Relevance of Angiogenesis in Neuroendocrine Tumors

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Abstract:
While traditional cytotoxic drugs have shown limited efficacy in neuroendocrine tumors (NETs), their biological features have been characterized and can be exploited therapeutically. Their most prominent trait is an extraordinary vascularization in low-grade NETs and an hypoxia-dependent angiogenesis in high-grade NETs, which is associated to a significant expression of many pro-angiogenic molecules. Therefore, several antiangiogenic compounds have been tested in these malignancies and among these, sunitinib has demonstrated activity in pancreatic NET patients by dually targeting the VEGFR and PDGFR pathways. In spite of these efficacious clinical results, apparent resistance to antiangiogenic therapies has been described in NETs animal models and in clinical trials. Therefore, overcoming anti-angiogenic resistance is a crucial step in the subsequent development of antiangiogenic therapies. Several strategies have been postulated to fight resistance, but pre-clinical studies and clinical trials will investigate and address these therapeutic approaches in the coming years in order to overcome resistance anti-angiogenic therapies in NETs.

Keywords: Neuroendocrine tumor, angiogenesis, sunitinib, molecular target

Introduction

NETs are rare malignacies but their incidence and prevalence has increased in the last decades [1]. NETs comprise a heterogeneous family of tumors with a wide and complex spectrum of clinical behavior. The limited effectiveness of traditional DNA-damaging agents has led to the exploration of new targeted drugs based in the molecular features of these tumors, in order to improve their systemic treatment.

NETs have a number of biological features that can be exploited therapeutically, such as an extraordinary tumor vascularization with high expression of several proangiogenic molecules [2,3]. Indeed, most NETs are hypervascular, as it is characteristic of normal endocrine glands which have a dense vascular network that facilitates hormone secretion and dumping to the bloodstream. Specifically, NETs show a microvascular density ranging from 10 to 20 fold higher than in typical carcinomas. However, many studies have shown that in pancreatic NETs microvascular density is higher in benign, low-grade tumors than in malignant, high-grade tumors [4]. Furthermore, these studies have demonstrated that intratumor vessel density is associated with a good prognosis and prolonged survival [5], which is completely the opposite to other digestive epithelial tumors and most carcinomas in general. Thus, an intriguing characteristic of NETs is their physiologically derived high vessel density in low-grade tumors that is diminished over tumor progression and aggressiveness. Nevertheless, high-grade NETs typically show hypoxic areas and upregulation of hypoxia-response transcription factors (HIFs) and hypoxia-dependent genes (Carbonic Anhydrase, Glucose Transporters…) [6]. Thus, while vessel density is lower in high-grade NETs, they show a very robust pro-angiogenic response that is clearly observed by increased endothelial proliferation and vascular overgrowth.

The high vascularization of NETs has its molecular base on the specific repertoire of secreted molecules from neuroendocrine cells. Indeed, neuroendocrine cells physiologically express a high level of pro-angiogenic molecules, particularly in the pancreas, but also in peptidergic endocrine cells which constitutively synthesize several members of the VEGF family [7]. Consistently, NETs also typically express a variety of pro-angiogenic cytokines and growth factors, including vascular endothelial growth

factor (VEGF-A, VEGF-C), Fibroblast Growth Factors (FGF2…) amongst others. For example, neuroendocrine tumors and their derived cell lines demonstrate a high capacity to synthesize and secrete high levels of several VEGF family members [2].

Overall, NETs not only show a dense vascular structure, but also have an angiogenic capacity that is characteristic of vessel-dependent tumors and thus evidencing a strong rationale for the use of anti-angiogenic therapies in this type of malignancies. Therefore, several antiangiogenic compounds are currently undergoing clinical evaluation in NETs, either as monotherapy or in combination with chemotherapy or other targeted drugs. We will mention the biology of each of this mechanisms of angiogenesis and discuss the clinical data that is available to date.

Early days, "Early" Anti-angiogenic Drugs

Experimental evidence of the sensitivity of NETs to anti-angiogenic drugs is based on preclinical studies in animal models, where promising results were described in the mid and late 90-ties with strikingly efficacious effects ranging from tumor stabilization to tumor regression depending on the model used [8]. In particular, several antiangiogenic drugs have been evaluated in a transgenic mouse model of insulinoma, the RIP-Tag2, developed by Douglas Hanahan [9]. Early studies with the aminopeptidase inhibitor TNP-470, minocycline and interferon α/β demonstrated an antiangiogenic effect together with an effective tumor growth impairment [10]. Further studies utilized the naturally occurring antiangiogenic molecules angiostatin and endostatin which demonstrated both antiangiogenic and antitumoral effects in different stages of islet cell tumor progression [8].

These preclinical results are associated to the clinical use of Thalidomide in NETs. Thalidomide is an orally bioavailable immunomodulatory drug with antiangiogenic properties due to its capacity to inhibit tumor necrosis factor alpha (TNF-α) production and also VEGF and basic fibroblast growth factor (bFGF) pathways. The first small clinical study (n=18) in NETs with thalidomide in monotheray didn't show objective responses [11]. Nevertheless, the combination of thalidomide and temozolomide was evaluated in another phase II study with a radiological response rate of 45% in pancreatic NETs and 7% in carcinoid tumors, respectively. However, a high incidence of grade 3-4 of lymphopenia was reported and 10% of the patients had opportunistic infections [12].

The use of Endostatin in the clinic has also demonstrated some benefit. *Endostatin* is a 20-kDa proteolytic fragment of collagen XVIII with antiangiogenic and antitumor activity in preclinical studies. The anti-angiogenic function of endostatin has been well documented during the past decade. However, the exact mechanism that endostatin executes its anti-angiogenic functions remains elusive. Both preclinical and human phase I studies of recombinant human endostatin (rhEndostatin) indicated activity in NETs. However, the phase II study performed in 40 patients with advanced NETs showed a high rate of stable disease (80%) but did not result in significant tumor regression. The toxicity was minimal[13].

The VEGF- VEGFR axis

The key mediator of angiogenesis is the VEGF and VEGF signaling inhibition has been shown to result in significant tumor growth delay in a wide range of animal models [14]. Consistently, several antiangiogenic therapies targeting the VEGF-VEGFR2/KDR signaling axis have shown to be effective in mouse models of NETs. In particular, a monoclonal antibody that blocks VEGF-A ligand (AF-493-NA) and a blocking antibody of the VEGFR2 (DC101) have been tested in the RIP-Tag2 mouse model of insulinoma with consistent antiangiogenic effects in microvessel density, endothelial cell proliferation and antitumor activity with increased apoptosis [3, Sennino and McDonald, personal comunication].

Bevacizumab, a humanized monoclonal antibody that recognizes and blocks VEGF, failed to inhibit growth NETs cells in vitro, but reduced their angiogenic potential by blocking the cells' ability to stimulate endothelial cell tube formation and proliferation and impaired tumor growth in animals [15].

Clinically, the activity of bevacizumab in NETs was tested in a randomized phase II study [16]. Forty-four patients on stable doses of octreotide were randomly assigned to 18 weeks of treatment with bevacizumab or PEG interferon alfa-2b. At disease progression (PD) or at the end of 18 weeks (whichever occurred earlier), patients received bevacizumab plus PEG interferon until progression. In the bevacizumab arm, four patients (18%) achieved confirmed partial response (PR), 17 patients (77%) had stable disease (SD), and one patient (5%) had PD. No objectives responses were observed in PEG interferon arm. Progression-free survival (PFS) rates after 18 weeks of monotherapy were 95% in bevacizumab arm versus 68% on the PEG interferon arm. Bevacizumab therapy also resulted in a significant reduction of tumor blood flow measured by functional CT scans.

A larger randomized phase III in patients with unresectable metastatic or locally advanced carcinoid tumors comparing depot octreotide acetate and interferon alfa-2b versus depot octreotide acetate and bevacizumab is being conducted since 2007 (SWOG S0518, clinicaltrials.gov NCT00569127). The results of this study are awaited in the near future.

Bevacizumab has also been tested in combination with cytotoxic drugs. Kulke and cols explored the efficacy and safety of the combination of bevacizumab plus temozolomide in a small phase II trial [17]. The combination showed an objective response rate of 24% in pancreatic NETs but 0% in carcinoid tumors. A phase II study of capecitabine, oxaliplatin and bevacizumab for metastatic or unresectable neuroendocrine tumors were reported in 2010 ASCO Annual Meeting. PR were observed in 7 pts (23%), SD in 22 pts (71%), and PD in 2 pts (6%). Of the patients who achieved a PR, 6 had pancreatic NETs [18]. The combination with FOLFOX (oxaliplatin, leucovorin and 5-fluorouracil) has also been tested with similar results [19]. Further phase III trials are warranted to establish the efficacy of adding bevacizumab to chemotherapy in NETs.

Other vascular players: PDGFR axis and the pericytes

Another critical cellular component of the blood vessels, the perivascular cells or pericytes, have shown to be relevant targets for effective anti-angiogenesis. These cells mediate the stabilization of the vessels based on synthesis of new basement membrane and tight association of endothelial and pericyte junctions. Molecularly, a specific crosstalk between endothelial cells and pericytes that implicates VEGF and PDGF is key for the vascular formation and maintenance, and creates a crucial therapeutic opportunity that has been exploited [20]. For its supportive cooperative function aiding the endothelial cell stabilization and function, PDGFR inhibition has been developed in the context of dual inhibition of VEGFR and PDGFR [8]. Indeed, experimental studies with the RIP-Tag2 transgenic mouse model demonstrate a significant synergy when both endothelial cells and pericytes are dually blocked with VEGFR and PDGFR small molecule inhibitors such as Sunitinib [21,22].

On the clinical side, PDGFRs have been characterised in human pancreatic NET samples. PDGFR-α and PDGFR-β are commonly expressed both on tumor cells and tumor stroma [23]. The clinical approach to dually inhibit both VEGFR and PDGFR in NETs has been developed using several small molecule compounds such as sunitinib, sorafenib, vatalanib and pazopanib.

Sunitinib is the only antiangiogenic drug tested in a randomized phase III placebo-controlled trial [24] in patients with progressive well differentiated pancreatic NETs which is stadistically positive in progression-free survival (11.4 months in sunitinib arm vs 5.5 months in placebo arm). Sunitinib 37.5 mg/day was administered orally in a continuous schedule. The objective response rate was 9.3% in the sunitinib group versus 0% in the placebo group. This study was the first positive phase III trial with anti-angiogenic drugs in the field and has changed the daily clinical practice in NETs. In a previous phase II study 107 patients (41 carcinoid tumors and 66 pancreatic NETs) with documented disease progression were treated with repeated six-week cycles of sunitinib 50 mg/day, four weeks on and two weeks off. The overall objective response rate was 16.7% in pancreatic NETs and 2.4% in carcinoid tumors [25].

Sorafenib is an orally active, multikinase inhibitor with selectivity for the VEGFR-2, VEGFR-3,PDGFR-β , FLT3, c-kit, RET and RAF kinases. Sorafenib monotheraphy has been evaluated in a phase II trial in 93 patients with NETs. The overall response rate was 10% in both pancreatic and carcinoid NETs [26].

Vatalanib inhibits all known VEGFRs, with particular selectivity for VEGFR-2. At higher concentrations vatalanib also inhibits PDGFR- β and c-kit. Two phase II studies were reported in 2008 in NETs but both showed no significant radiological responses [27,28]. Finally, pazopanib, another potent inhibitor of VEGFR, PDFGR-α/β and c-kit, has been tested in combination with octeotride LAR in pancreatic NETs with 17% of PR and a PFS of 11.7 months [29].

Indirect anti-angiogenic mechanisms

Certain anti-angiogenic drugs operate by targeting a tumor cell capacity of production of a particular pro-angiogenic growth factor. Such angiogenesis inhibitors have been termed "indirect anti-angiogenic inhibitors" as they do not target the effectors of angiogenesis (direct anti-angiogenic inhibitors), but rather indirectly regulate the pro-angiogenic capacities of the tumor cells. The most relevant of these indirect anti-angiogenic therapies in NETs is the inhibition of phosphatidylinositol 3-kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) pathway, which is clearly activated in NETs [30].

The PI3K/AKT/mTOR pathway is activated in the majority of human cancers and this pathway is known to play a key role in numerous cellular functions, additionally to angiogenesis, which include proliferation, adhesion, migration, invasion, metabolism and survival. Activation of the PI3K/AKT pathway in tumor cells is a prominent enhancer of VEGF secretion but also modulates the expression of other angiogenic factors such as nitric oxide and angiopoietins. Numerous inhibitors targeting the PI3K/AKT/mTOR pathway have shown to decrease VEGF secretion and angiogenesis [31]. Among these, everolimus has demonstrated antiproliferative activity in several mammalian cell lines and clinically, this drug is the second targeted therapy in the field, besides sunitinib, that has shown efficacy and has become practice changing in the management of patients with progressive advanced pancreatic

NETs.(ENETS guidelines paper number 6 Pavel et al Neuroendocrinology In Press) The results of the randomized phase III placebo-controlled trial of everolimus in this population showed a median progression-free survival of 11.0 months with everolimus as compared with 4.6 months with placebo ($p<0.001$), representing a 65% reduction in the estimated risk of progression or death. Everolimus also showed a good tolerance with a low rate of severe adverse events [33].

Antiangiogenic Resistance

Clinical results using anti-angiogenic drugs demonstrate only moderate gains in time to progression, and scarce benefits in overall survival, despite the long-term treatment. Why are there such modest and short-lasted benefits of anti-angiogenic therapies in the clinic? The initial hypothesis was that anti-angiogenesis therapy would not induce resistance ("resistant to resistance") because it targeted endothelial cells instead of the tumor cell itself [34]. Nevertheless, clinical and experimental evidence indicates that resistance to anti-angiogenic therapy does indeed occur. VEGF inhibition produces vascular triming and hypoxia, which leads to upregulation of multiple proangiogenic molecules, including VEGFs, FGFs and angiopoietins, which can contribute to eventual resistance [3, 35]. Furthermore, studies in the RIP-Tag2 model have described progression of NETs in course of anti-angiogenic therapies targeting the VEGF/VEGFR signaling axis. Thus, potent angiogenesis inhibition can alter the natural history of tumors by trigering resistance to therapy and increasing invasion and lymphatic or distant metastasis [36].

Strategies to overcome this resistance mechanism are warranted. Yao and Phan [37] have proposed some stratagies to overcome the anti-angiogenic resistance that are based in preclinical studies. Dual targeted therapies have been tested in xenografts. The combination of bevacizumab and HIF-1 or Sp1 inhibitors may increase the therapeutic efficacy of anti-angiogenic treatment [38, 39]. In another study, Allen and cols [40] suggest that cotargeting of VEGF and FGF signaling pathways can improve efficacy and overcome adaptive resistance to VEGF inhibition in the RIP-Tag2 model of pancreatic NETs. They tested the dual FGFR/VEGFR tyrosine kinase inhibitor brivanib in both first and second line following the failure of anti-VEGFR2 antibody (DC101) or sorafenib showing promising results in overcoming resistance to VEGF-selective therapy.

On the clinical side, some phase II studies have tested the combination of antiangiogenic drugs. 2-methoxyestradiol (2ME2) administered in combination with bevacizumab has been evaluated in a prospective study in thirty-one patients with metastatic carcinoid tumors [41]. No confirmed radiologic responses by RECIST were observed. However, 68% of the radiologically evaluable patients experienced at least some degree of tumor reduction, and the median PFS time was 11.3 months. The results of a study [42] with the combination of sorafenib and bevacizumab were reported in 2011 ASCO Annual meetitng. The overall response ratio was 9.8% and the disease control rate at 6 months was 95.1%. Median progression free survival was 12.4 months. The most common grade 3-4 toxicities were hand-foot syndrome and asthenia which occurred in 20.5% and 15.9% of patients, respectively. Another trial have tested the combination of Bevacizumab and Everolimus in NETs. Addition of Everolimus to Bevacizumab was associated with further decrease in tumor blood flow (15%; $p=0.02$) than Bevacizumab alone. By intention to treat (ITT) analyses, there were 26% of PR and 27% of SD. The median PFS was 14.4 months [43].

On the other hand, the identification of biomarkers for response or resistance to a particular anti-angiogenic regimen is imperative. A study in the RIP-Tag2 model of pancreatic NETs described that tumors refractory to therapy following long-term treatment with a vascular endothelial growth factor receptor-2 blocking antibody contained blood vessels with a prolific investment of pericytes expressing α-smooth muscle actin. Further studies are warranted to validate the occurrence of pericytes expressing α-smooth muscle actin as a biomarker for tumors refractory to therapy [44].

A perspective

Morphological, histological and molecular features of NETs strongly support the notion that angiogenesis is a promising target in these malignancies. Indeed, several anti-angiogenic drugs have been clinically validated and two of those have been recently approved and are being incorporated in the daily clinical practice of pancreatic NETs. Nevertheless, not all patients respond to these therapies demonstrating upfront refractoriness to therapy or *intrinsic resistance*. This patient population has to be carefully studied and detected in the future to find the most appropriate patient selection marker or charateristic in order to effectively treat these refractory patients. On the other hand, anti-angiogenic drugs demonstrate clinical efficacy in many NETs patients, but these clinical benefits are overshadowed by apparent *acquired resistance* to antiangiogenic therapies emerging in NETs. Therefore, overcoming antiangiogenic resistance is a crucial step in the future development of antiangiogenic therapies. Several strategies have been postulated to fight resistance, including multi-pathway inhibitors or multi-

combination of anti-angiogenic drugs that target different pathways that can revert resistance. In this sense, clinical studies that investigate and address these approaches in the coming years are warranted.

Neverhteless, preclinical data in the RIP-Tag2 model indicates that many of these mechanisms of resistance show reversibility after anti-angiogenic therapy has been stopped (Pàez-Ribes & Casanovas, unpublished observations). This confirms that these forms of resistance may reflect adaptations to therapy rather than irreversively acquired capabilities, and thus suggest that swithching to a non-angiogenic drug in these resistant patients could revert their angiogenesis dependance and re-sensitize these patients to anti-angiogenic drugs. Following this hypothesis, sequential treatment with an anti-angiogenic drug followed by a non-anti-angiogenic drug (i.e. another targeted therapy or chemotherapy) could resensitize patients to another anti-angiogenic drug as a third line of treatment. Obviously, many studies are warranted to unravel the preclinical basis and clinical potential of this hypothetical sequential treatment and to finally determine its clinical benefit for NETs patients.

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References

1- Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey JN, Rashid A, Evans DB. 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;26:3063-72.

2- Terris B, Scoazec JY, Rubbia L, Bregeaud L, Pepper MS, Ruszniewski P, Belghiti J, Fléjou J, Degott C. Expression of vascular endothelial growth factor in digestive neuroendocrine tumours. Histopathology 1998; 32: 133–138.

3- Casanovas O, Hicklin DJ, Bergers G, Hanahan D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell 2005; 8: 299–309.

4- Couvelard A, O'Toole D, Turley H, Leek R, Sauvanet A, Degott C, Ruszniewski P, Belghiti J, Harris AL, Gatter K, Pezzella F. Microvascular density and hypoxia-inducible factor pathway in pancreatic endocrine tumours: negative correlation of microvascular density and VEGF expression with tumour progression. Br J Cancer. 2005;92(1):94-101.

5- Marion-Audibert AM, Barel C, Gouysse G, Dumortier J, Pilleul F, Pourreyron C, Hervieu V, Poncet G, Lombard-Bohas C, Chayvialle JA, Partensky C, Scoazec JY. Low microvessel density is an unfavorable histoprognostic factor in pancreatic endocrine tumors. Gastroenterology 2003; 125: 1094-1104.

6- Couvelard A, Deschamps L, Rebours V, Sauvanet A, Gatter K, Pezzella F, Ruszniewski P, Bedossa P. Overexpression of the oxygen sensors PHD-1, PHD-2, PHD-3, and FIH Is associated with tumor aggressiveness in pancreatic endocrine tumors. Clin Cancer Res 2008; 14: 6634-6639.

7- Konstantinova I, Lammert E. Microvascular development: learning from pancreatic islets. Bioessays 2004; 26:1069-1075.

8- Bergers G, Javaherian K, Lo KM Folkman J, Hanahan D. Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. Science 1999; 284: 808–812.

9- Hanahan D. Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. Nature 1985; 315: 115–122.

10- Parangi S, Dietrich W, Christofori G Holmgren L, Grosfeld J, Folkman J, Hanahan D. et al. 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.

11- Varker KA, Campbell J, Shah MH. Phase II study of thalidomide in patients with metastatic carcinoid and islet cell tumors. Cancer Chemother Pharmacol. 2008;61:661-8.

12- Kulke MH, Stuart K, Enzinger PC, Ryan DP, Clark JW, Muzikansky A, Vincitore M, Michelini A, Fuchs CS. Phase II study of temozolomide and thalidomide in patients with metastatic neuroendocrine tumors. J Clin Oncol. 2006 Jan 20;24(3):401-6.

13- Kulke MH, Bergsland EK, Ryan DP, Enzinger PC, Lynch TJ, Zhu AX, Meyerhardt JA, Heymach JV, Fogler WE, Sidor C, Michelini A, Kinsella K, Venook AP, Fuchs CS. Phase II study of recombinant human endostatin in patients with advanced neuroendocrine tumors. J Clin Oncol. 2006 Aug 1;24(22):3555-61.

14- Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, Ferrara N. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth *in vivo*. Nature.1993;362:841–844.
15- Zhang J, Jia Z, Li Q, Wang L, Rashid A, Zhu Z, Evans DB, Vauthey JN, Xie K, Yao JC. Elevated expression of vascular endothelial growth factor correlates with increased angiogenesis and decreased progression-free survival among patients with low-grade neuroendocrine tumors. Cancer. 2007 Apr 15;109(8):1478-86.
16- Yao JC, Phan A, Hoff PM, Chen HX, Charnsangavej C, Yeung SC, Hess K, Ng C, Abbruzzese JL, Ajani JA. 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;26:1316-23.
17- Kulke MH, Stuart K, Earle CC, Bhargava P, Clark JW, Enzinger PC, Meyerhardt JA, Attawia M, Lawrence C, Fuchs CS. A phase II study of temozolomide and bevacizumab in patients with advanced neuroendocrine tumors. J Clin Oncol 2006;24, No. 18S (June 20 Supplement):4044.
18- Kunz PL, Kuo T, Zahn JM, Kaiser HL, Norton JA, Visser BC, Longacre TA, Ford JM, Balise RR, Fisher GA. A phase II study of capecitabine, oxaliplatin, and bevacizumab for metastatic or unresectable neuroendocrine tumors. J Clin Oncol 28:15s, 2010 (suppl; abstr 4104).
19- Venook AP, Ko AH, Tempero MA, Uy J, Weber T, Korn M, Bergsland EK. Phase II trial of FOLFOX plus bevacizumab in advanced, progressive neuroendocrine tumors. J Clin Oncol 2008; 26:abstr.
20- Franco M, Roswall P, Cortez E, Hanahan D, Pietras K. (2011) Pericytes promote endothelial cell survival through induction of autocrine VEGF-A signaling and Bcl-w expression. Blood 2011; 118(10):2906-17.
21- Bergers G, Song S, Meyer-Morse N et al. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J Clin Invest 2003; 111: 1287–1295.
22- Pietras K, Hanahan D. A multitargeted, metronomic, and maximum-tolerated dose ‘‘chemo-switch’’ regimen is antiangiogenic, producing objective responses and survival benefit in a mouse model of cancer. J Clin Oncol 2005; 23:939–952.
23- Fjallskog ML, Lejonklou MH, Oberg KE, Eriksson BK, Janson ET: Expression of molecular targets for tyrosine kinase receptor antagonists in malignant endocrine pancreatic tumors. Clin Cancer Res 2003; 9: 1469–1473.
24- Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, Valle J, Metrakos P, Smith D, Vinik A, Chen JS, Horsch D, Hammel P, Wiedenmann B, Van Cutsem E, Patyna S, Lu DR, Blanckmeister C, Chao R, Ruszniewski P. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. N Engl J Med 2011;364:501-13.
25- Kulke MH, Lenz HJ, Meropol NJ, Posey J, Ryan DP, Picus J, Bergsland E, Stuart K, Tye L, Huang X, Li JZ, Baum CM, Fuchs CS. Activity of sunitinib in patients with advanced neuroendocrine tumors. J Clin Oncol. 2008 Jul 10;26(20):3403-10.
26- Hobday TJ, Rubin J, Holen K, et al: MC044h, a phase II trial of sorafenib in patients (pts) with metastatic neuroendocrine tumors (NET): a Phase II Consortium (P2C) study. J Clin Oncol 2007; 25:abstr.
27- Pavel ME, Bartel C, Heuck F, Neumann F, Tiling N, Pape UF, Plöckinger U, Wiedenmann B. Open-label, non-randomized, multicenter phase II study evaluating the angiogenesis inhibitor PTK787/ ZK222584 (PTK/ZK) in patients with advanced neuroendocrine carcinomas (NEC). J Clin Oncol 2008; 26:abstr
28- Anthony L, Chester M, Michael S, O’Dorisio TM, O’Dorisio MS. Phase II open-label clinical trial of vatalanib (PTK/ZK) in patients with progressive neuroendocrine cancer. J Clin Oncol 26: 2008 (May 20 suppl; abstr 14624).
29- A. T. Phan, J. C. Yao, D. R. Fogelman, K. R. Hess, C. S. Ng, S. A. Bullock, P. Malinowski, E. Regan, M. Kulke. A prospective, multi-institutional phase II study of GW786034 (pazopanib) and depot octreotide (sandostatin LAR) in advanced low-grade neuroendocrine carcinoma (LGNEC). J Clin Oncol 28:7s, 2010 (suppl; abstr 4001).
30- Vignot S, Faivre S, Aguirre D, Raymond E. mTOR-targeted therapy of cancer with rapamycin derivatives. Ann Oncol 2005; 16: 525–537.
31- Karar J, Maity A.PI3K/AKT/mTOR Pathway in Angiogenesis. Front Mol Neurosci. 2011;4:51.
32- Pavel M, Baudin E, Couvelard A, Krenning E, Öberg K, Steinmüller T, Anlauf M, Wiedenmann B, Salazar R. ENETS Consensus Guidelines for the Management of Patients with Liver and Other Distant Metastases from Neuroendocrine Neoplasms of Foregut, Midgut, Hindgut, and Unknown Primary. Neuroendocrinology 2012;95:157–176 In press.

33- Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG, Tomassetti P, Pavel ME, Hoosen S, Haas T, Lincy J, Lebwohl D, Öberg K; RAD001 in Advanced Neuroendocrine Tumors, Third Trial (RADIANT-3) Study Group. Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med. 2011 Feb 10;364(6):514-23.
34- Boehm, T., Folkman, J., Browder, T., and O'Reilly, M.S. 1997. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature 390:404-407.
35- Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer. 2008; 8(8):592-603.
36- Pàez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Viñals F, Inoue M, Bergers G, Hanahan D, Casanovas O. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell. 2009 Mar 3;15(3):220-31.
37- Yao JC, Phan A. Overcoming antiangiogenic resistance. Clin Cancer Res. 2011 Aug 15;17(16):5217-9.
38- Rapisarda A, Hollingshead M, Uranchimeg B, Bonomi CA, Borgel SD, Carter JP, et al. Increased antitumor activity of bevacizumab in combination with hypoxia inducible factor-1 inhibition. Mol Cancer Ther 2009;8:1867–77.
39- Jia Z, Zhang J, Wei D, Wang L, Yuan P, Le X, et al. Molecular basis of the synergistic antiangiogenic activity of bevacizumab and mithramycin. Cancer Res 2007;67:4878–85.
40- Allen E, Walters IB, Hanahan D. Brivanib, a Dual FGF/VEGF inhibitor, is active both first and second line against mouse pancreatic neuroendocrine tumors developing adaptive/ evasive resistance to VEGF inhibition. Clin Cancer Res 2011;17:5299–310.
41- Kulke MH, Chan JA, Meyerhardt JA, Zhu AX, Abrams TA, Blaszkowsky LS, Regan E, Sidor C, Fuchs CS. A prospective phase II study of 2-methoxyestradiol administered in combination with bevacizumab in patients with metastatic carcinoid tumors. Cancer Chemother Pharmacol 2011;68:293-300.
42- Castellano DE, Capdevila J, Salazar R, Sastre J, Alonso V, Llanos M, Garcia-Carbonero R, Abad A, Sevilla I, Duran I. Sorafenib and bevacizumab combination targeted therapy in advanced neuroendocrine tumor: A phase II study of the Spanish Neuroendocrine Tumor Group (GETNE0801). J Clin Oncol 2011;29 (suppl; abstr 4113).
43- Yao JC, Phan AT, Fogleman D, Ng CS, Jacobs CB, Dagohoy CD, Leary C, Hess KR. Randomized run-in study of bevacizumab (B) and everolimus (E) in low- to intermediate-grade neuroendocrine tumors (LGNETs) using perfusion CT as functional biomarker. J Clin Oncol 28:15s, 2010 (suppl; abstr 4002).
44- Franco M, Pàez-Ribes M, Cortez E, Casanovas O, Pietras K. Use of a mouse model of pancreatic neuroendocrine tumors to find pericyte biomarkers of resistance to anti-angiogenic therapy. Horm Metab Res. 2011 Nov;43(12):884-9. Epub 2011 Sep 29.