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MINI REVIEW

Role of biological targeted therapies in gastroenteropancreatic neuroendocrine tumours

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Abstract Approximately two-thirds of neuroendocrine tumours (NET) occur in the gastrointestinal tract and over 60% present with metastases. With greater insight into molecular pathways involved in tumour progression, opportunities are presented for the use of targeted therapies in NET. Although a wide array of targeted agents has been investigated, only a handful has emerged as forerunners from recent clinical trials. This literature review focuses on the use of anti-angiogenic monoclonal antibody bevacizumab, as well as small molecule inhibitors sunitinib and everolimus.

Keywords Bevacizumab · Carcinoid · Everolimus · Neuroendocrine · Pancreatic · Sunitinib

Introduction

Gastroenteropancreatic neuroendocrine tumours (GEP-NET) are divided into those which originate in the pancreas (PNET), and those outside as carcinoid tumours. Tumours are highly vascular with high expression of vascular endothelial growth factor receptors (VEGFR) which have been demonstrated to correlate with the risk of tumour progression [1–4]. Over 96% of tumours also over-express epidermal growth factor receptors (EGFR) [5].

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Anti-angiogenic therapies and tyrosine kinase inhibitors

The use of anti-angiogenic therapies in NET has been one major area of research in finding a way to inhibit tumour growth. These include an array of drugs which bind to VEGF or antagonise tyrosine kinase-triggered intracellular growth signalling. Bevacizumab is a recombinant humanised monoclonal antibody that binds to VEGF-A (Fig. 1). In a phase II trial, 44 patients with metastatic carcinoid tumours were randomised to receive 18 weeks of treatment using octreotide in combination with 3-weekly bevacizumab at 15 mg/kg or weekly pegylated interferon α-2b (PIF) at 0.5 mcg/kg [6]. After a predetermined time point at 18 weeks or disease progression, patients then received a combination of all the three drugs. Those in the bevacizumab arm demonstrated better partial response (PR) and disease stabilisation rates of 18 versus 0% and 77 versus 68%, respectively with the lower rates of progression (5 vs. 27%). There was also a statistically significant improvement in progression free survival (PFS) at 18 weeks (95 vs. 68%, $P = 0.02$) (Table 1). In addition to response according to Response Evaluation Criteria In Solid Tumours (RECIST) (Table 2), the trial also assessed tumour blood flow, tumour blood volume and permeability using functional CT, reporting a significant reduction in tumour blood flow at baseline compared to week 18 and blood volume (49 vs. 28%, 34 vs. 24%, respectively) for patients treated with bevacizumab. As expected, toxicity profiles of both groups were different, and whereas no patients on bevacizumab encountered grade 3–4 granulocytopaenia (0 vs. 27%), a higher rate of grade 3–4 hypertension (36 vs. 0%) was seen.

Tyrosine kinase inhibitors (TKI) introduced an alternate target by disrupting intracellular signalling known to propagate tumour progression. Sunitinib malate is a TKI with activity against a number of receptors, including

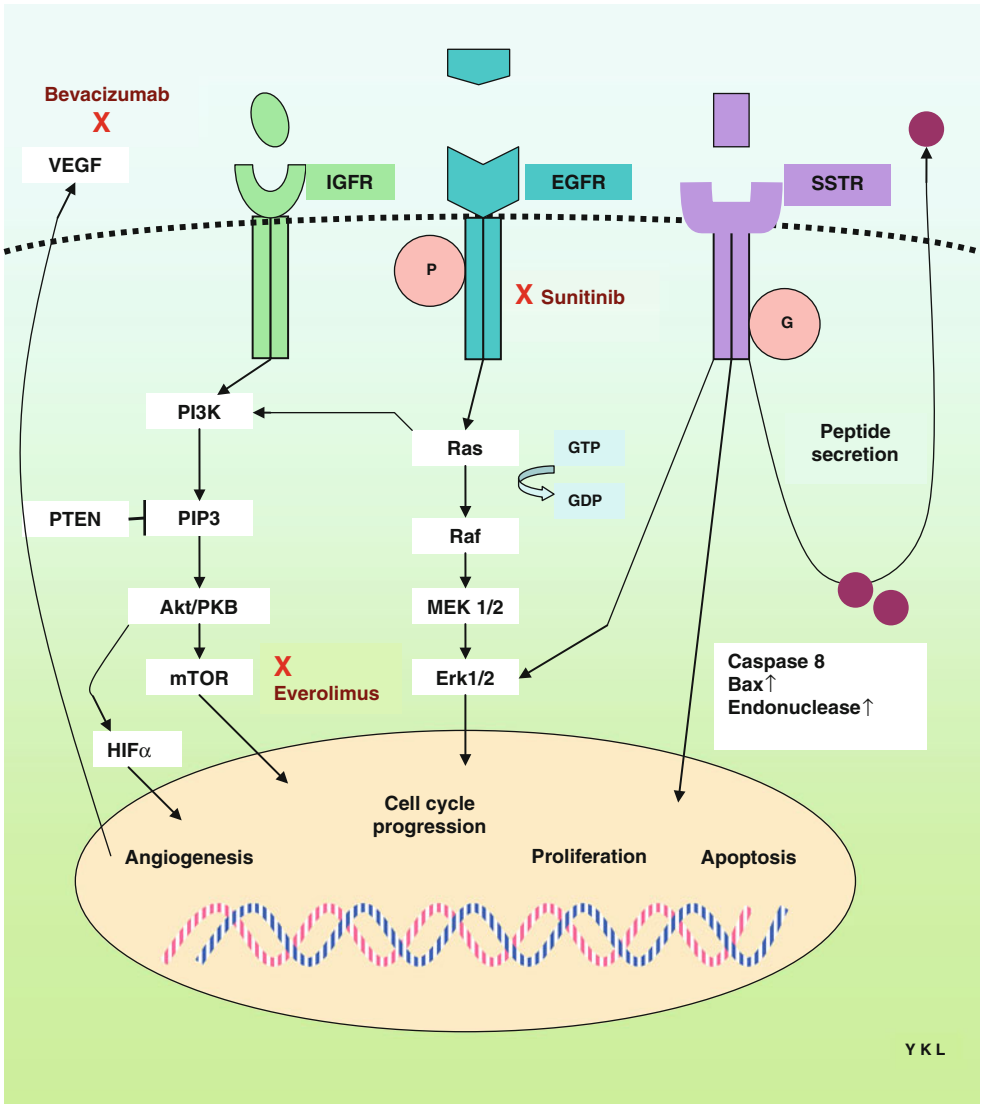

Fig. 1 Simplified diagram of cell surface receptors, intracellular signalling pathways and drug targets

Table 1 A selection of clinical studies investigating biological targeted drug therapies in gastroenteropancreatic neuroendocrine tumours

Study author	Tumour type	Treatment	PR	SD	Outcome
Yao et al. 2008 [6]	Carcinoid	Octreotide with bevacizumab (B) or interferon α-2b (PIF)	18% (+B) vs. 0% (+PIF)	77% (+B) vs. 68% (+PIF)	18 week PFS 95% (+B) vs. 68% (+PIF)
Yao et al. 2010 RADIANT 1 [11]	NET	Everolimus (E) ± octreotide (O)	9.6% (E) vs. 4.4% (E + O)	67.8% (E) vs. 80% (E + O)	9.7 months (E) vs. 16.7 months (E + O)
Yao et al. 2010 [15]	NET	Everolimus with bevacizumab	26%	69%	mPFS 14.4 months
Raymond et al. 2011 [8]	PNET	Sunitnib (S) vs. placebo (P)	9.3% (2CR, 6 PR) (S) vs. 0% (P)	–	mPFS 11.4 (S) vs. 5.5 (P) months
Yao et al. 2011 RADIANT 3 [12]	PNET	Everolimus (E) vs. placebo (P)	5% (E) vs. 2% (P)	–	mPFS 11 months (E) vs. 4.6 (P) months

CR complete response, *mPFS* median progression-free survival, *mTTP* median time to progression, *NET* neuroendocrine tumour, *PIF* pegylated interferon α-2b, *PNET* pancreatic neuroendocrine tumour, *PR* partial response, *SD* stable disease

Table 2 Combined abbreviated reference for RECIST [25, 26]

Definition	Criteria
Complete response (CR)	Disappearance of all target lesions. Any pathological lymph nodes must have reduction in short axis to <10 mm
Partial response (PR)	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters
Progressive disease (PD)	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study with an absolute increase of at least 5 mm. (The appearance of one or more new lesions is also considered progression)
Stable disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

VEGFR-1, VEGFR-2, VEGFR-3, platelet-derived growth factor receptors (PDGFR-α and PDGFR-β). An abstract reported on a phase II trial of 107 patients with progressive advanced NET (41 carcinoid and 66 PNET) treated with 6-weekly cycles of sunitinib at 50 mg o.d. (4 out of every 6 weeks of each cycle) [7]. PR was reported in 17% (11/66) of the PNET and 2% (1/41) of the carcinoid cases. After a median follow up of 13.4 months, disease stabilisation was seen in 68 and 83%, respectively. Hypertension was the predominant side effect and occurred more commonly in carcinoid patients (19.7 vs. 9.8%). Reported median time to progression (TTP) was 7.7 months for PNET and 10.2 months for carcinoid tumours. Encouraging data led to the development of a multi-centre randomised, double-blinded placebo-controlled phase III trial for advanced PNET progressing within 12 months of previous initial therapy [8]. Sunitinib 37.5 mg o.d. was compared to placebo, and patients were treated until progression or development of unacceptable side effects. In patients who progressed, treatment was unblinded and those on placebo were allowed to continue with sunitinib. The study ended early due to more deaths in the placebo group and after a median 4.6 months of treatment in 154 evaluable patients, the published report quoted a PFS of 11.4 months in the sunitinib group versus 5.5 months in the placebo group (HR 0.42; 95% CI: 0.26–0.66; $P < 0.001$). While no patients in the placebo group responded, 8 (9.3%) patients treated with sunitinib achieved objective response based on RECIST (2 complete, 6 partial). Exploratory analysis showed efficacy in categories including age (<65 or ≥65 years), race, gender, ECOG performance status (0, 1 or 2), number of metastatic sites and history of previous treatment (including surgery, chemoembolisation, radiofrequency ablation and somatostatin analogue therapy). The greatest benefit was found in low grade tumours with ki-67 of ≤5%, and multivariate analysis only found interval between diagnosis and randomisation (<3 or ≥3 years) as a significant independent prognostic variable for PFS. Sunitinib incurred expected side-effects of diarrhoea (59%), nausea (45%), increased grade 3 or 4 neutropaenia (12 vs. 0%) and hypertension (10 vs. 0%). Similar encouraging result was also demonstrated with sorafenib, which targets RAF kinase, PDGFR, VEGFR-2, VEGFR-3 and c-Kit. In a study involving progressive advanced NET (50 carcinoid and 43 PNET), 93 patients received sorafenib 400 mg b.d., the abstract reported a 10% PR in both groups, but 6-month PFS was lower in carcinoid patients (40%, 8/20) compared to PNET (61%, 14/23) [9].

mTOR inhibition

A separate strategy focuses on disrupting peptides involved in intracellular signalling pathways which lead to cell growth, proliferation, protein synthesis and angiogenesis. One of the main pathways involve mTOR (mammalian target of rapamycin), an important regulator of PI3K within the PI3K-PIP3-AKT/PKB cascade. Temsirolimus is an early mTOR inhibitor which reported initial activity, followed more recently by everolimus which demonstrated clinical benefit in a recent randomised phase III trial. In a non-randomised phase II study published in 2006, second line weekly temsirolimus 25 mg was used in 36 patients (21 carcinoid and 15 PNET) [10]. Although it achieved PR in only 2 patients (5.6%), median TTP was 10.6 months in the PNET group compared to 6 months in the carcinoid group. Interestingly, the investigators found that an increase of pAKT and decrease of pmTOR after 2 weeks of therapy were significantly associated with a longer TTP ($P = 0.041$ and $P = 0.048$, respectively). With the introduction of a newer generation of mTOR inhibitor, a pilot study assessed everolimus at 5 mg o.d. versus 10 mg o.d. with octreotide LAR 30 mg every 28 days as second line therapy in patients with carcinoid and PNET [11]. From 60 evaluable cases, an overall PR rate of 22% was achieved. However when results between the two subgroups were compared, more partial responses and fewer cases of progression occurred in the carcinoid compared to the PNET group (17 vs. 27% and 3 vs. 13%, respectively). Better response was achieved with the higher dose of everolimus (PR 30 vs. 13%), but grade 3–4 haematological toxicities were more frequent (6 vs. 3%). The results of this initial study led to the same investigators taking forward the everolimus 10 mg o.d. dose into the non-randomised RADIANT 1 study recruiting a bigger cohort of patients with progressive PNET [12]. In order to further investigate the impact of concurrent octreotide therapy, cases were stratified into those who received octreotide in addition to

everolimus at 10 mg o.d ($n = 115$) and those who did not ($n = 45$ cases). PR rate was 9.6 versus 4.4% in favour of the everolimus monotherapy group and although concurrent octreotide did not improve tumour response, better PFS was achieved after a follow-up period of over 16 months (median 9.7 vs. 16.7 months). As a follow up, the RADIANT 3 was developed as a multi-centre randomised, double-blinded placebo-controlled phase III trial which recruited 410 patients with progressive PNET. Patients were randomised to best supportive care with everolimus 10 mg o.d. or placebo and treated until progression or development of unacceptable side effects [13]. In addition, those who progressed on placebo were allowed to continue on everolimus after unblinding. After a median follow-up period of 17 months and median duration of treatment of 8.8 months, the authors reported a significant difference in the primary end point of PFS in the treated group of 11 months compared to 4.6 months on placebo with a 65% reduction in risk of progression or death (HR 0.35; 95% CI: 0.27–0.45; $P < 0.001$). Benefit was irrespective of age, gender, race, WHO performance status, prior treatment (chemotherapy or somatostatin analogue) or tumour grade (well vs. moderately differentiated), and treatment with everolimus confirmed better tumour response and stabilisation (PR 5 vs. 2%, stabilisation 73 vs. 51%). Grade 3 or 4 toxicities were more common in the everolimus group and the most frequent side effects encountered were stomatitis (7% vs 0%), anaemia (6 vs. 0%), and hyperglycaemia (5 vs. 2%) [14]. Although more deaths were seen in the everolimus compared to the placebo group (6 vs. 2%), only 1 was considered to be drug related.

The corresponding randomised double-blinded phase III trial assessing the use of everolimus in progressive advanced carcinoid tumours was the RADIANT-2 trial where 429 patients on octreotide were randomised to receive everolimus or placebo [15]. Similarly, treatment was with everolimus at 10 mg o.d. administered with octreotide LAR 30 mg every 28 days versus octreotide LAR alone. Median PFS in the everolimus arm was 16.4 months compared to 11.3 months in the placebo group, with an associated 23% reduction in risk of progression (HR = 0.77; 95% CI: 0.59–1.00). Although it did not meet its statistical endpoint, a 5.5 month improvement in PFS was reported ($P = 0.0014$).

mTOR inhibition plus bevacizumab

As we have seen, therapies targeted directly towards angiogenesis via inhibition of VEGF with bevacizumab and mTOR inhibitors have shown efficacy in clinical trials. In a reported abstract, investigators studied the combination of everolimus and bevacizumab in 39 patients. Functional CT was used as a surrogate marker and the study included tumours measuring ≥3 cm [16]. Patients were treated with either everolimus or bevacizumab for an initial cycle before receiving a combination of both drugs. Functional imaging at multiple time points demonstrated treatment with bevacizumab alone resulted in a significant (32%) decrease in blood flow and everolimus alone resulted in a significant (13%) increase in mean blood transit time. However, combination treatment showed synergistic anti-tumour activity where a further decrease in blood flow and increase in mean transit time was seen. This translated to an overall PR and stabilisation rate of 26 and 69%, respectively with a reported median PFS of 14.4 months. Further separate trials are recruiting patients to everolimus in combination with other targeted agents.

Future perspectives of biological targeted therapy in GEP-NET

On the horizon, a number of other biological strategies have been considered. Interferon-β introduced to cell lines promoted cell cycle arrest and apoptosis [17], and its mechanism has been linked to modulation of insulin-like growth factor (IGF) intra-cellular signalling responsible for tumorigenesis and proliferation [18]. In a phase I trial investigating IGF-1R inhibition in 53 patients with various solid tumours, PR was seen in 1 of the 5 cases of NET [19]. However, no responses were reported in a follow-up dedicated phase II study of 25 patients with GEP-NET [20]. New generation somatostatin analogues including pasireotide are also being investigated [21, 22]. Correspondingly, multi-targeting studies are underway, including in vitro assessment of interferon combined with TKIs [23]; and in a recent study brivanib, a dual fibroblast growth factor/VEGF inhibitor demonstrated in vivo tumour stasis after progression on sorafenib [24].

Conclusion

Among the vast array of targeted therapies, bevacizumab, sunitinib and everolimus have set the milestones in improving tumour control and clinical outcome in patients with advanced NET validated through clinical trials. However, optimal timing of the use of these agents has yet to be fully defined. Single agent therapy with sunitinib or everolimus has demonstrated efficacy in improving PFS at second line. The choice of targeted agent may thus depend on associated side effects. Raymond et al. [8] reported predominant diarrhoea, nausea, neutropaenia and hypertension with sunitinib, while stomatitis, anaemia and hyperglycaemia were typical of everolimus. The impact of these drugs as initial therapy is currently unknown. The strategy of sequential targeting with

TKIs followed by mTOR inhibitors or vice versa, as well as potential synergistic effect of combined multi-targeted therapy will be interesting to further explore. Likewise, the way response is measured has to be re-evaluated. Novel imaging techniques investigating more relevant endpoints like tumour blood flow for bevacizumab may pave the way for more reliable methods of assessment which could better correlate with drug efficacy, tumour control and survival. Perhaps predictive molecular biomarkers like pAKT and pmTOR on treatment with mTOR inhibitors demonstrated by Duran et al. [10] could also prove to be useful. In conclusion, data from recent important clinical trials investigating targeted therapies have set a new trend in improving clinical outcome in patients with GEP-NET. These studies will provide the framework for future research and the full potential of targeted therapies in the management of NET is yet to be realised.

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Conflict of interest The author has no conflict of interest to declare.

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