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10. Perspectives in the development of novel treatment approaches

James C Yao, Laura Catena, Annamaria Colao, and Giovanni Paganelli

Role of mTOR inhibitor in the management of NETs: RADIANT studies

James C Yao

Associate Professor and Deputy Chair, Gastrointestinal Medical Oncology, University of Texas MD Anderson Cancer Center, USA

Introduction

Dysregulation of the mammalian target of rapamycin (mTOR) pathway has been observed in many human tumors and has been found to be implicated in the promotion of cancer cell growth and survival. Hence, the mTOR pathway is considered an important target of anticancer therapy. This paper focuses on the role of mTOR inhibition and on the recent developments of the mTOR inhibitor everolimus (RAD001) in this field.

The role of mTOR inhibition in the management of neuroendocrine tumors

The mammalian target of rapamycin (mTOR) is an intracellular, evolutionary-conserved serine/threonine-protein kinase that acts as a central regulator of the cell cycle and metabolism in response to environmental cues. It exerts its effects primarily by turning on and off the cell's translation machinery, which includes the ribosomes, and is responsible for the synthesis of proteins that are essential for cell growth and proliferation, angiogenesis, and bioenergetics[1-3]. Specifically, mTOR lies at the interface of multiple signal transduction pathways and performs its regulatory function in response to activating or inhibitory signals transmitted through these pathways, which are located upstream of mTOR in the cell. mTOR-mediated signals include stimulation by growth factors such as vascular endothelial growth factors (VEGFs), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1), hormones (estrogen, progesterone), and the presence or absence of nutrients (glucose, amino acids) or oxygen[4,5]. Growth factors stimulate mTOR through the PI3K (PI3-kinase)/Akt pathway by binding to RTKs (receptor tyrosine kinases). PTEN (phosphatase and tensin homolog) regulates the activity of Akt and, once activated, Akt mediates the activity of TSC2, resulting in the activation of mTOR. Activated mTOR increases the rate of protein synthesis for mRNA by activating proteins that direct DNA translation, such as S6K1 and 4E-BP1[6]. This results in an increase in the production of proteins that stimulate cell growth and proliferation, cellular metabolism, and angiogenesis.

Recently, an increasing body of evidence has indicated that the mTOR pathway is involved in the patoghenesis of neuroendocrine tumors (NETs). In effect, mTOR can be inappropriately "switched on" by many activating mutations found in NETs. Several genetic syndromes associated with NETs, such as tuberous sclerosis complex, neurofibromatosis, von Hippel-Lindau syndrome and multiple endocrine neoplasia type 1) involve signaling through the mTOR pathway[7]. For instance, tuberous sclerosis complex (TSC)1/2 is an inhibitor of mTOR and is present in normal neuroendocrine cells[8]. The loss of TSC2 at chromosome 16p13 is known to be associated with the development of pancreatic islet cell tumors[9,10]. The neurofibromatosis (*NF1*) gene also regulates the activity of mTOR, and the loss of NF1 (neurofibromin 1) protein leads to constitutive mTOR activation and is associated with the development of carcinoid tumors in the ampulla of Vater, duodenum, and mediastinum[11-14]. Similarly, approximately 12% of patients presenting with von Hippel-Lindau syndrome, which is due to a germline deletion of the *VHL* gene, develop islet cell carcinoma[15]. In addition, sporadic loss of the VHL loci has been found in both carcinoid and islet cell tumors[16]. Several reports have showed increased EGF and IGF signalling upstream of mTOR in NETs[17-20]. Interestingly, the role of the PI3K/Akt/mTOR pathway in pancreatic endocrine tumors (PETs) has been recently confirmed by Missaglia *et al.*, who demonstrated that TSC2 and PTEN, two key inhibitors of the Akt/mTOR pathway, were downregulated in most of the primary tumors, and that their low expression was significantly associated with shorter disease-free survival and overall survival[21].

Given the association between mTOR activation and human cancer, the mTOR pathway has become an important target for cancer drug development, and in the recent years significant efforts have been made to synthesize specific and effective inhibition of this pathway. Rapamycin analogs form a complex with the intracellular immunophilin FKBP12 and the resultant complex binds to the FK-rapamycin binding domain of mTOR, in turn leading to inhibition of the function of mTOR in mTORC1 and the mTORC1-mediated signal pathway, thereby preventing phosphorylation of S6K1 and 4EBP1. Among the recently developed rapamycin analogues, everolimus (RAD001) has shown promising antitumor activity, in combination with octreotide long-acting repeatable (LAR), in a phase II study on 60 pa-

tients with metastatic, unresectable, low-to-intermediate grade carcinoids and islet cell tumors[22]. The intent-to-treat (ITT) response rate was 20%. In the protocol population, there were 13 patients with confirmed partial responses (PRs, 22%), 42 patients with stable disease (SD; 70%), and 5 patients with progressive disease (PD, 8%). Among 30 carcinoid patients, there were 5 confirmed PRs (17%), 24 SDs (80%), and one PD (3%). Among 30 islet cell patients, there were eight PRs (27%), 18 SDs (60%), and four PDs (13%). Overall median progression-free survival (PFS) was 60 weeks. Stratified by tumor group, median PFS of patients with carcinoid and islet cell tumors was 63 weeks and 50 weeks, respectively. Based on these encouraging results, everolimus has been subsequently evaluated in a larger, phase II trial.

Antitumor activity of everolimus in advanced pancreatic NETs: the RADIANT-1 study

RADIANT-1 (RAD001 in Advanced Neuroendocrine Tumors) was a multinational, open-label, phase II trial conducted to evaluate the antitumor activity of everolimus in patients (n = 160) with advanced pancreatic NETs experiencing progression according to the Response Evaluation Criteria in Solid Tumors (RECIST) on or after cytotoxic chemotherapy[23]. Enrolment was stratified based on ongoing octreotide therapy at study entry: patients who were not on octreotide therapy at study entry were assigned to stratum 1 (n = 115) and were treated with oral everolimus 10 mg daily, whereas patients who were on octreotide LAR for at least 3 consecutive months at study entry were assigned to stratum 2 (n = 45) and received everolimus 10 mg daily orally and octreotide LAR intramuscularly every 28 days at prestudy dose (≤30 mg). Treatment was continued until disease progression or unacceptable toxicity. The primary end point of the study was objective response rate (ORR) in stratum 1. Secondary end points included ORR in stratum 2 and PFS, duration of response, OS, safety, and pharmacokinetics in both strata. Exploratory analyses were also performed on biomarkers, including serum chromogranin A (CgA) and neuron-specific enolase (NSE).

Efficacy was evaluated according to RECIST (computed tomography or magnetic resonance imaging) at baseline and every 3 months. All radiographic images were reviewed locally at the study site and centrally by two independent reviewers.

By central radiology review, in stratum 1 there were 11 partial responses (PRs, 9.6%), 78 patients (67.8%) with stable disease (SD), and 16 patients (13.9%) with progressive disease (PD). The best overall response was unknown in 10 patients (8.7%), while a clinical benefit (PR + SD) was reported in 89 patients (77.4%). In stratum 2, there were 2 partial responses (4.4%), 36 patients (80.0%) with SD, and no patients with PD; the best overall response was unknown in 7 patients (15.6%), and a clinical benefit (PR + SD) was observed in 38 patients (84.4%). Median PFS by central radiology review was 9.7 months (95% confidence interval [CI], 8.3 to 13.3 months) in stratum 1, and 16.7 months (95% CI, 11.1 months to not available [NA]) in stratum 2. Median OS in stratum 1 was 24.9 months (95% CI, 20.2 to 27.1 months), whereas it had not been reached for stratum 2 at the time of data cutoff. The 24-month survival rate for stratum 2 was 54.7% (95% CI, 21.7% to 87.8%). Tumor shrinkage was observed in 64 patients (59.3%) in stratum 1 and in 32 patients (84.2%) in stratum 2.

CgA and NSE levels were evaluated monthly if elevated at baseline. Interestingly, an early CgA response, defined as normalization or ≥30% decrease at week 4 in CgA levels, was associated with significantly longer PFS and OS. Specifically, an early CgA response was observed in 46.5% of evaluable patients (33 of 71 patients) in stratum 1. Median PFS in early CgA responders was 13.3 months compared with 7.5 months in patients who did not demonstrate an early CgA response ($P = .00004$; hazard ratio [HR] = 0.25; 95% CI, 0.13 to 0.51). Similarly, in early CgA responders of stratum 1 median OS was 24.9 months compared with 12.7 months in early CgA nonresponders ($P = 0.01092$; HR = 0.25; 95% CI, 0.21 to 0.83). The same association between an early response and prolonged PFS was observed also for NSE. Median PFS was 8.6 months in early NSE responders who achieved normalization or ≥30% decrease at week 4 in NSE levels, compared with 2.9 months in patients who did not demonstrate an early NSE response in stratum 1 ($P = 0.00062$; HR = 0.25; 95% CI, 0.10 to 0.58). PFS data by CgA or NSE in stratum 2 were not evaluable because of the small numbers of patients.

Treatment with everolimus, with or without concomitant octreotide LAR, was generally well tolerated, with the majority of adverse events that were mild to moderate in severity.

Taken together, these findings have confirmed the antitumor activity of everolimus in pancreatic NETs reported in the previously mentioned phase II trial, and have paved the way to the development of large-scale, confirmatory phase III trials. The randomized, placebo-controlled, RADIANT-2 and the RADIANT-3 trials have completed accrual, and are currently evaluating everolimus plus octrotide LAR *versus* octreotide LAR plus placebo in advanced NETs and everolimus plus best supportive care *versus* best supportive care plus placebo in advanced pancreatic NETs, respectively.

The significant association between early CgA or NSE response and longer PFS suggest that these markers are a promising tool for selecting patients most likely to benefit from everolimus. However, their predictive and prognostic value warrant further evaluation in ongoing phase III clinical trials.

Potential effects of everolimus on the glycemic control in insulinoma patients

Refractory hypoglycaemia is a severe complication of malignant insulinoma and its management is challenging. Currently available treatments include dietary modification, diazoxide, and the use of intravenous dextrose infusion or enteral feedings. Patients with advanced, unresectable insulinomas often have prolonged hospitalizations and may have fatal complications from this disease.

In a recent report, Kulke *et al.*[24] evaluated the clinical responses of four consecutive patients with functioning insulinomas who were treated with everolimus. Multiple therapies had failed in all four patients, and they all required aggressive management of hypoglycaemia. After receiving everolimus, all four patients had substantial improvement in glycemic control. Some possible explanations for this improvement have been proposed, but further confirmation is needed. In effect, while in the two patients with radiological evidence of tumor regression the observed clinical benefit may have been the result of the antitumor effect of everolimus, the improvement of the two other patients who did not have tumor regression seems to suggest a direct effect of the drug on glycemic control.

Available evidences suggest that functional insulin receptors are present on beta cells and mediate insulin stimulated insulin production and release, and that mTOR inhibition downstream of insulin receptors may decrease insulin production and release[25,26]. Other studies have indicated that mTOR inhibition may either suppress or increase insulin output, depending on the dose and schedule[27]. It is also possible that everolimus induces peripheral insulin resistance, as suggested by the fact that long-term treatment with rapamycin in renal-transplant recipients induced peripheral insulin resistance by impairing AKT activation and signaling through the insulin-receptor substrate pathway[28]. Further investigations are warranted to define the effect of everolimus on glycemic control.

Conclusions

In the recent years, the mTOR pathway has become an important target for cancer drug development, and specific and effective inhibitors of this pathway have been synthesized and investigated. The novel mTOR inhibitor everolimus, alone or in combination with octreotide LAR, has demonstrated antitumor activity and has been well tolerated in phase II studies on patients with advanced NETs. Large-scale, confirmatory phase III trials are ongoing and, hopefully, they may lead to further advances in the treatment of NETs. Further studies are warranted to define combination strategies with everolimus and other biological agents.

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Targeted therapy

Laura Catena

Medical Oncology Unit 2, Fondazione IRCCS Istituto Nazionale dei Tumori of Milan, Italy

Introduction

The recent interest in the development of molecularly targeted therapy as a treatment modality for neuroendocrine tumors (NETs) had led to investigate the potential role of molecules such as imatinib, bevacizumab, sunitinib, temsirolimus, and everolimus (RAD001). However, the extreme heterogeneity and complexity of NETs and the availability of small number of patients on one hand, together with the fact that clinical experience sometimes goes ahead of our knowledge on molecular biology of NETs on the other, may result in misinterpretation of the potential of these drugs.

Recombinant human endostatin

Endostatin is a 20-kd proteolytic fragment derived from the carboxy-terminal region of collagen XVIII, a proteoglycan which is a major constituent of blood vessels throughout the body[1]. In preclinical studies, endostatin has been shown to inhibit the migration and proliferation of vascular endothelial cells and cause tumor regression[2-5]. Four phase I studies of recombinant human endostatin (rhEndostatin) suggested activity in neuroendocrine tumors (NETs), which are known to be hypervascular[6-9].

Based on these premises, a multicenter phase II study of rhEndostatin was performed in patients (n = 42) with both metastatic carcinoid (53%) or pancreatic NETs (47%)[10]. Nearly all patients (90%) had histologically well differentiated tumors, and the majority (64%) had received some form of prior therapy other than surgery including systemic chemotherapy, interferon alfa, and embolization of hepatic metastases. Patients received rhEndostatin at the initial dose of 60 mg/m^2/day. After completion of 4 weeks of therapy, 52% of patients were found to have subtherapeutic trough endostatin levels and underwent dose escalation to 90 mg/m^2/day. Notably, patients receiving octreotide at study entry were allowed to continue therapy at stable doses throughout study treatment.

In this study, no patients experienced a partial or complete radiologic response to therapy, 32 (80%) had stable disease (SD) as their best response to therapy, and eight patients (20%) experienced disease progression (PD). The median progression-free survival (PFS) was 5.8 months (range, 1.9 to 13.5 months) for patients with pancreatic endocrine tumors and 7.6 months (range, 5.3 to 19.2 months) for patients with carcinoid tumors. The median overall survival (OS) was 17.2 months (range, 8.1 to 27.2 months) for patients with pancreatic endocrine tumors and 22.6 months (range, 17.8 to >27.4 months) for patients with carcinoid tumors. The main treatment-emergent adverse events (AEs) included dyspnea (36%), fatigue (30%), abdominal pain (29%) and diarrhea (26%). Grade 3 toxicities were developed by 34% of patients.

This study demonstrated that treatment with rhEndostatin was associated with low toxicity, but did not result in a significant antitumor activity in patients with advanced NETs. After this first phase II evaluation, rhEndostatin has not been further evaluated in this indication. However, this trial had some limitations, namely

the inclusion of a heterogeneous patient population, including both carcinoid and pancreatic NETs, the lack of firm data regarding the optimal therapeutic dose of rhEndostatin. Finally, it is worth noting that patients receiving octreotide at study entry continued their therapy throughout the study period, which can be a point of reflection in light of the results of the PROMID study.

Temsirolimus

Temsirolimus (sirolimus 42-ester 2,2-bis hydroxymethyl propionic-acid; CCI-779) is a more water-soluble ester derivative of its parent compound sirolimus, selected for development as an anticancer agent based on its more favourable pharmaceutical characteristics and superior therapeutic index. Temsirolimus has been tested in phase I and II trials with rather promising activity on different tumor types, including advanced renal cell carcinoma (RCC) and advanced breast cancer[11-14].

Based on these previous experiences, a phase II study was undertaken to evaluate the efficacy, safety and pharmacodynamics of temsirolimus 25 mg per week as intravenous infusion in a mixed population of patients (n = 37) with pretreated, well differentiated neuroendocrine carcinomas comprising carcinoid tumors and pancreatic islet cell carcinomas[15]. Efficacy results suggested only modest activity in advance NETs: no complete response (CR) was reported, 5% of patients experienced a partial response (PR) and 27% of patients showed PD. However, 54% of patients achieved SD. The intent-to-treat response rate was 5.6% (95% CI 0.6-18.7%), median time to progression (TTP) was 6 months and 1-year OS rate was 71.5%. Interestingly, pharmacodynamic analyses in paired biopsies, obtained before and 2 week after initiation of temsirolimus, confirmed that temsirolimus effectively downregulates the phosphorylation of S6, and that higher baseline levels of pS6 and phosphorylated mTOR seem to predict for a better response. In this study, significant toxicity was observed, with the most frequently reported adverse events including fatigue (78%), hyperglycaemia (69%) and rash/desquamation (64%).

The little activity demonstrated by temsirolimus in this cohort, together with the unfavourable toxicity profile shown, did not warrant further investigation of this drug as single agent in this patient population. However, one could argue if the results obtained in a very small sample size of patients with mixed tumors should be taken for granted. Furthermore, it may be conceivable that the use of a different dose should have resulted in lower toxicity.

Imatinib mesylate

Imatinib mesylate is a phenylaminopyrimidine derivative which inhibits protein tyrosine kinases, abl, platelet derived growth factor (PDGF) receptor (PDGFR), and c-kit. In diseases associated with activating mutations in abl, c-kit and PDGFβ, such as chronic myelogenous leukaemia and gastrointestinal stromal tumor, imatinib has demonstrated significant clinical activity and has led to significant improvement in clinical outcome[16,17].

Based on the promising results observed in these diseases and on the fact that carcinoid tumors express both PDGF ligand and receptors, it was decided to investigate the activity of imatinib also in this setting. With this intent, a phase II study was designed to assess the response rate and the safety profile of imatinib 400 mg twice daily in a patient population (n = 31) with metastatic carcinoid tumors[18]. Concurrent use of octreotide was allowed. According to the results, no patient achieved a CR, 4% of patients experienced a PR, 83% had SD and 33% had PD. The median PFS was 5.9 months (95% confidence interval, 2.1-9.7 months), whereas the median OS was 36 months (95% confidence interval, 18-54 months). Notably, patients receiving concurrent octreotide therapy during the study also had a significantly improved PFS compared with those not on octreotide therapy (49 weeks compared with 14 weeks; $P = 0.03$), which highlights the importance of planning the addition of octreotide in clinical trials on this population. Immunoistochemical analysis were also performed on available tumor tissue to screen for expression of imatinib targets on tumors. Interestingly, these analyses revealed that most carcinoid tumors expressed abl, PDGF and PDGFR, but the expression did not predict outcome. In addition, none of the cases tested expressed c-kit. As far as toxicity is concerned, the most common grade 3/4 adverse event associated with imatinib was fatigue, which occurred in 26% of patients, followed by hypophosphatemia and diarrhea which were observed in 20% and 11% of patients, respectively.

Bevacizumab

Bevacizumab is a humanized monoclonal antibody that recognizes and blocks vascular endothelial growth factor A (VEGF-A). Carcinoid tumors are vascular and are known to express vascular endothelial growth factor (VEGF)[19,20], and in low-grade neuroendocrine tumors VEGF expression has been associated with metastases and shortened PFS[21].

Based on these observations, a phase II, randomized study was conducted to assess the activity of bevacizumab *versus* pegylated (PEG) interferon alfa-2b as monotherapy, followed by the combination of the two agents, in metastatic carcinoid tumors[22]. Specifically, the study population consisted of 44 carcinoid patients on a stable dose of depot octreotide for 2 months before study entry. One prior cytotoxic chemotherapy was allowed, while prior interferon was not allowed. Patients

were randomized to 18 weeks of treatment with bevacizumab 15 mg/kg intravenously once every 3 weeks or PEG interferon alfa-2b 0,5 mcg/kg subcutaneously once per week. All of them continued depot octreotide at the prestudy dosage. After the completion of the 18-week therapy or at disease progression (whichever occurred first), patients received bevacizumab plus PEG interferon, always in association to octreotide, until progression.

The results obtained in this study look promising and indicate that bevacizumab may have a role in the treatment of carcinoid tumors. In the bevacizumab arm, 18% of patients achieved a confirmed PR, 77% experienced SD and 5% had PD. In the PEG interferon arm, no patient achieved PR, 68% had SD and 27% had PD. Most interestingly, PFS was 16.5 months in patients treated with bevacizumab compared to 14.0 months in patients treated with PEG interferon. Median OS has not been reached. The advantage in PFS observed in the bevacizumab arm is even higher compared to that observed with octreotide in the PROMID study, where the duration of PFS was 14.3 months. Another interesting observation of this study regards the effect of treatment on tumor blood flow, which was evaluated by means of functional computed tomography (CT) scans obtained at baseline, 2 days after the first dose of bevacizumab, 9 weeks after the first dose of PEG interferon, and 18 weeks after the start of initial treatment. Compared with paired baseline measurements on functional CT scans, a decrease in tumor blood flow was observed in 49% (P <.01) and 28% (P <.01) of patients treated with bevacizumab at day 2 and week 18, whereas no significant changes in tumor blood flow were observed following PEG interferon. However, due to the small number of patients who had functional CT scans in each arm, the ability to correlate functional CT scan findings with clinical outcome was limited, and the only two responders having functional CT scans did not show any statistically significant correlation between blood flow parameters and tumor responses.

The most frequent toxicities were hypertension in the bevacizumab arm, which occurred in 36% of patients, and granulocytopenia, which occurred in the 27% of the PEG interferon arm.

The favourable PFS obtained in the bevacizumab arm warrants further evaluation in larger randomized trials. However, in future studies the use of PEG interferon as comparator should probably be abandoned.

Sunitinib malate

Sunitinib malate is a small molecule kinase inhibitor with activity against a number of tyrosine kinase receptors, including VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, PDGFR-β, stem-cell factor receptor, glial cell line-derived neurotrophic factor receptor, and FMS-like tyrosine kinase-3[23-26]. The antitumor activity of sunitinib in both renal call carcinoma (RCC) and GI stromal tumors (GIST) has been demonstrated in several studies, and today this molecule is approved for use in patients with these tumor types[27-30].

The highly vascular nature of neuroendocrine tumors, as well as the fact that they express vascular endothelial growth factor (VEGF) and its receptor (VEGFR), have led to assess the activity of sunitinib also in this disease. Specifically, the molecule has been assessed in a phase II, open-label, multicenter study on patients (109) with pretreated carcinoid and pancreatic neuroendocrine tumors[31]. Sunitinib was administered in repeated 6-week treatment cycles at the oral dose of 50 mg once daily for 4 weeks, followed by 2 weeks off treatment. Patients receiving stable doses of octreotide at baseline (53% with carcinoid tumors and 27% with pancreatic NETs) were allowed to continue therapy during the study period. Treatment was continued until disease progression, unacceptable toxicity, or withdrawal of consent.

In pancreatic endocrine tumor patients, PR rate was 16.7%, and SD rate was 68%. Among carcinoid patients, PR rate was 2.4%, and SD rate was 83%. No CR was reported in either group of tumors. Median TTP was 7.7 months in pancreatic neuroendocrine tumor patients and 10.2 months in carcinoid patients. PD was observed in 7.6% and 10.2% of pancreatic neurondocrine tumor and carcinoid patients, respectively. One-year survival rate was 81.1% in pancreatic neuroendocrine tumor patients and 83.4% in carcinoid patients.

These results demonstrate that treatment with sunitinib is associated with antitumor activity in patients with advanced NETs. However, toxicity was considerable, with 88.8% of patients experiencing fatigue and 70% of patients experiencing anemia.

On the basis of these phase II results, a phase III, multicenter, randomized, double-blind trial (NCT00428597) investigated the efficacy and safety of sunitinib *versus* placebo in patients (n = 171) with well-differentiated pancreatic islet cell tumors and documented disease progression within the past 12 months[32]. The primary endpoint was PFS. The results of this study, which have been presented at the ASCO GI this years, confirms the antitumor activity of the molecule, as demonstrated by the median duration of PFS of 11.1 months in the sunitinib arm compared to 5.5 months in the placebo arm (hazard ratio 0.397; 95% CI: 0.243, 0.649; P <0.001).

Conclusions

Molecular-targeted drugs may have a potential role as a treatment strategy for NETs. In particular, bevacizumab and sunitinib malate have demonstrated to have antitumor activity and must be further evaluated in future investigations. However, to fully appreciate the poten-

tial of these drugs in the treatment of NETs, it is of paramount importance to perform clinical trials on well-defined, homogeneous patients' population of adequate size. Furthermore, particular attention must be paid to the toxicity profile of these drugs. In fact, an accurate estimation of the risk-benefit ratio is warranted, especially because NETs patients are typically long-term survivors. Knowledge on molecular biology of NETs are essential to guide clinical experience and to allow a correct evaluation of treatment response.

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Dopastatins: somatostatin-dopamine chimeric molecules

Annamaria Colao

Department of Molecular & Clinical Endocrinology & Oncology, University "Federico II" of Naples, Naples, Italy

Introduction

Neuroendocrine tumors (NETs) express somatostatin receptors, which are considered targets for therapy. The development of somatostatin analogs for treatment of functioning NETs was a revolution in the management of these patients, and recently these drugs have also demonstrated an anti-tumor effect, with stabilization of tumor growth over long periods of time. However, a number of patients may display tachyphylaxis or resistance to treatment with the available compounds. In the last decades, new insights in the field of somatostatin as well as dopamine receptor pathophysiology have opened a new scenario for the management of tumors expressing these receptors. Indeed, new analogues, which may drastically change the current therapeutic modalities of treatment of NETs, have been already developed and are currently under investigation in preclinical and clinical studies. Among these new compounds with larger binding properties to different receptors subtypes compared to the currently available drugs, the chimeric molecules are capable of binding to both somatostatin and dopamine receptors, and appear to be a promising therapeutic tool in patients resistant to "classical" dopamine agonists and somatostatin analogs.

Rational basis for the clinical use of dopastatins

The presence of a high density of somatostatin receptors (SSRs) on human neuroendocrine tumors (NETs) and pituitary adenomas forms the rationale for the successful application of octapeptide somatostatin analogs such as octreotide and lanreotide in these diseases. Indeed, currently available somatostatin analogs effectively control hypersecretory syndromes associated with several forms of NETs and, as recently demonstrated, they can also inhibit tumor cell proliferation leading most frequently to disease stabilization for long periods of time. Nevertheless, the effects of somatostatin analogs are often partial and of limited duration, and a rather high number of patients may either display tachyphylaxis or resistance to the available compounds. Several possible explanations for this thachyphylaxis have been proposed, including the heterogeneous expression of SSRs or the expression of SSR subtypes with low affinity for the ligand or other causes, as subsequently detailed.

The majority of SSR-positive tumors simultaneously express multiple SST subtypes, but there is a considerable variability in the SST subtype expression patterns not only among the different tumor types, but also among tumors of the same type. Indeed, this differential expression of SSR subtypes among tumors may partly account for the differences observed among patients with respect to the efficacy of treatment. Among the 5 subtypes of SSRs ($sst_{1\text{-}5}$), the subtype 2 (sst_2) is the most abundantly expressed in human neuroendocrine gastroenteropancreatic tumors (GEP NETs), pituitary ade-

nomas and non neuroendocrine tumors[1]. The predominant expression of the sst_2 receptors in human tumors forms the basis for the successful clinical application of the sst_2 preferential ligands currently in use. In fact, octreotide and lanreotide present with a binding affinity mainly directed towards sst_2 and comparable to the affinity of native somatostatin (SRIF-14). However, their binding affinities for sst_5 and sst_3, which are strongly expressed in most tumors as well, are respectively 10 to 15 and 60-160 times less than that of SRIF-14, and this lower affinity for SSR subtypes other than sst_2 may explain, at least in part, the escape from somatostatin analog therapy observed in a proportion of patients.

Other mechanisms underlying thachyphylaxis to somatostatin analogs may include a down-regulation of SSRs on the tumor cells and the selection of SSR-negative cell clones[1].

Importantly, these observations have reopened the potential role of dopamine receptors (DRs) and of dopaminergic drugs in the treatment of pituitary adenomas as well as in other tumors expressing DRs. Nowdays dopamine agonists, including cabergoline, bromocriptine, quinagolide, terguride and lisuride, represent the medical treatment of choice for prolactinomas. Through preferential binding to the subtype 2 of DRs (D_2), they can effectively decrease prolactin (PRL) secretion as well as the size of the tumor in patients with prolactinoma, with rates of control as high as 80-90% for microprolactinomas and of 60-75% for macroprolactinomas[2].

In most growth hormone (GH)-secreting pituitary adenomas, both sst_2, sst_5 and the subtype 2 of DRs (D_2) are significantly coexpressed, at both mRNA and protein levels, although half of GH tumours also coexpress sst_3 and sst_1, particularly mixed GH/PRL adenomas[3-9]. The vast majority of prolactinomas express high numbers of D_2 receptor together with sst_1 and particularly sst_5, which are also notably present, whereas sst_2 is only expressed in a minority of them[3,4,8-10]. Again, the D_2 receptor is preferentially coexpressed in association with sst_3 and sst_2 in most clinically non-functioning pituitary adenomas (NFPAs)[6,11-12], and is significantly coexpressed with sst_5 in corticotroph adenomas, where sst_2, sst_1 and sst_3 are also expressed but at lower levels[4,12-14]. With regard to the GEP NETs, a pivotal study by O'Toole *et al.* demonstrated the coexpression of sst_2 and D_2 in 100% of cases, and of sst_5 in 89%[15]. D_2 expression has been found as well in adrenocorticotropic hormone (ACTH)-secreting ectopic neuroendocrine tumors associated with the ectopic ACTH syndrome (EAS). Pivonello *et al.* were the first to demonstrated the presence of D_2, as well as of D_4, in 6 patients with ACTH-secreting ectopic tumors, including four lung, one thymic, and one pancreatic carcinoids[16]. In particular, specific D_2 immunostaining was found in five (83.3%) of the six cases, among which the four lung and the thymic carcinoids, and the expression of this receptor was confirmed at quantitative reverse-transcriptase-polymerase chain reaction (RT-PCR) in all the three cases of lung carcinoids that were tested with this technique. Interestingly, this study demonstrated also for the first time a possible effectiveness of the dopamine agonist, preferentially D_2 ligand cabergoline in ACTH–secreting ectopic tumors. In two of the three patients with persistent EAS after surgery, treatment with cabergoline resulted in a significant suppression of plasma ACTH and urinary cortisol levels after 1 month, and in a complete normalization of both clinical parameters after 3 months. However, treatment escape occurred in one of these patients afterward[16].

In a subsequent report published on the New England Journal of Medicine, the same authors described the case of a patient with ectopic ACTH or Cushing's syndrome due to a lung carcinoid tumor[17]. Since somatostatin analogs had been shown to be effective in controlling carcinoid corticotropin secretion, after surgery therapy with lanreotide was begun. At six months, urinary cortisol levels decreased, but then stopped responding to treatment, and lanreotide therapy was stopped after one year. When RT-PCR analysis of SSR and DR expression was performed in a tumor sample, the dopamine D_2 receptor was found in addition to somatostatin receptor subtype 5, and dopamine-agonist therapy with cabergoline was initiated. After six months, cortisol secretion normalized but then stopped responding again, and the administration of cabergoline was stopped as well after one year. On the basis of the documented interaction between the D_2 and the sst_5 receptors, combined treatment with cabergoline and lanreotide was then instituted. Combination treatment with a somatostatin analog and a dopamine agonist resulted in a rapid and more prolonged normalization of urinary cortisol levels. This case documents the long-term effectiveness of combined treatment with a somatostatin analog and a dopamine agonist in a patient who no longer had a response to either agent alone, and, most interestingly, supports the hypothesis of an interaction between somatostatin and dopamine receptors resulting in a somehow enhanced, synergistic action. In fact, since this tumor expressed both D_2 and sst_2 receptors, escape from single agent treatment most likely was not due to a lack of expression of the specific receptor targeted by the single ligand[17].

The hypotesis of an interaction between SSRs and DRs was indeed demonstrated by Rocheville M and coworkers[18], who showed that these receptors, when coexpressed at the membrane level and simultaneously bound by appropriate ligands, come close one to the other forming homo- and hetero-dimers. These dimers constitute novel receptor entities which may activate alternative pathways and potentiate intracellular signal transduction, resulting in more pronounced apoptotic mechanisms[18].

The improved characterization of tumor receptor profiles, together with the discovery of a functional interac-

tion between SSRs and DRs at the cell membrane level, have opened a new scenario for the management of tumors expressing these receptors, and have led to a new chemical approach consisting in the development of a new class of hybrid, chimeric molecules which combine structural elements of both somatostatin and dopamine. These molecules have been called dopastatins or chimeras to highlight their capacity to target simultaneously both SSRs and DRs. For instance, the chimeric compound BIM-23A387 targets simultaneously sst_2 and D_2, whereas BIM-23A760 binds with high affinity to sst_2 and D_2 and with lower affinity to sst_5. In an experiment performed on a pituitary adenoma cell line (AtT20 cells), the group of Los Angeles guided by Shlomo Melmed immunocytochemically localized the sst_2 and the D_2, and found that both receptors were present on the same cell membranes (Ben-Shlomo, Melmed/Culler, Cedars-Sinai/IPSEN, personal communication). The complete overlap of these receptors on the same cell forms the rational basis for the clinical usage of dopastatins.

Preclinical and clinical evidences on dopastatins

Apart from BIM-23A760, which has already reached the experimental clinical phase II, all the other chimeric molecules are currently under investigation in preclinical studies *in vitro* and *in vivo*. So far, preliminary data seem to confirm the higher potency of these new chimeric molecules compared with the clinically available dopamine agonists and somatostatin analogs, particularly in inhibiting hormone secretion in cell cultures of selected clinically nonfunctioning adenomas (NFAs) and GH-secreting pituitary adenomas. Thus, BIM-23A387 and BIM-23A760 have been recently tested *in vitro* in primary cultures derived from different series of NFAs, showing a suppression of the α-subunit concentration (the biological marker of the tumor activity) that was higher of about 50% compared to that induced by traditional drugs (Ferone *et al.*, unpublished observations).

A larger study with dopastatins in primary cell cultures of GEP NETs has been started by Pivonello and coworkers, to evaluate the effect of sst_2/sst_5 and D_2 selective chimeric molecules on cell proliferation measured by ^{3}H-thymidine incorporation. Data collected so far indicate that BIM-23A387 and BIM-23A760 have been significant more potent in inhibiting cell proliferation compared to lanreotide and cabergoline, either used alone or in combination (Pivonello *et al.*, unpublished observations).

Similarly, in a TT cell line derived from medullary thyroid carcinoma (MTC), BIM-23A387 and BIM-23A760 result in a more potent suppression of calcitonin compared to lanreotide, cabergoline and their combination. However, in this setting BIM-23A387 achieves a higher inhibition of calcitonin secretion compared to BIM-23A760. In fact, sst_5, which represents a preferential target of BIM-23A760, but not of BIM-23A387, is mainly involved in cell proliferation, while it exerts only minimal effects on the control of hormonal secretion. Notably, this observation confirms that the effects of the different chimeric compounds vary according to the diverse receptor expression patterns and, above all, to the specific role of receptor types in each tumor (Pivonello *et al.*, unpublished observations).

Moreover, results from a study on normal primates seem to suggest that the administration of BIM-23A760 is not associated with the risk of insulin suppression and hyperglycemia, which are often observed in patients treated with somatostatin analogs. In humans, GH secretion is inhibited by somatostatin and is stimulated by dopamine. Interestingly, in cynomolgous monkeys (*Macaca fascicularis*), which are the species closest to humans from the evolutionary point of view, BIM-23A760 at escalating doses induces significant, dose-related suppression of GH and prolactin, but no change in either insulin or glucose (Culler, IPSEN, personal communication).

As previously mentioned, BIM-23A760 has been the first chimeric molecule to have reach experimental phase II. Specifically, in a recent multicenter, open-label, phase 2, single-dose study, seven men and four postmenopausal women with acromegaly were allocated to BIM 23A760 dosing cohorts (1 mg and 4 mg) according to their sensitivity to octreotide[19]. The first four patients showing octreotide sensitivity received a single subcutaneous dose of 1 mg. After the safety data had been examined by a clinical safety committee, and if the dose was considered safe and well tolerated, a further four patients with octreotide sensitivity received a single subcutaneous dose of 4 mg. Patients not showing sensitivity to octreotide received the dose being administered at the time their eligibility for the trial was confirmed. The 1-mg cohort (n = 5) had a baseline GH level of 5.1 µg/L, compared with 29.1 µg/L in the 4-mg cohort (n = 6). The lower dose achieved a maximum GH suppression that averaged 66.4%, while the higher dose reduced baseline levels of GH by an average of 74%. Nine of 11 patients had GH suppression greater than 50%, and the median time to maximum GH suppression was 48 to 49 hours with both doses. IGF-1 levels remained elevated throughout follow-up, but all patients in both groups had reductions from baseline. Notably, GH suppression was maintained up to 2 weeks after single-dose administration, which suggests that this compound has an intrinsic long-acting activity. A significant suppression of PRL levels was observed as well in both cohorts. As far as safety is concerned, 6 patients experienced adverse events (AEs), without significant differences between the two cohorts. Five of these AEs were considered drug-related, including two cases of mild hypotension and one each of abdominal distension, diarrhea,

and injection-site erythema. All adverse effects were mild or moderate in severity, and none led to withdrawal from the study. No clinical changes were observed at ECG, gallbladder echography or other clinical laboratory safety tests. No specific antibodies were detected in any of the samples assayed[19].

Conclusions

The advent of new chimeric molecules that bind both somatostatin and dopamine receptors may provide a new therapeutic option in the management of NET patients. In different NETs, dopastatins seem to be more effective than traditional somatostatin analogs and dopamine agonists, used alone or in combination, and hopefully they may allow to overcome resistance to these "classical" compounds which is still observed in a rather high percentage of patients. Currently available evidences, derived mostly from preclinical studies and in part also from phase II clinical trials, have shown encouraging results. In particular, dopastatins have been shown to inhibit hormone secretion and cell proliferation in experimental settings in different NET cell lines or cell cultures, and to induce or stabilize somatostatin-dopamine receptor dimers in tumor cell lines. Furthermore, data from the first clinical phase II trial on acromegaly suggest that these compounds inhibit GH, IGF-I and PRL secretion from GH-secreting pituitary tumors in a dose-dependent and prolonged fashion. However, further studies are needed to elucidate the potential role of dopastatins in the future therapeutic armamentarium of NETs. A clearer understanding of the expression of dopamine and somatostatin receptors at the cellular level, with particular emphasis on the co-expression of the different receptors subtypes in each tumor, is also strongly warranted.

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Peptide receptor radionuclide therapy

Giovanni Paganelli

Nuclear Medicine Division, European Institute of Oncology, Milan, Italy

Introduction

The improved knowledge of the molecular characteristics and processes characterizing neuroendocrine tumors (NETs) has paved the way to the exploration of new treatment modalities. One of this modality is peptide receptor radionuclide therapy (PPRT), which relies upon the overexpression of somatostatin receptors on tumor cells. This paper provides a brief overview of the main events which led to the development of PRRT, summaries the state of the art of this therapeutic approach and lingers on new perspectives.

Rational basis of peptide receptor radionuclide therapy

In the early 1970s it was found that the small cyclic peptide hormone somatostatin (SST) exerts inhibitory effects on the physiological secretion of various hormones and on the overproduction of hormones from certain tumor types. This discovery has been of paramount importance and has marked the beginning of a new era in the treatment and management of neuroendocrine tumors (NETs). Since the majority of NETs express somatostatin receptors (SSTRs), they represent an ideal target for therapeutic and diagnostic strategies based on the use of SST analogs. The approved analogs octreotide and lanreotide are currently used for the symptomatic control of hypersecretory syndromes, while their antiproliferative efficacy is still under debate. Octreotide was the first analog to be developed and applied in the clinical practice in the 1980s. It is a synthetic peptide which exerts most of the biological actions of the native peptide somatostatin, but has a longer plasma half-life (2 min *vs* ~120 min following subcutaneous administration) being resistant to plasma degradation[1]. Octreotide binds to SSTR subtype 2 (sst_2) with high affinity, to sst_5 with moderately high affinity and to sst_3 with intermediate affinity, whereas binding to subtypes 1 and 4 is extremely weak.

Radiopharmaceuticals are molecules labelled with a radioisotope. The covalent link of the radiolabelled molecule to a chelator ensures the stability of the complex. Radiopharmaceuticals can be employed for the *in vivo* visualization and localization of SSTR-positive NETs due to their ability to bind suitable ligands[2]. Interestingly, these substances offer an imaging modality which is based on the physiological (ie, the presence of functioning receptors) rather than the anatomical characteristics of the tumors[3]. Specifically, binding of the radiolabelled compound to the receptor after injection constitutes the molecular basis for the use of radiolabelled SST analogs for the identification of SSTR-positive tumors. After binding, receptor-mediated internalization of the receptor-ligand complex can occur, resulting in degradation to metabolites in the lysozomes[4-7]. These metabolites are retained in the lysozomes with consequent longer retention of radioactivity in sst_2 positive cells. Following binding or internalization, a therapeutic radionuclide can exert its radiation effects to tumor cell DNA and other cell structures.

The first radiolabelled somatostatin analog was ^{123}I-labelled Tyr^3-octreotide[8]. However, this compound showed limited power in abdominal NETs because of hepatic clearance and short retention in tumor cells[9]. Octreotide was subsequently labelled with ^{111}In, via the attachment of diethylenetriamine-N,N,N',N'N-pentaacetic acid (DTPA) as chelator. [^{111}In-DTPA^0]-octreotide displayed superior pharmacokinetic characteristics as compared to [^{123}I-Tyr^3]octreotide[9,10]. Somatostatin receptor scintigraphy with [^{111}In-DTPA^0]-octreotide (OctreoScan®) has proven to have great potential for the visualization of SSTR-positive tumors, and has become an imaging modality of choice in patients with gastroenteropancreatic (GEP) tumors[11,12]. Furthermore, it provides a more accurate staging of the disease by demonstrating tumor sites that were not shown by conventional imaging[13]. Subsequently, ^{99m}Tc-labelled tetra-amine [Tyr^3]octreotate (Demotate) and the positron emission tomography (PET) tracer [^{68}Ga-DOTA^0, Tyr^3]octreotide were also introduced for SSTR scintigraphy[14,15]. Labelled lanreotide was developed as well, but the images obtained with this compound were generally of inferior quality compared to those achieved by using radiolabelled octreotide[16,17].

Peptide receptor radionuclide therapy (PRRT) represents the logical extrapolation of the experience with radiolabelled octreotide for the diagnosis of SSTR expressing tumors. In effect, coupling a radioisotope to a molecule which specifically binds to tumor cells can deliver an effective radiation dose to the tumors without damaging healthy tissues, thus limiting adverse effects[18]. The first attempts to perform PRRT were made in 1990s in a multicenter study which used high activities of the molecule already used in diagnostic imaging, ^{111}In-[DTPA]^0-octreotide. At the basis of the choice of this compound there was the knowledge that the Auger electrons and internal conversion electrons emitted by ^{111}Ind in addition to the γ-radiation decay in close proximity to the cell nucleus after the peptide/receptor complex internalization. Treatment of patients with metastasized NETs with high activity of ^{111}In-[DTPA]^0-octreotide resulted in promising effects, but partial remissions were observed only in exceptional cases[19]. In effect, although ^{111}Ind emits some therapeutic particles, it is not an optimal ra-

dionuclide for radiotherapy and higher-energy and longer-range -particle emitters such as ^{90}Y (E_{max} 2.3 MeV, R_{max} 11 mm, $T_{1/2}$ 64 hrs) are more suitable for therapeutic purposes. Therefore a new analogue, Tyr^3-octreotide, was labelled with ^{90}Y and bound to the bifunctional chelator DOTA (1,4,7,10-tetra-azacyclododecane-*N*,*N*′,*N*′′,*N*′′′-tetra-acetic acid), resulting in ^{90}Y-[DOTA]^0-Tyr^3-octreotide [^{90}Y-DOTATOC]. This new analogue displays a similar pattern of affinity for SSRs, but is characterized by an higher hydrophilicity compared to ^{111}In-[DTPA]^0-octreotide[20]. Finally, another radiolabelled somatostatin analog was developed: [^{177}Lu-DOTA^0, Tyr^3]octreotate (^{177}Lu-DOTATATE). This analog differs from [DOTA]^0-Tyr^3-octreotide for the replacement of the C-terminal threoninol with threonine, and has a 9-fold higher affinity for sst_2[21].

At the moment, ^{90}Y-DOTATOC and ^{177}Lu-DOTATATE are the radiopeptides most frequently used for the treatment of NETs by PRRT, and to date they have provided highly appealing results in terms of achievement of objective responses in patients with otherwise untreatable, metastatic disease. Unfortunately, these radiopeptides are not commercially available and must be synthesized *de novo*. For this reason, PRRT is currently performed only at a few centers of excellence, including Rotterdam University, Basel University and the European Institute of Oncology in Milan.

Pretreatment considerations

The radioactive concentration at the tumor site is crucial for the success of PRRT and can be modulated. In fact, the probability of tumor shrinkage increases proportionally with the increase of the radioactivity concentration in the tumor. The various factors that can influence the amount of uptake of radiolabelled SST analogs include the kinetics characteristics of the radiopeptide used, the density of SSTR expression on the tumor, the type of SSTRs expressed by the tumor, the affinity of the radioligand for the SSTRs, and the efficiency of SSTR-mediated internalization and reclycling[1]. ^{90}Y-DOTATOC and ^{177}Lu-DOTATATE show a remarkably favourable pharmacokinetic profile, with rapid plasma clearance after administration and relevant renal excretion[22]. Furthermore, as previously mentioned, they possess a high affinity for sst_2, the most widely expressed receptor in NETs. The receptor density on tumor *versus* normal organs plays also a critical role. The higher is the density, the greater will be the amount of radiopeptide that may be conveyed inside the tumor cells. In clinical practice, the density is generally evaluated by means of receptor scintigraphy. Tumor remission, in fact, is positively correlated with a high uptake at receptor scintigraphy[23]. Nevertheless, tumor radiation dose does not only depend directly on the administered activity and the uptake *versus* time, but also on the tumor mass. Since tumor radiation dose represents the ratio between delivered radioactivity and tumor mass, smaller masses have higher chances of mass reduction, owing to a higher absorbed dose in the tumor. For this reason, in order to optimize outcome it is highly recommended that PRRT is administered when metastatic lesions are still of limited size.

With regard to the radionuclide, the choice of the most appropriate option must be guided by the different kind of lesions to be treated as well as by the energy emission of the radionuclide itself. The energy of lutetium (Lu) is low (E_{max} 0.5 Mev), so that this energy will be absorbed in a small area, up to 1.8 mm from the decay point. For this reason, ^{177}Lu is generally used for the treatment of superficial area. ^{90}Y has more energetic emission (E_{max} 2.3 Mev) and the maximum range of the particles is of 11 mm. Therefore, ^{90}Y finds application for more extended lesions. An important implication of the long range of each emitted electron is the production of crossfire from the radioisotope localized on receptor-positive tumor cells. In fact, even though tumor heterogeneity can cause incomplete responses, the production of crossfire can kill the nearby receptor-negative tumor cells, a circumstance which avoids the need to target every cell within the tumor and allows to overcome a certain degree of heterogeneity[24-27].

Biodistribution and dosimetry

PRRT aims to deliver the highest possible dose to the tumor while sparing normal tissues from damage[26]. However, radiopharmaceuticals are taken up by both tumor cells and normal tissues, with the highest predicted absorbed doses to the spleen, the kidneys and the tumor (Figure 1)[28,29].

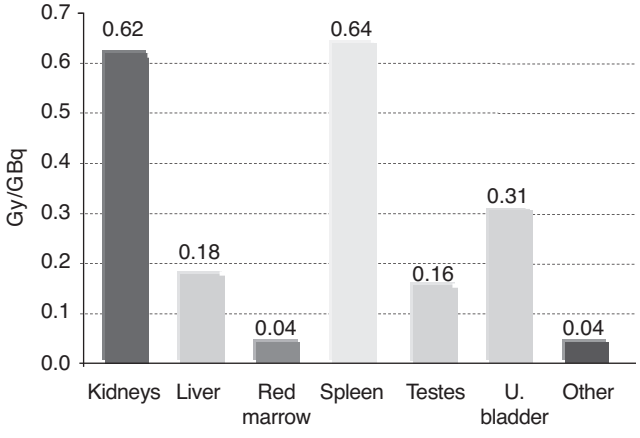

Figure 1 - Median absorbed doses after administration of ^{177}Lu-DOTATATE.

Given the marked radiosensitivity of the renal parenchyma, the kidneys must be considered the principal dose-limiting organs in PRRT with ^{177}Lu-DOTATATE and especially with ^{90}Y-DOTATOC. These radiopharmaceuticals are predominantly cleared by the kidneys. The small peptides are filtered by the glomerulus and most of the activity is excreted into the urine. However, part of the injected activity is taken up in the proximal tubular cells. After transport to the lysozomes, about 2% of the radioligand is retained in the lysozomes[8], resulting in prolonged kidney irradiation. Pre-existing risk factors such as hypertension, diabetes or previous chemotherapy with platinum-based regimes, considerably augment nephrotoxicity. However, dosimetry can provide reliable dose estimates to the kidneys and to tumoral tissues before therapy, leading to identify patients who would benefit most from therapy and patients unsuitable for therapy as well[26,30]. Specifically, dosimetric analyses suggest a BED threshold of 28 Gy in patients with risk factors, and a BED threshold of 40 Gy in patients without risk factors. A potential reduction in the renal irradiation dose can be achieved by the use of positively charged amino acids, such as L-lysine or L-arginine, which competitively inhibit the proximal tubular re-absorption of the radiopeptide.

Efficacy of PRRT with 90Y-DOTATOC and 177Lu-DOTATATE

PRRT with ^{90}Y-DOTATOC and ^{177}Lu-DOTATATE has been so far evaluated in several clinical phase I-II trials. Although all these studies were largely inhomogeneous with regard to patient selection, inclusion criteria, treatment schedules and dosages, their results indicate that this that this treatment modality is a promising tool in the management of patients with otherwise untreatable, metastatic NETs.

The radiopeptide that has been most extensively evaluated is ^{90}Y-DOTATOC. In a first report by Otte *et al.*[31], 29 patients received four or more cycles of ^{90}Y-DOTATOC according to a dose-escalating scheme with cumulative activities of 6.120 ± 1.347 GBq/m^2. Twenty of these patients showed disease stabilisation, two had partial remission, four minor remission and three progression. In a subsequent study[32], 39 patients were treated with four equal intravenous injections, for a total of 7.4 GBq/m2 of ^{90}Y-DOTATOC, administered at 6-week intervals. The objective response rate according to WHO criteria was 23%, with complete remissions in 5% of the patients, partial remissions in 18%, stable disease in 69% and progressive disease in 8%. Interestingly, objective response rates in endocrine pancreatic tumors were 38%. A significant reduction of clinical symptoms was reported as well[32].

Between 1997 and 2002, our group treated 141 patients affected mainly with NETs with a cumulative activity of 7.4-26.4 GBq of ^{90}Y-DOTATOC, divided into 2-16 cycles, 4-6 weeks apart. Objective response rate was 26%. Disease stabilization was observed in 55% of the patients and disease progression in 20%. The mean duration of response ranged between 2 and 59 months (median, 18 months). Most of the patients who responded (69.7% of cases) had gastro-entero- pancreatic neuroendocrine tumors[30]. Figure 2 shows an example of a partial response in a patient affected by a pancreatic endocrine tumor[33]; Figure 3 provides an example of a good objective response in a patient with liver metastases from a pancreatic insulinoma[34].

With regard to ^{177}Lu-DOTATATE, the Rotterdam group treated 35 patients affected by gastroenteropancreatic NETs with 3.7, 5.6, or 7.4 GBq, up to a final cumulative dose of 22.2-29.6 GBq. Complete and partial responses were observed in 38% of patients[35].

More recently, an evaluation of 504 patients treated with ^{177}Lu-DOTATATE up to a cumulative dose of 27.8-29.6 GBq, 310 of which evaluated for efficacy, reported the occurrence of complete and partial remissions in 2 and 28% of cases, respectively, and demonstrated a survival benefit of 40 to 72 months from time of diagnosis compared with historical controls[36].

Interestingly, our group of Milan has terminated the first phase I-II study with ^{177}Lu-DOTATATE on 51 patients with neuroendocrine and non-neuroendocrine tumors, including bronchial, duodenum, ileum, appendix, rectum, pancreatic endocrine carcinomas as well as endocrine carcinomas of unknown origin, paragangliomas and meningiomas. Patients were treated with 3.7 - 7.4 GBq/cycle (maximum cumulative activity of 3.7-28.9 GBq in 1-4 cycles). Treatment has been completed in September 2008 and patients' evaluation is still ongoing. To date, objective responses has been observed in 20% of patients, disease stabilization in 44% and disease progression in 18%.

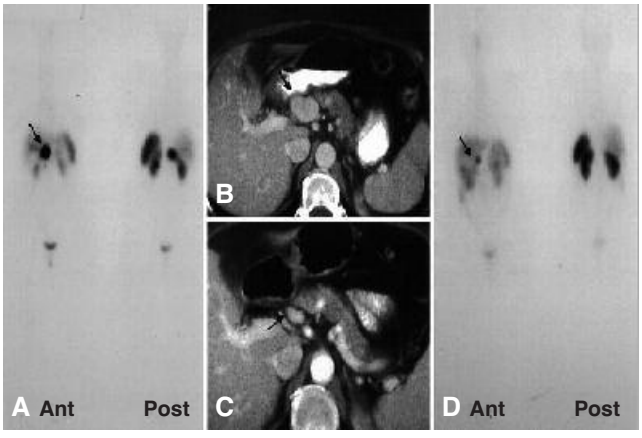

Figure 2 - Partial response to ^{90}Y-DOTATOC in a patient affected by an endocrine pancreatic tumor, as detected by OctreoScan scintigraphy and CT scan, performed respectively before (A, B) and after (C, D) treatment.

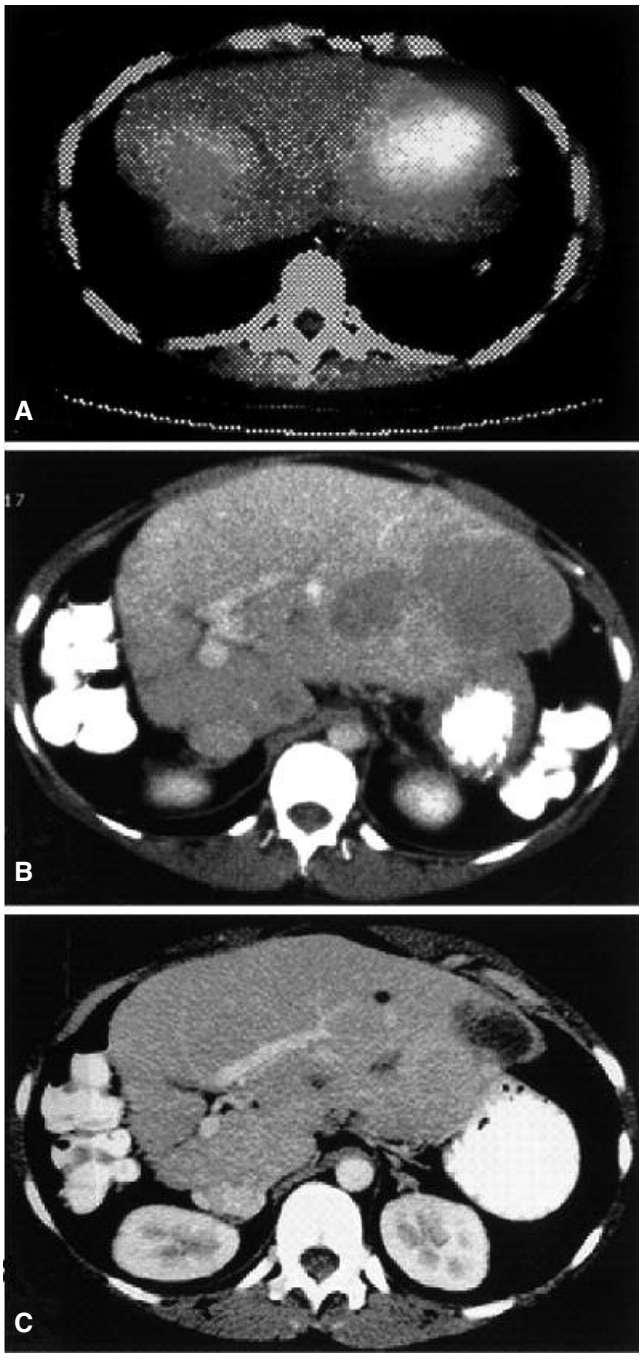

Figure 3 - Liver metastases from pancreatic insulinoma before and after ^{90}Y-DOTATOC therapy in a 38-year-old patient with good uptake. A) OctreoScan prior to treatment superimposed on CT. B) CT prior to treatment. C) CT 8 months after treatment: a remarkable reduction in liver lesions is evident.

Future perspectives

Present knowledge and experience indicate the high potential of PRRT for the treatment of advanced NETs. However, new, homogeneous phase II and III trials are needed to confirm initial efficacy data and definitively pass from experimental to standardized therapy. Furthermore, we need Good Manufacturing Practice facilities for the centralized production and delivery of radiopeptides. Finally, since patients with NETs benefit most from a multidisciplinary therapeutic approach, the integration of PRRT with radiolabelled somatostatin analogs with other treatment modalities, for example chemotherapy, must be explored in order to further increase response rate. In Australia, where the legislation on experimental studies is less restrictive, various studies with ^{177}Lu-DOTATATE in combination with capecitabine, capecitabine plus temozolomide or everolimus have already been performed. In line with this trend, our group has started a phase I-II trial (LUX study) to assess PRRT with ^{177}Lu-DOTATATE associated with metronomic capecitabine in patients affected by aggressive gastroenteropancreatic NETs (positive ^{18}FDG/^{68}Ga-DOTATOC).

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