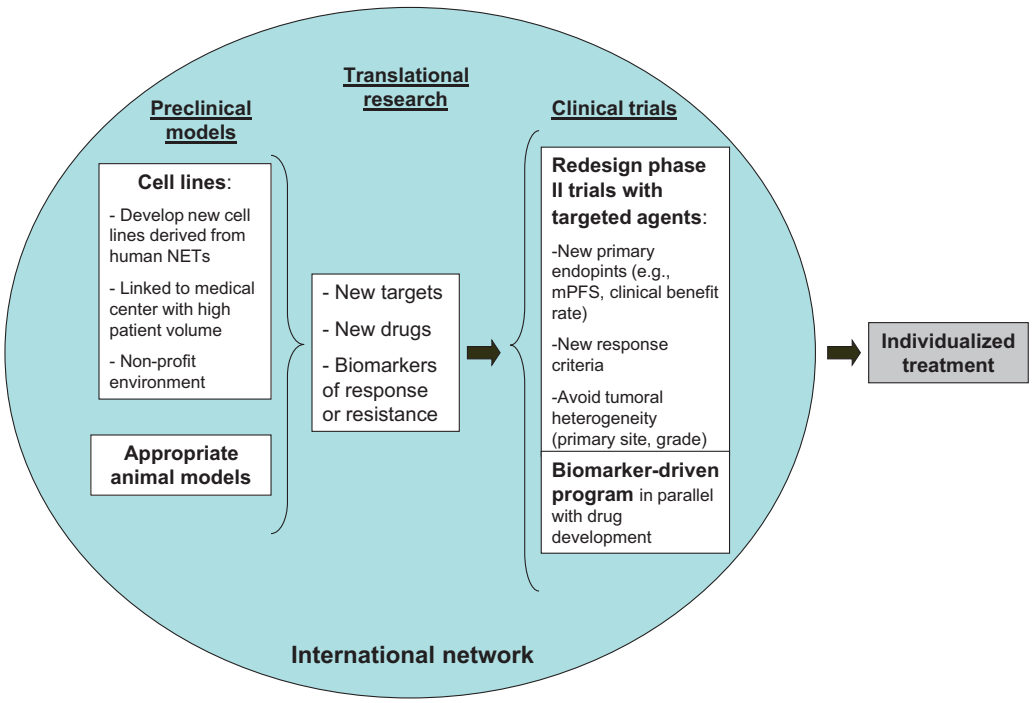

Figure 1. Translational research in NETs.

multiple endocrine neoplasia type 1 (MEN1), the gene encoding menin, a component of the histone methyltransferase complex, in 44% of tumors and of two subunits of a transcription/chromatin remodeling complex comprising DAXX (death-domain-associated protein) and ATRX (alpha thalassemia/mental retardation syndrome X-linked) in 43% of pNETs. Although the finding of MEN1 mutations was not totally unexpected, given the known role of this gene in the NET-causing hereditary MEN type 1 syndrome, the identification of DAXX and ATRX mutations was unprecedented and has attracted much attention. Further detailed analyses of these genes and their related regulatory partners indicate that they work together to stabilize chromatin and, particularly, to contribute to alternative lengthening of telomeres, a telomerase-independent mechanism for maintaining telomere growth through homologous recombination.[6,10,11] Notably, these three genes (MEN1, DAXX and ATRX), which were found to be the most frequently mutated genes in pNETs, are closely linked to epigenetic regulation. As epigenetic regulation is a reversible mechanism, it might be an attractive target for therapeutic intervention.[12,13]

Interestingly, the study by Jiao *et al*.[9] also revealed that 14% of the tumors had mutations in genes directly involved in the PI3K/Akt/mTOR pathway, a signaling cascade that regulates multiple, critically important cellular functions—such as cell growth and proliferation, metabolism, motility and apoptosis—and is the target of everolimus, an mTOR inhibitor currently used to treat patients with advanced pNETs.[14,15] Some of the genes identified in these sporadic tumors were already known to be associated with familial cancer syndromes causing NETs, such as tuberous sclerosis (TSC2). These and other results, such as the finding that PTEN and TSC2 are downregulated in 75% of pNETs,[16] emphasize the pivotal role of this complex pathway in NET tumorigenesis and its potential as a therapeutic target.

Recently, exome sequencing was used to define the genomic landscape of small intestinal NETs (SI-NETs).[17] Analysis of 48 tumors (mostly G1 (70%) and G2 (30%) ileal and small bowel carcinoids) revealed 197 non-synonymous mutations and/or disruptions in protein-encoding gene regions and 14 mutations in splice sites. These mutations affected many genes, including MEN1, VHL,[18] FGF receptor 2 (FGFR2) and BRAF. Notably, an integrated analysis that combined mutational and copy-number data indicated that the PI3K/Akt/mTOR pathway is by far the most frequently altered pathway in SI-NETs (33%). In addition to the genes known to be related to NET development in hereditary syndromes (MEN1, TSC2 and VHL), the study identified a number of additional candidate genes residing on chromosomal regions found to be altered in NETs (CDKN2A, CDKN2C, RASSF1, MET, PTEN, WT1, CDH1, TP53 and ERBB2). Specific analysis of some of these candidates has already provided evidence regarding their precise actions and potential contributions to NET biology.[19] For instance, work on CDKN2A (p16) and RASSF1 has demonstrated that they are relevant to the carcinogenesis of gastroenteropancreatic neuroendocrine tumors (GEP-NETs) by showing that these known tumor suppressor genes are often subject to gene silencing by promoter DNA hypermethylation in these tumors.[6] Similarly, another recent exome-sequence and genome-sequence analysis of SI-NETs identified recurrent somatic mutations and deletions in the cyclin-dependent kinase inhibitor gene CDKN1B, which encodes p27.[20] Results showing that SI-NETs frequently contain CDKN1B frameshift mutations (14 of 180 SI-NETs) and hemizygous deletions encompassing CDKN1B (7 of 50 SI-NETs) suggest that p27 acts as a cell cycle-related tumor suppressor in SI-NETs.[20]

To date, few studies have attempted to apply high-throughput molecular 'omics' approaches to NETs. Two recent studies have used proteomics to analyze protein expression in NETs.[21,22] One used an orthotopic xenograft model of NETs to identify proteins differentially expressed in NETs and subsequently tested and confirmed that these proteins were differentially expressed in human SI-NETs compared with non-tumor tissue. These differentially expressed proteins were mostly cytoskeleton-associated proteins, and they were either downregulated (CRMP2, TCP1ε, TPM2, vimentin, desmin) or upregulated (14-3-3γ, CK8).[21] A second study compared the proteomics of microdissected cells from six metastatic (malignant) and six non-metastatic (benign) insulinomas and identified 16 differentially expressed proteins; this differential expression was then confirmed in 62 insulinomas. Of these 16 proteins, two were present at higher levels in malignant than in benign insulinomas (aldehyde dehydrogenase 1A1 and voltage-dependent anion-selective channel protein 1), whereas the poorly known tumor protein D52 (TPD52) binding protein was present at lower levels in malignant insulinomas and the level of this protein correlated with recurrence-free and overall disease-related survival.[22] The fact that these studies led to the identification of proteins that had not been previously implicated in NETs with other methodologies underscores the informative potential of proteomics and the power of using complementary approaches to discover novel NET biomarkers (Figure 2). Similarly, one of the few transcriptome-related approaches to studying SI-NETs reported to date used a re-analysis of two publically available SI-NET transcriptomes to identify and validate a series of candidate genes related to neuroendocrine secretion, the nervous system and neuroendocrine development, and hypoxia and cyclin/CDK4 regulation,[23] which deserve future detailed studies.

Epigenetic regulatory mechanisms are emerging as important and powerful drivers of tumorigenesis and are thus receiving increasing attention from researchers interested in NETs.[6,13,24] As noted earlier, genes that are frequently mutated in NETs, such as MEN1, DAXX and ATRX, are tightly related to chromatin remodeling, which reinforces the idea that NETs are tightly regulated by epigenetic mechanisms. Indeed, as nicely reviewed by Karpathakis *et al*.[24] second-generation epigenomic tools are revealing that the key mechanisms of epigenetic regulation, including histone modification, DNA methylation and microRNA production, are active in NET pathobiology. A prominent example is the common hypermethylation of the promoter region of RASSF1 (the Ras-association domain gene family 1 tumor suppressor gene, which induces cell cycle arrest) in pNETs and SI-NETs; similarly, the CDKN2A promoter and the CTNNB1 promoter are often hypermethylated in pNETs and metastatic SI-NETs, respectively.[6,24,25] In addition, the finding that the IGF2 gene shows a unique differential hypermethylation signature in insulinomas but is hypomethylated in other types of NETs is of special interest due to the relation of epigenetic changes in insulin like growth factor and insulinoma.[26] Similarly, specific sets of upregulated and downregulated microRNAs are included in epigenetic changes and are likely to provide tools to refine NET diagnosis and prognosis in the near future and might reveal new points of intervention for diagnosis, prognosis or therapy, potentially including miR-21 in pNETs.[6,13,24]

Like epigenetics, alternative splicing can alter the expression and activity of proteins.[26] Defects in alternative splicing represent an additional emerging pathobiological mechanism in cancer,[27] including NETs.[28] Aberrantly truncated variants of somatostatin receptor subtype 5 (sst5TMD4) have recently been identified that can disrupt normal functioning of the key inhibitory receptor sst2 and decrease the responses of different tumor cells and tissues, *in vivo* and *in vitro*, to somatostatin analogs.[29] Interestingly, sst5TMD4 is overexpressed in GEP-NETs, where it is associated to enhanced features of aggressiveness (for example, it is upregulated in lymph-node metastases and in tumor tissue from patients with residual disease, it correlates with angiogenic markers and its overexpression in cell models increases cell proliferation and migration).[30] These findings point towards the potential value of sst5TMD4 as a novel biomarker and research target in NETs.[28,31,32]

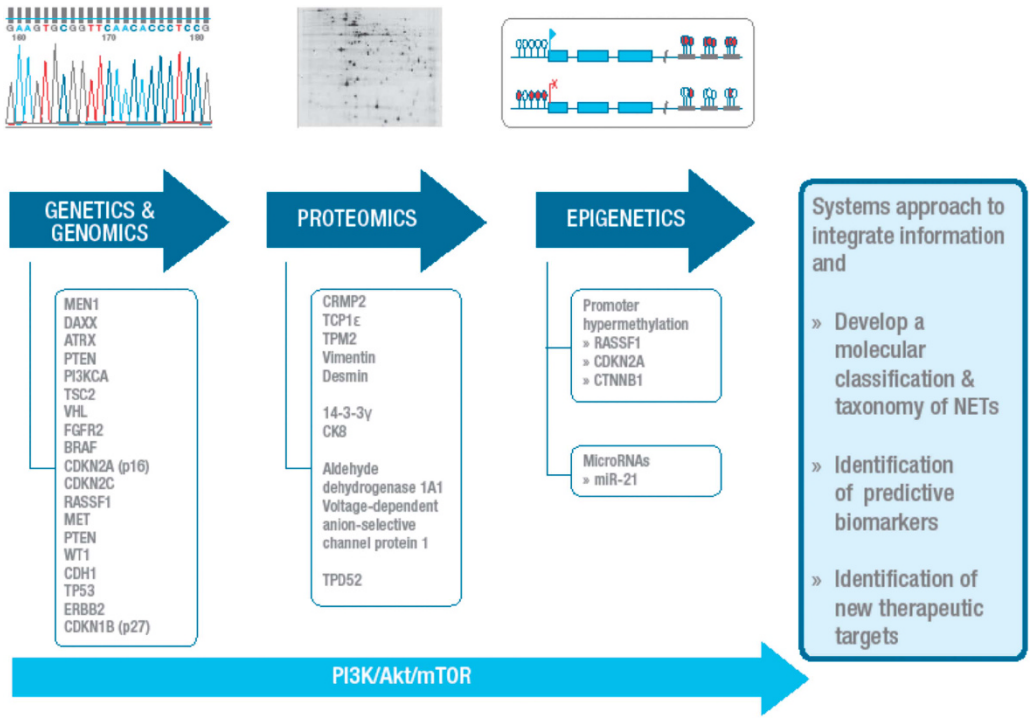

Figure 2. Trends in the molecular analysis of neuroendocrine tumors (NETs). Progress in the molecular understanding of NETs has followed the development and successful application of novel techniques and methodologies, which have progressively allowed access to new levels of information, from genetics to genomics, proteomics and epigenetics. The list of altered genes, proteins, promoter regions and microRNAs (see text for further details and references) continues to grow and thereby provides an increasingly complex and more complete picture of the molecular landscape of NETs and their pathogenesis. Some key markers are recurrently identified in this molecular quest, such as those related to the PI3K/Akt/mTOR signaling pathway. The remaining challenges to develop this picture fully will probably require the application of multidimensional integration of molecular analyses at genomic, epigenetic, proteomic and metabolomic levels through systems approaches. This integration would provide the tools to develop a molecular classification/taxonomy of NETs and would help to identify predictive biomarkers and novel therapeutic targets.

When viewed as a whole, the molecular evidence gathered to date increasingly indicates that NETs are a highly heterogeneous group of tumors. Because of their common neuroendocrine origin and other shared features, NETs have classically been considered to be similar to each other; however, we now know that they differ substantially in a number of other characteristics. For example, the number of genetic alterations in SI-NETs (and well-differentiated sporadic lung NETs) is higher than in well-differentiated pNETs, and, despite their commonalities, pNETs and SI-NETs show considerable divergences in their specific chromosomal alterations.[6,7,13] Unfortunately, an integrated, systematic classification for NETs based on molecular phenotyping, such as has been generated for other cancers, is not yet available.[29] The development of such a valuable taxonomical reference will require the combined efforts of multiple laboratories providing a large number of carefully annotated NET samples, as well as the integration of data from multiple 'omics' platforms (also known as integromics) and currently accepted histological, pathological and clinical variables.[4]

ANIMAL MODELS OF NETS

The development and use of appropriate animal models is essential for developing the next generation of cancer drugs and bringing them to clinical trials. The value of an animal model depends on its validity, selectivity, predictive value and reproducibility,[30] but there is no perfect tumor model for any type of human cancer, including NETs. When selecting the best model, one must consider the heterogeneity and genetic stability of the cancer cells in the selected animal and choose a suitable and clinically meaningful biological end point (for example, local growth, metastasis or survival) (Figure 3).

Xenotransplant models of NETs

The development of xenograft models of NETs has been hampered by a shortage of cultured human cell lines derived from this group of tumors. The development of such cell lines has been a challenge because of the difficulties resulting from their slow growth. Nevertheless, two cell lines are available and are frequently used: the BON1 line, derived from a human pancreatic carcinoid,[33,34] and the GOT1 line, derived from a liver metastasis of a human midgut carcinoid.[35,36] The KRJ1 line was derived from a human carcinoid more recently and is not yet widely used.[37] By contrast, various rodent neuroendocrine cell lines have been developed, including MIN6, TC6, TC3, NIT1 and HIT-T15 derived from mice and RIN-B2, RIN-B6 and RIN-38A derived from rats. All of these cell lines, although useful in some studies, have limitations for understanding human disease because of their animal origins.

The scarcity of cultured cell lines derived from human GEP-NETs is a major limitation in the study of these tumors and the development of therapies for them. Therefore, it is crucial to develop new cell lines derived from human NETs for use in both animal models and *in vivo* and *in vitro* studies on the biology, biochemistry and genetics of these rare tumors. To meet this need, a cell culture unit linked to medical centers with high surgical volume will be needed.

Induced spontaneous NET models

Induced spontaneous tumor models attempt to closely mimic the clinical context. These are usually models in which tumors arise

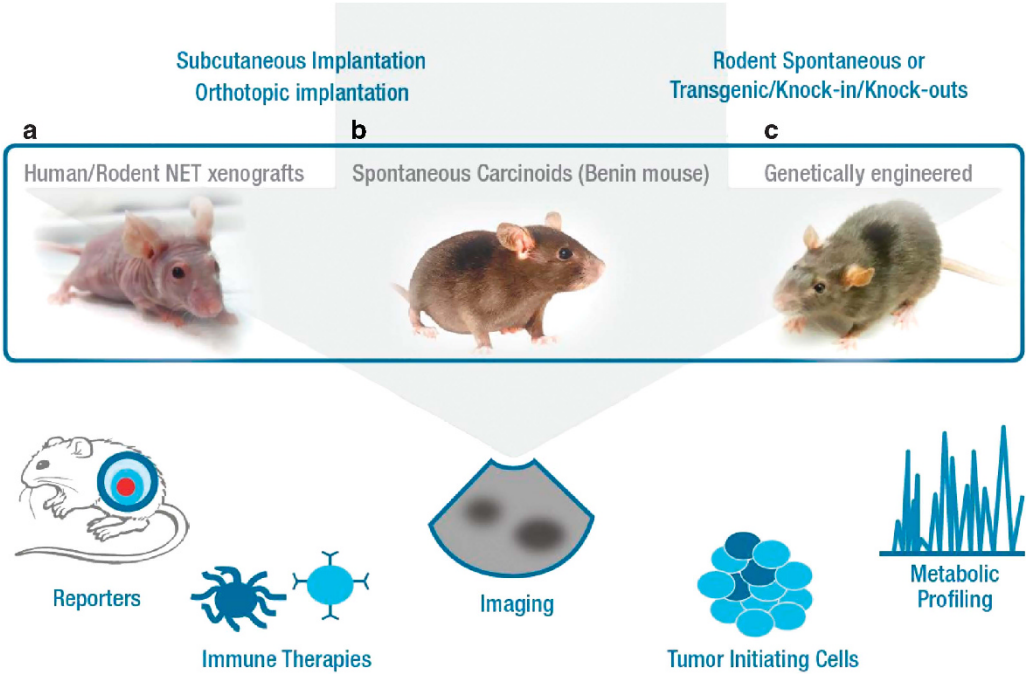

Figure 3. Animal models of NETs. Several different animal models of NETs have been used for basic research and drug development. (**a**) Human or rodent NET cell lines implanted in immunocompromised mice (xenografts) have been extensively used. (**b**) A unique spontaneous rodent model of small intestine exists (*Mastomys* mice, called Benin mice), although it is rarely used. (**c**) Genetic engineering of mice has also been exploited to develop NET models, including transgenic (RIPTag2), knock-in (active CDK4) or knock-out (p18p27 double knock-out) mice. Although no perfect tumor model for NETs exists, the appropriate use of these models is essential for drug discovery and the development of a new generation of cancer drugs.

after animals are treated with carcinogens or exposed to viruses.[38] A spontaneous model of NETs was described in the 1990 s,[39] in which rodents of the genus *Mastomys*, particularly those of the *Mastomysnatalensis* species (called Benin mice), spontaneously develop gastric carcinoids. However, this model is rarely used nowadays, as the genome of *Mastomys* is not well known and the molecular causes of NETs in this mouse species are not well understood.

Genetically modified NET models

The first animal model of pancreatic NETs was developed in 1985 by Douglas Hanahan at Cold Spring Harbor Laboratory in the USA.[40] The expression of SV40 T antigen under the control of the promoter of the gene that encodes insulin results in specific tumorigenesis localized to the beta cells of pancreatic islets. Since its development, this model has provided valuable information on some of the cellular and genetic events that are involved in the progression of NETs.[41–48] However, the main deficiency of the model is that T antigen is not a driving force in human NETs, so this model cannot provide insight into tumor initiation in humans. More recently, targeted deletion of the menin gene in mice has been seen to recapitulate many of the key features of the MEN type 1 syndrome in humans.[49–53] The genetic mutation present in this knock-out mouse model is also found in some patients with sporadic NETs and, therefore, these mice are an important and clinically relevant model of pancreatic NETs.

Other genetically modified mice have unexpectedly shown neuroendocrine phenotypes. For example, knock-in mice with a constitutively active CDK4 gene reproducibly develop pancreatic NETs.[54,55] Although no mutations have been identified in CDK4 in human NETs, this observation implies that the p16/cyclin D1/CDK4/pRb pathway and cell cycle regulation have important roles in the pathogenesis of NETs. Similarly, mice in which the genes for both p18 and p27 (cell cycle inhibitory proteins) had been knocked-out developed pituitary adenomas and, less frequently, parathyroid adenomas and hyperplasia of the pancreatic islets.[56] These are all endocrine tumors and could have the same biological background.

DEVELOPMENT OF THERAPIES FOR NETS

Limitations

In the past decade, many novel targeted agents have been tested in different types of NETs. It is difficult to compare results among these phase II studies because the patients enrolled varied in terms of the primary site and aggressiveness of NETs and in their prognosis. In addition, the primary end points of these trials differ, which results in a scenario in which dozens of phase II clinical trials with several targeted agents have been performed but there is no consensus on which agents are most promising for specific groups of patients and a biomarker-driven program in a specific subgroup of patients is lacking.

The classic evolution of drug development involves preliminary evidence of clinical activity in phase I studies, followed by one or more confirmatory phase II studies, in which the response rate is usually the primary end point, and finally a confirmatory placebo-controlled phase III trial. This path has been followed for developing treatments for NETs, with varying success. One of the first targeted agents tested in NETs was the mTOR inhibitor temsirolimus; a phase II study failed to reach its primary end point of response rate based on the RECIST criteria and development of this drug to treat NETs was stopped.[57] A similar trial of the mTOR inhibitor everolimus, however, showed clinically relevant improvements in tumor response and progression-free survival (PFS) in the same range as those observed with temsirolimus, and was considered clinically meaningful.[58] This conclusion led to two phase III studies and to the subsequent approval of everolimus for the management of advanced pNETs.[58,59] The different outcomes of similar drugs in phase II studies should raise questions about

how we design phase II studies and define the activity of a targeted agent in this field.

NETs are highly heterogeneous and, so far, no clinically meaningful driver mutations for NETs have been identified that could lead to a targeted therapy with high tumor response rates. However, new drugs (everolimus and sunitinib) have been shown to decrease the probability of disease progression in placebo-controlled phase III studies, which provided sufficient evidence on the efficacy of these agents to support their inclusion in guidelines for the management of advanced NETs. Therefore, there is an urgent need to redesign phase II clinical trials of targeted agents for NETs, ideally to include some of the following concepts: (1) moving from the classic primary end points of response rate based on RECIST criteria to survival parameters (such as median PFS, clinical benefit rate, PFS rates at certain time points or changes in tumor growth rate); (2) searching for different criteria to define response rates (for example, antiangiogenic effects such as CHOI criteria or metabolic responses assessed with nuclear medicine techniques); (3) implementing randomized designs to avoid both single-arm phase II studies, which yield limited data on drug efficacy and require complicated interpretation, and inappropriate comparisons between different phase II studies, and to improve predictions of the outcomes of eventual phase III trials; and (4) using predictive biomarkers to select patients.

One of the main limitations of clinical trials in patients with NETs has been the lack of a potent biomarker program to run alongside drug development. As a result, we are still using serum biomarkers that could have some predictive value for new targeted agents, such as everolimus in pNETs. For example, an association between a rapid decrease in chromogranin A levels and tumor response to everolimus has been reported.[59] However, measuring chromogranin A levels is not helpful for selecting patients before the treatment decision has been made and thus non-responder patients are likely to be exposed to possible drug side effects. Most importantly, this approach does not exclude those patients without rapid decreases in chromogranin A levels can still benefit from the drug. We need pretreatment biomarkers to be used as predictive factors of response, in order to improve patient selection. In this line, the recent development of transcript blood profiles in NETs (NETest) has demonstrated a higher sensibility than classical biomarkers, such as chromogranin A and even tumor grade, and a better predictive value of response to several treatment approaches in this field, such as somatostatin analogs, peptide receptor radionucleotide therapy, surgical resections and locoregional ablation procedures. The blood NET transcript analyses also allow the monitoring of the disease evolution favoring a real-time dynamic assessment and may have an impact in decision-making process.[60] Several ongoing clinical trials have included the NETest validation and would probably substitute the classical monoanalyte strategies for prognostic and predictive value of blood biomarkers in NETs setting.

If we consider one of the most well-defined pathways involved in NET pathogenesis, the PIK3CA/Akt/mTOR pathway, and search for predictive biomarkers for everolimus treatment, we find that the data are far from clear and informative. Over the past 2 years, studies of the genomic landscape and protein expression in advanced pNETs have revealed that mutations in the mTOR pathway are present in up to 15% of patients and that the downregulation of the tumor suppressor genes within the pathway, PTEN and TSC2, occurs in up to 80% of patients and loss of expression is associated with poor prognosis in such settings.[9,16] More recent data have also confirmed that mTOR pathway activation is a factor associated with poor prognosis in NET patients and that there is a significant correlation between high Ki67 values and the expression of mTOR, PIK3CA and p-4EBP1, which means that high Ki67 values are associated to poor prognosis.[61] However, no correlation has been reported between the activation of the mTOR pathway and the response to mTOR inhibitors in a clinical setting. Preclinical data have suggested that PIK3CA and PTEN aberrations and high p-Akt levels can predict sensitivity to rapamycin in NET cell lines, and this correlation was confirmed in NET patients treated with rapalogs.[62] A single clinical trial suggested that mTOR pathway activation was a potentially predictive biomarker for response to an mTOR inhibitor (temsirolimus).[57] However, no similar correlation has been reported for everolimus, even though it has been used to treat more than a thousand patients with NETs in prospective, randomized and placebo-controlled clinical trials.[59,63–65] It might be possible to shed light on this apparent contradiction by comparing the efficacy of everolimus in patients in the RADIANT program with the mTOR pathway activation status of these patients. Protein/mRNA expression would be a better indicator of mTOR pathway activation than mutational status as it would avoid errors related to epigenetic changes that could affect protein expression in the absence of direct gene mutations. Data from these analyses will allow researchers to design confirmatory prospective clinical trials in which biomarkers are used to predict responses to rapalogs in NETs, eventually leading to better selection of patients for this type of treatment.

Another targeted agent that has been approved for the treatment of advanced pNETs is the tyrosine kinase inhibitor (TKI) sunitinib. Although many clinical trials have tested the efficacy of sunitinib and other TKIs with a similar mechanism of action in patients with NETs,[3] we currently do not have any biomarkers that can predict responses to these drugs. The first clinical trial to show that sunitinib was a promising treatment for NETs also identified several potential biomarkers that might be used to predict clinical benefit following sunitinib therapy: higher baseline sVEGFR2-3 and VEGF levels, lower baseline IL-8 levels, greater decrease in sVEGFR2-3 levels and greater increase in IL-8 levels during treatment than in patients that did not respond to the treatment.[66] However, these biomarkers were not analyzed in the regulatory phase III study of sunitinib in patients with pNETs, and it is not possible to retrospectively investigate the predictive power of these biomarkers because no archival tumor samples were required to participate in the trial. Evidence on the correlation between the levels of soluble biomarkers and the response to TKIs was also reported in a phase II study of pazopanib in patients with NETs and the ability of biomarkers to predict responses to TKIs in patients with NETs warrants future assessment in phase II–III clinical trials.[67]

The low incidence of NETs and the high heterogeneity of these tumors have limited research in this field for many years. The relative lack of treatment options has led to the inclusion of procedures whose efficacy is not supported by strong evidence in most NET treatment guidelines.[68] An example of such a procedure is temozolomide-based chemotherapy.[68] Several retrospective analyses have suggested that temozolomide is mainly effective in pNETs and that it produces significant response rates as a first-line therapy.[69] However, no prospective data have been collected using a phase III design to demonstrate that temozolomide-based chemotherapy improves survival in patients with advanced NETs. Several prospective phase II studies evaluating the efficacy of temozolomide-based chemotherapy in patients with advanced NETs are currently registered at www.clinicaltrials.gov and are ongoing. However, classic mistakes are being repeated: for instance, the potential use of a biomarker to predict responses to temozolomide it has been evaluated only retrospectively and the predictive value is not enough clear to recommend using it in a prospective way before making treatment decisions in clinical trials.[70] Currently, *post hoc* analyses of prospective phase II studies with temozolomide-based chemotherapy are evaluating *O6-methylguanine-DNA methyltransferase* (MGMT) expression by different techniques to determine a more reliable correlation with temozolomide response. A deep restructuring of the design of clinical trials is urgently needed so that biomarkers are used in

Table 1. Phase II and III clinical studies in NETs

Study	*Phase*	*Drug*	*Drug class*	*Setting*	*Primary end point met*	*Retrospective biomarker analysis*	*Prospective biomarker utilization*
RADIANT-1[59]	II	Everolimus	mTOR inh	pNETs	Yes (response rate)	Yes	No
RADIANT-2[63]	III	Everolimus		GI–lung NETs	No (PFS)	Yes	No
RADIANT-3[64]	III	Everolimus		pNETs	Yes (PFS)	Yes	No
RADIANT-4[65]	III	Everolimus		GI and lung NETs	Yes (PFS)	Yes	No
Raymond[71]	III	Sunitinib	TKI	pNETs	Yes (PFS)	No	No
Ruszniewski	III	177Lu-DOTATATE	Radionucleotide	GI NETs	Yes (PFS)	NA	NA
Duran[57]	II	Temsirolimus	mTOR inh	pNETs	No (response rate)	Yes	No
COOPERATE-II[72]	II	Everolimus+pasireotide	mTOR+somatostatin analog	pNETs	No (PFS)	Yes	No
RAMSETE[73]	II	Everolimus	mTOR	GI NETs	No (response rate)	Yes	No
Kulke[74]	II	Sunitinib		All	Yes (response rate)	Yes	No
Grande[75]	II	Pazopanib	TKI	All	Yes (clinical benefit rate)	Yes	No
Phan[76]	II	Pazopanib		pNET GI NETs	Yes in pNET No in GI NETs	No	No
Reidy-Lagunes[77]	II	MK-0646	IGFR inh	All	No (response rate)	Yes	No
Castellano[78]	II	Bevacizumab+Sorafenib	VEGFA inh+TKI	All	No (PFS & Toxicity)	No	No
Yao[79]	II	Bevacizumab		Carcinoids		No	No
Yao[80]	III	IFN-a2b+octreotide vs bevacizumab+octreotide	Immunomodulator and antiantiogenic (IFN), somatostatin analog, VEGFA inh	Gastrointestinal NETs	NO (PFS)	No	No
Kulke[81]	II	Everolimus vs Everolimus+bevacizumab		pNETs	Yes (PFS)	No	No
Chan[82]	II	Bevacizumab+Temozolomide	VEGFA inh+Chemo	All	Yes (response rate)	No	No
Strosberg[69]	II*	Temozolomide+Capecitabine	Chemo	pNETs	Yes (response rate)	No	No

Abbreviations: *, retrospective; NET, neuroendocrine tumor; PFS, progression-free survival; pNET, pancreatic NETs; TKI, tyrosine kinase inhibitor.

decision-making criteria and to avoid the previous mistakes that have jeopardized the development of drugs that were potentially effective as treatments for NETs (Table 1).

CONCLUSIONS

In spite of the advances in our understanding of the molecular basis of NET development and the discovery of new targeted agents for the treatment of advanced NETs that have occurred over the past decade, we are still far from being able to implement personalized oncology in patients with NETs. It will be essential to improve preclinical models and combine clinical and translational research methods in order to make progress in treating this prevalent and highly heterogeneous disease. Taking as an example recent phase III studies, which were able to recruit large numbers of patients in a fairly short period, the international networks involved in these studies should continue their collaboration to make further progress and modify their approaches to clinical research on the basis of the increasing basic and translational knowledge about NETs. The commonly observed slow growth of NETs, the easily accessible locations of metastatic tumors and recent improvements in liquid biopsy techniques have created a unique opportunity to obtain samples from tumors at all stages of the evolution of the disease and these opportunities should be exploited to adapt therapies to changes in tumor biology.

CONFLICT OF INTEREST

JC: advisory, speaker role and investigational grants from Pfizer, Novartis and Ipsen. JPC: advisory, speaker fees and investigational grants from Novartis and Ipsen. AS: speaker role and investigational grants from Pfizer, Novartis and Ipsen. RS: advisory, speaker role from Novartis and Ipsen.

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REFERENCES

1 Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE *et al.* One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol* 2008; **26**: 3063–3072.
2 Modlin IM, Lye KD, Kidd M. A 5-decade analysis of 13,715 carcinoid tumors. *Cancer* 2003; **97**: 934–959.
3 Capdevila J, Tabernero J. A shining light in the darkness for the treatment of pancreatic neuroendocrine tumors. *Cancer Discov* 2011; **1**: 213–221.
4 Doroshow JH, Kummar S. Translational research in oncology-10 years of progress and future prospects. *Nat Rev Clin Oncol* 2014; **11**: 649–662.
5 Mendelsohn J, Baselga J. The EGF receptor family as targets for cancer therapy. *Oncogene* 2000; **19**: 6550–6565.
6 Meeker A, Heaphy C. Gastroenteropancreatic endocrine tumors. *Mol Cell Endocrinol* 2014; **386**: 101–120.
7 Zikusoka MN, Kidd M, Eick G, Latich I, Modlin IM. The molecular genetics of gastroenteropancreatic neuroendocrine tumors. *Cancer* 2005; **104**: 2292–2309.
8 Asa SL. Pancreatic endocrine tumors. *Mod Pathol* 2011; **24**: S66–S77.
9 Jiao Y, Shi C, Edil BH, de Wilde RF, Klimstra DS, Maitra A *et al.* DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science* 2011; **331**: 1199–1203.
10 Heaphy CM, de Wilde RF, Jiao Y, Klein AP, Edil BH, Shi C *et al.* Altered telomeres in tumors with ATRX and DAXX mutations. *Science* 2011; **333**: 425.
11 De Wilde RF, Edil BH, Hruban RH, Maitra A. Well-differentiated pancreatic neuroendocrine tumors: from genetics to therapy. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 199–208.
12 Elsässer SJ, Allis CD, Lewis PW. Cancer. New epigenetic drivers of cancers. *Science* 2011; **331**: 1145–1146.

13 Oberg K, Casanovas O, Castaño JP, Chung D, Delle Fave G, Denèfle P *et al.* Molecular pathogenesis of neuroendocrine tumors: implications for current and future therapeutic approaches. *Clin Cancer Res* 2013; **19**: 2842–2849.
14 Chan J, Kulke M. Targeting the mTOR signaling pathway in neuroendocrine tumors. *Curr Treat Options Oncol* 2014; **15**: 365–379.
15 Yao J, Pavel M, Lombard-Bohas C, van Cutsem E, Lam D, Kunz T *et al.* Everolimus (EVE) for the treatment of advanced pancreatic neuroendocrine tumors (pNET): final overall survival (OS) results of a randomized, double-blind, placebo (PBO)-controlled, multicenter phase III trial (RADIANT-3). *Ann Oncol* 2014; **25**: iv394–iv405.
16 Missiaglia E, Dalai I, Barbi S, Beghelli S, Falconi M, della Peruta M *et al.* Pancreatic endocrine tumors: expression profiling evidences a role for AKT-mTOR pathway. *J Clin Oncol* 2010; **28**: 245–255.
17 Banck MS, Kanwar R, Kulkarni AA, Boora GK, Metge F, Kipp BR *et al.* The genomic landscape of small intestine neuroendocrine tumors. *J Clin Invest* 2013; **123**: 2502–2508.
18 De Dosso S, Grande E, Barriuso J, Castellano D, Tabernero J, Capdevila J. The targeted therapy revolution in neuroendocrine tumors: in search of biomarkers for patient selection and response evaluation. *Cancer Metastasis Rev* 2013; **32**: 465–477.
19 Capurso G, Festa S, Valente R, Piciucchi M, Panzuto F, Jensen RT *et al.* Molecular pathology and genetics of pancreatic endocrine tumours. *J Mol Endocrinol* 2012; **49**: R37–R50.
20 Francis JM, Kiezun A, Ramos AH, Serra S, Pedamallu CS, Qian ZR *et al.* Somatic mutation of CDKN1B in small intestine neuroendocrine tumors. *Nat Genet* 2013; **45**: 1483–1486.
21 Couderc C, Bollard J, Couté Y, Massoma P, Poncet G, Lepinasse F *et al.* Mechanisms of local invasion in enteroendocrine tumors: Identification of novel candidate cytoskeleton-associated proteins in an experimental mouse model by a proteomic approach and validation in human tumors. *Mol Cell Endocrinol* 2015; **399**: 154–163.
22 Alkatout I, Friemel J, Sitek B, Anlauf M, Eisenach PA, Stühler K *et al.* Novel prognostic markers revealed by a proteomic approach separating benign from malignant insulinomas. *Mod Pathol* 2014; **28**: 69–79.
23 Kidd M, Modlin IM, Drozdov I. Gene network-based analysis identifies two potential subtypes of small intestinal neuroendocrine tumors. *BMC Genomics* 2014; **15**: 595.
24 Karpathakis A, Dibra H, Thirlwell C. Neuroendocrine tumours: cracking the epigenetic code. *Endocr Relat Cancer* 2013; **20**: R65–R82.
25 House MG, Herman JG, Guo MZ, Hooker CM, Schulick RD, Lillemoe KD *et al.* Aberrant hypermethylation of tumor suppressor genes in pancreatic endocrine neoplasms. *Ann Surg* 2003; **238**: 423–431; discussion 431–432.
26 Luco RF, Allo M, Schor IE, Kornblihtt AR, Misteli T. Epigenetics in alternative pre-mRNA splicing. *Cell* 2011; **144**: 16–26.
27 Oltean S, Bates DO. Hallmarks of alternative splicing in cancer. *Oncogene* 2013; **33**: 5311–5318.
28 Castaño JP, Sundin A, Maecke HR, Villabona C, Vazquez-Albertino R, Navarro E *et al.* Gastrointestinal neuroendocrine tumors (NETs): new diagnostic and therapeutic challenges. *Cancer Metastasis Rev* 2014; **33**: 353–359.
29 Guedj M, Marisa L, de Reynies A, Orsetti B, Schiappa R, Bibeau F *et al.* A refined molecular taxonomy of breast cancer. *Oncogene* 2012; **31**: 1196–1206.
30 DeVita VT, Schein PS. The use of drugs in combination for the treatment of cancer: rationale and results. *N Engl J Med* 1973; **288**: 998–1006.
31 Durán-Prado M, Gahete MD, Hergueta-Redondo M, Martínez-Fuentes AJ, Córdoba-Chacón J, Palacios J *et al.* The new truncated somatostatin receptor variant sst5tmd4 is associated to poor prognosis in breast cancer and increases malignancy in MCF-7 cells. *Oncogene* 2012; **31**: 2049–2061.
32 Sampedro-Nuñez M, Luque RM, Ramos-Levi A, Gahete MD, Serrrano-Somavilla A, Villa-Osaba A *et al.* Presence of sst5TMD4, a truncated splice variant of the somatostatin receptor subtype 5, is associated to features of increased aggressiveness in pancreatic neuroendocrine tumors. *Oncotarget* 2015; **7**: 6593–6608 In press.
33 Musunuru S, Carpenter JE, Sippel RS, Kunnimalaiyaan M, Chen H. A mouse model of carcinoid syndrome and heart disease. *J Surg Res* 2005; **126**: 102–105.
34 Parekh D, Ishizuka J, Townsend CM, Haber B, Beauchamp RD, Karp G *et al.* Characterization of a human pancreatic carcinoid *in vitro*: morphology, amine and peptide storage, and secretion. *Pancreas* 1994; **9**: 83–90.
35 Kölby L, Bernhardt P, Johanson V, Schmitt A, Ahlman H, Forssell-Aronsson E *et al.* Successful receptor-mediated radiation therapy of xenografted human midgut carcinoid tumour. *Br J Cancer* 2005; **93**: 1144–1151.
36 Kölby L, Bernhardt P, Ahlman H, Wängberg B, Johanson V, Wigander A *et al.* A transplantable human carcinoid as model for somatostatin receptor-mediated and amine transporter-mediated radionuclide uptake. *Am J Pathol* 2001; **158**: 745–755.
37 Modlin IM, Kidd M, Pfragner R, Eick GN, Champaneria MC. The functional characterization of normal and neoplastic human enterochromaffin cells. *J Clin Endocrinol Metab* 2006; **91**: 2340–2348.
38 Corbett TH, Roberts BJ, Leopold WR, Peckham JC, Wilkoff LJ, Griswold DP *et al.* Induction and chemotherapeutic response of two transplantable ductal adenocarcinomas of the pancreas in C57BL/6 mice. *Cancer Res* 1984; **44**: 717–726.
39 Nilsson O, Wängberg B, Johansson L, Modlin IM, Ahlman H. Praomys (Mastomys) natalensis: a model for gastric carcinoid formation. *Yale J Biol Med* 1992; **65**: 741–751; discussion 827–829.
40 Hanahan D. Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. *Nature* 1985; **315**: 115–122.
41 Folkman J, Watson K, Ingber D, Hanahan D. Induction of angiogenesis during the transition from hyperplasia to neoplasia. *Nature* 1989; **339**: 58–61.
42 Blasco MA, Rizen M, Greider CW, Hanahan D. Differential regulation of telomerase activity and telomerase RNA during multi-stage tumorigenesis. *Nat Genet* 1996; **12**: 200–204.
43 Gocheva V, Zeng W, Ke D, Klimstra D, Reinheckel T, Peters C *et al.* Distinct roles for cysteine cathepsin genes in multistage tumorigenesis. *Genes Dev* 2006; **20**: 543–556.
44 Inoue M, Hager JH, Ferrara N, Gerber H-P, Hanahan D. VEGF-A has a critical, nonredundant role in angiogenic switching and pancreatic beta cell carcinogenesis. *Cancer Cell* 2002; **1**: 193–202.
45 Naik P, Karrim J, Hanahan D. The rise and fall of apoptosis during multistage tumorigenesis: down-modulation contributes to tumor progression from angiogenic progenitors. *Genes Dev* 1996; **10**: 2105–2116.
46 Parangi S, Dietrich W, Christofori G, Lander ES, Hanahan D. Tumor suppressor loci on mouse chromosomes 9 and 16 are lost at distinct stages of tumorigenesis in a transgenic model of islet cell carcinoma. *Cancer Res* 1995; **55**: 6071–6076.
47 Christofori G, Naik P, Hanahan D. A second signal supplied by insulin-like growth factor II in oncogene-induced tumorigenesis. *Nature* 1994; **369**: 414–418.
48 Perl AK, Wilgenbus P, Dahl U, Semb H, Christofori G. A causal role for E-cadherin in the transition from adenoma to carcinoma. *Nature* 1998; **392**: 190–193.
49 Crabtree JS, Scacheri PC, Ward JM, Garrett-Beal L, Emmert-Buck MR, Edgemon KA *et al.* A mouse model of multiple endocrine neoplasia, type 1, develops multiple endocrine tumors. *Proc Natl Acad Sci USA* 2001; **98**: 1118–1123.
50 Crabtree JS, Scacheri PC, Ward JM, McNally SR, Swain GP, Montagna C *et al.* Of mice and MEN1: Insulinomas in a conditional mouse knockout. *Mol Cell Biol* 2003; **23**: 6075–6085.
51 Bertolino P, Tong W-M, Galendo D, Wang Z-Q, Zhang C-X. Heterozygous Men1 mutant mice develop a range of endocrine tumors mimicking multiple endocrine neoplasia type 1. *Mol Endocrinol* 2003; **17**: 1880–1892.
52 Bertolino P, Tong W-M, Herrera PL, Casse H, Zhang CX, Wang Z-Q. Pancreatic beta-cell-specific ablation of the multiple endocrine neoplasia type 1 (MEN1) gene causes full penetrance of insulinoma development in mice. *Cancer Res* 2003; **63**: 4836–4841.
53 Biondi CA, Gartside MG, Waring P, Loffler KA, Stark MS, Magnuson MA *et al.* Conditional inactivation of the MEN1 gene leads to pancreatic and pituitary tumorigenesis but does not affect normal development of these tissues. *Mol Cell Biol* 2004; **24**: 3125–3131.
54 Sotillo R, Dubus P, Martín J, de la Cueva E, Ortega S, Malumbres M *et al.* Wide spectrum of tumors in knock-in mice carrying a Cdk4 protein insensitive to INK4 inhibitors. *EMBO J* 2001; **20**: 6637–6647.
55 Rane SG, Dubus P, Mettus R V, Galbreath EJ, Boden G, Reddy EP *et al.* Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in beta-islet cell hyperplasia. *Nat Genet* 1999; **22**: 44–52.
56 Franklin DS, Godfrey VL, O'Brien DA, Deng C, Xiong Y. Functional collaboration between different cyclin-dependent kinase inhibitors suppresses tumor growth with distinct tissue specificity. *Mol Cell Biol* 2000; **20**: 6147–6158.
57 Duran I, Kortmansky J, Singh D, Hirte H, Kocha W, Goss G *et al.* A phase II clinical and pharmacodynamic study of temsirolimus in advanced neuroendocrine carcinomas. *Br J Cancer* 2006; **95**: 1148–1154.
58 Yao JC, Phan AT, Chang DZ, Wolff RA, Hess K, Gupta S *et al.* Efficacy of RAD001 (everolimus) and octreotide LAR in advanced low- to intermediate-grade neuroendocrine tumors: results of a phase II study. *J Clin Oncol* 2008; **26**: 4311–4318.
59 Yao JC, Lombard-Bohas C, Baudin E, Kvols LK, Rougier P, Ruszniewski P *et al.* Daily oral everolimus activity in patients with metastatic pancreatic neuroendocrine tumors after failure of cytotoxic chemotherapy: a phase II trial. *J Clin Oncol* 2010; **28**: 69–76.
60 Kidd M, Drozdov I, Modlin I. Blood and tissue neuroendocrine tumor gene cluster analysis correlate, define hallmarks and predict disease status. *Endocr Relat Cancer* 2015; **22**: 561–575.
61 Qian ZR, Ter-Minassian M, Chan JA, Imamura Y, Hooshmand SM, Kuchiba A *et al.* Prognostic significance of MTOR pathway component expression in neuroendocrine tumors. *J Clin Oncol* 2013; **31**: 3418–3425.

62 Meric-Bernstam F, Akcakanat A, Chen H, Do K-A, Sangai T, Adkins F *et al.* PIK3CA/PTEN mutations and Akt activation as markers of sensitivity to allosteric mTOR inhibitors. *Clin Cancer Res* 2012; **18**: 1777–1789.
63 Pavel ME, Hainsworth JD, Baudin E, Peeters M, Hörsch D, Winkler RE *et al.* Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study. *Lancet* 2011; **378**: 2005–2012.
64 Yao JC, Shah MH, Ito T, Lombard-Bohas C, Wolin EM, Van Cutsem E *et al.* Everolimus for advanced pancreatic neuroendocrine tumors. *N Engl J Med* 2011; **364**: 514–523.
65 Yao JC, Fazio N, Singh S, Buzzoni R, Carnaghi C, Wolin E *et al.* Everolimus for the treatment of advanced, non-functional neuroendocrine tumours of the lung or gastrointestinal tract (RADIANT-4): a randomised, placebo-controlled, phase 3 study. *Lancet* 2015; **387**: 968–977.
66 Zurita AJ, Khajavi M, Wu HK, Tye L, Huang X, Kulke MH *et al.* Circulating cytokines and monocyte subpopulations as biomarkers of outcome and biological activity in sunitinib-treated patients with advanced neuroendocrine tumours. *Br J Cancer* 2015; **112**: 1199–1205.
67 Grande E, Casanovas O, Earl J, Castellano DE, Garcia-Carbonero R, Teule A *et al.* sVEGFR2 and circulating tumor cells to predict for the efficacy of pazopanib in neuroendocrine tumors (NETs): PAZONET subgroup analysis. *ASCO Meet Abstr* 2013; **31**: 4140.
68 Oberg K, Knigge U, Kwekkeboom D, Perren A, Group EGW. Neuroendocrine gastro-entero-pancreatic tumors: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2012; **23**: vii124–vii130.
69 Strosberg JR, Fine RL, Choi J, Nasir A, Coppola D, Chen DT *et al.* First-line chemotherapy with capecitabine and temozolomide in patients with metastatic pancreatic endocrine carcinomas. *Cancer* 2011; **117**: 268–275.
70 Kulke MH, Hornick JL, Frauenhoffer C, Hooshmand S, Ryan DP, Enzinger PC *et al.* O6-methylguanine DNA methyltransferase deficiency and response to temozolomide-based therapy in patients with neuroendocrine tumors. *Clin Cancer Res* 2009; **15**: 338–345.
71 Raymond E, Dahan L, Raoul J-L, Bang Y-J, Borbath I, Lombard-Bohas C *et al.* Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. *N Engl J Med* 2011; **364**: 501–513.
72 Kulke MH. A Randomized Open-label Phase II Study of Everolimus Alone or in Combination with Pasireotide LAR in Advanced, Progressive Pancreatic Neuroendocrine Tumors (pNET): COOPERATE-2 Trial. ENETS Conference 2015. 2015, vol. 102, p 131.
73 Pavel ME, Wiedenmann B, Capdevila J, Reed N, Valle JW, Caglio S *et al.* RAMSETE: A single-arm, multicenter, single-stage phase II trial of RAD001 (everolimus) in advanced and metastatic silent neuro-endocrine tumours in Europe. *ASCO Meet Abstr* 2012; **30**: 4122.
74 Kulke MH, Lenz H-J, Meropol NJ, Posey J, Ryan DP, Picus J *et al.* Activity of Sunitinib in Patients With Advanced Neuroendocrine Tumors. *J Clin Oncol* 2008; **26**: 3403–3410.
75 Grande E, Capdevila J, Castellano D, Teule A, Duran I, Fuster J *et al.* Pazopanib in pretreated advanced neuroendocrine tumors: a phase II, open-label trial of the Spanish Task Force Group for Neuroendocrine Tumors (GETNE). *Ann Oncol* 2015; **26**: 1987–1993.
76 Phan AT, Halperin DM, Chan JA, Fogelman DR, Hess KR, Malinowski P *et al.* Pazopanib and depot octreotide in advanced, well-differentiated neuroendocrine tumours: a multicentre, single-group, phase 2 study. *Lancet Oncol* 2015; **16**: 695–703.
77 Reidy-Lagunes DL, Vakiani E, Segal MF, Hollywood EM, Tang LH, Solit DB *et al.* A phase 2 study of the insulin-like growth factor-1 receptor inhibitor MK-0646 in patients with metastatic, well-differentiated neuroendocrine tumors. *Cancer* 2012; **118**: 4795–4800.
78 Castellano D, Capdevila J, Sastre J, Alonso V, Llanos M, García-Carbonero R *et al.* Sorafenib and bevacizumab combination targeted therapy in advanced neuro-endocrine tumour: a phase II study of Spanish Neuroendocrine Tumour Group (GETNE0801). *Eur J Cancer* 2013; **49**: 3780–3787.
79 Yao JC, Phan A, Hoff PM, Chen HX, Charnsangavej C, S-CJ Yeung *et al.* Targeting vascular endothelial growth factor in advanced carcinoid tumor: a random assignment phase II study of depot octreotide with bevacizumab and pegylated interferon alpha-2b. *J Clin Oncol* 2008; **26**: 1316–1323.
80 Yao JC, Guthrie K, Moran C, Strosberg JR, Kulke MH, Chan JA *et al.* SWOG S0518: Phase III prospective randomized comparison of depot octreotide plus interferon alpha-2b versus depot octreotide plus bevacizumab (NSC #704865) in advanced, poor prognosis carcinoid patients (NCT00569127). *J Clin Oncol* 2015; **33**(suppl; abstr 4004).
81 Kulke MH, Niedzwiecki D, Foster NR, Fruth B, Kunz PL, Kennecke HF *et al.* Randomized phase II study of everolimus (E) versus everolimus plus bevacizumab (E+B) in patients (Pts) with locally advanced or metastatic pancreatic neuroendocrine tumors (pNET), CALGB 80701 (Alliance). *J Clin Oncol* 2015; **33**(suppl; abstr 4005).
82 Chan JA, Stuart K, Earle CC, Clark JW, Bhargava P, Miksad R *et al.* Prospective study of bevacizumab plus temozolomide in patients with advanced neuroendocrine tumors. *J Clin Oncol* 2012; **30**: 2963–2968.