Neuroendocrine tumours - Models for Rare Tumour Management

David L Chan,1 David Goldstein2 and John R Zalcberg3
1. Royal North Shore Hospital, Northern Clinical School, University of Sydney, St Leonards, New South Wales, Australia.
2. University of New South Wales and Prince of Wales Hospital, Randwick, New South Wales, Australia.
3. Division of Cancer Medicine, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia.
Email: dlhchan1@gmail.com

Abstract

Neuroendocrine tumours have posed a challenge for the development of research and management strategies because of their rarity and heterogeneity, both from clinical and molecular perspectives. Classification and increased understanding of the molecular landscape has been made possible by the use of reproducible pathological measures and the study of familial forms of the disease. Clinical trials have shown the importance of multi-centre collaboration, appropriate patient selection and stratification by site. These strategies can be applied to other rare malignancies, hopefully resulting in better molecular understanding and improved treatments.

Neuroendocrine tumours (NET) were first described in the medical literature more than a century ago, with the term ‘carcinoid tumour’ being coined by Obendorfer in 1907. They are relatively rare, but are increasing in incidence, from 1.7/100,000 in 1980-89 to 3.3/100,000 in 2000-06 in Australia – which may be due to increased awareness and detection of these tumours.1

Although NETs share a common origin in enterochromaffin cells, this cell type is located in various organs throughout the gastrointestinal tract, as well as the bronchi. Classically, NETs have been grouped embryologically by their site of origin (foregut, midgut or hindgut). Considered as a group, they display striking variation in clinical presentation. Some tumours may be slow-growing and relatively asymptomatic, some may cause symptoms due to the production of biologically active hormones and yet others grow rapidly with an aggressive clinical course. This has complicated attempts to design and interpret clinical trials in this area and hampered attempts to formalise a treatment strategy for all NETs.

The great variance in clinical course has driven the need for a classification system to predict risk, culminating in the publication of the World Health Organisation (WHO) classification in 2010. The study of specific pathogenic mutations underlying familial NETs has also helped elucidate molecular pathogenesis in sporadic cases. While the exact criteria for grading NETs remains the subject of much discussion, the principles behind the classification and treatment of NETs by histological features and ultimately molecular pathways, rather than by anatomic location alone, provide valuable insights into the treatment and investigation of other rare tumours.

Table 1: 2010 World Health Organisation (WHO) classification of neuroendocrine tumours

<table>
<thead>
<tr>
<th>Grade</th>
<th>Mitotic count (mitoses per 10 high power fields)</th>
<th>Ki-67 index</th>
<th>Traditional nomenclature</th>
<th>WHO/ENETS nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>&lt;2mit/10HPF</td>
<td>&lt;3%</td>
<td>Carcinoid, islet cell tumour</td>
<td>Neuroendocrine tumour, Grade 1</td>
</tr>
<tr>
<td>Grade 2</td>
<td>2-20mit/10HPF</td>
<td>3-20%</td>
<td>(Atypical) Carcinoid, islet cell tumour</td>
<td>Neuroendocrine tumour, Grade 2</td>
</tr>
<tr>
<td>Grade 3</td>
<td>&gt;20mit/10HPF</td>
<td>&gt;20%</td>
<td>Small cell carcinoma, large cell neuroendocrine carcinoma</td>
<td>Neuroendocrine carcinoma (large cell or small cell type)</td>
</tr>
</tbody>
</table>

Mixed adenoneuroendocrine carcinoma (MANEC)

Hyperplastic and pre-neoplastic lesions

Mit: Mitoses HPF: High power fields ENETS: European Neuroendocrine Tumour Society
Molecular heterogeneity and classification

NETs display molecular heterogeneity – having a wide variation in the degree of histological aggressiveness, as characterised by the number of mitoses in the pathological specimen, as well as by determination of the proliferation marker Ki67. WHO published its first classification of neuroendocrine tumours in 1980 with four categories - carcinoid tumours, mucuscarcinoïd tumours, mixed forms of carcinoid adenocarcinoma and pseudotumour lesions. The subsequent revisions in 2000 and 2010 reflected a shift away from purely morphological classifications towards histological grading by mitotic rate and Ki67 labelling (see table 1) – proven prognostic factors in gastrointestinal but also pulmonary NETs.2,3

The classification of gastroenteropancreatic NETs (GEPNETs) by mitotic rate and Ki67 labelling provides valuable lessons in the management of other rare tumours. The paucity of cases may make it difficult to develop pathological markers of risk. As a result, mitotic count and Ki67, being somewhat reproducible and feasible in all histological subtypes, are promising potential candidates for risk stratification. The development of objective, quantitative models of risk stratification are essential in order to develop both accurate prognoses and test new therapeutic modalities, and this would be an important starting point in the study of any rare tumour.

The study of families affected by NETs and the genetic syndromes underlying this phenotype led to research into genetic mutations underlying the pathogenesis of NETs. While some genetic mutations were largely confined to familial cases of NETs, some occurred with considerable frequency in sporadic NETs, paving the way to further understanding of pathogenesis and providing possible therapeutic targets.

MEN1 and VHL: genetic clues from familial neoplasia syndromes

Multiple endocrine neoplasia type 1 is an autosomal dominant disorder caused by mutation in the MEN1 gene on chromosome 11q13. It is classically associated with parathyroid and pituitary neoplasms, as well as GEPNETS. Patients with MEN1 germline mutations are more likely to develop functional NETs, particularly gastrinomas and insulinomas; conversely patients with Zollinger-Ellison syndrome have a high likelihood of germline MEN1 mutations (20-60%),4 and is in fact the first clinical symptom of MEN1 syndrome in 40% of affected patients.5 While menin, the protein product of MEN1, has been suggested to interact with various nuclear proteins involved in regulation of gene transcription, its exact function has not been fully defined.

Von Hippel Lindau disease is another autosomal dominant disorder caused by mutation of the VHL gene, resulting in a high lifetime incidence of various tumours, most commonly haemangioheloblastomas of the central nervous system, renal cell carcinomas (clear cell subtype) and phaeochromocytomas. It is also associated with pancreatic neuroendocrine tumours (pNETs) with a lifetime incidence of 9-17% in case series.6,7 The VHL gene is responsible for producing pVHL, a tumour suppressor protein which acts via degradation of HIF1 and HIF2, proteins active in the angiogenesis pathway. Thus, defective production of pVHL would remove normal physiological controls on cell growth and predispose to tumorigenesis.

The above association has led to hypotheses regarding the possible tumorigenic role of MEN1 and VHL in GEPNETS. Massive exome sequencing of 48 small intestinal NETs have confirmed deletions in MEN1, but also have pointed to other potential key mutations in FGFR2, HOOK3, VHL and BRAF among others.8 Other case series have detected MEN1 deletions in a majority of sporadic gastrinomas, as well as some insulinomas and pulmonary carcinoids.3,10

The above examples illustrate the importance of studying familial cases of rare malignancies, continuing a tradition that began with the study of retinoblastoma and the subsequent development of the Knudson two-hit hypothesis. This approach can lead to elucidation of the genomic structure underlying carcinogenesis and direct research towards relevant therapeutic targets.

Lessons in clinical trial design from GEPNETs

One of the lessons learnt from GEPNET trials is the difficulty in accruing the patient numbers needed for a phase III trial. Given the rarity of this disease, timely accrual in a trial context is aided by a multicentre, collaborative approach. For example, even though the PROMID trial which evaluated the role of long acting octreotide versus placebo reported significant results with only 85 randomised patients, this was achieved by 18 German academic centres pooling their referral base over seven years.11 The Raymond trial, which examined the role of sunitinib in pNETS (see below), improved on this by enlisting 42 centres in 11 countries,12 resulting in accrual of 171 patients over only two years. Rare tumours, more so than other types, require close collaboration to achieve sufficient power in a randomised study to advance the evidence base.

Secondly, heterogeneous rare diseases need to be stratified to define populations that will benefit from treatment. Differential efficacy has been observed in trials of targeted agents, showing benefit in pNETs, but not other GEPNETS. Sunitinib, a multitargeted tyrosine kinase inhibitor, is currently the only approved targeted therapy for GEPNETs. Trials of targeted agents, such as the Swede trial, which randomised 46 patients across two treatment arms, showed no statistically significant difference in progression free survival, but did show an improvement in overall survival in the treatment group.13 This highlights the importance of biomarkers to characterise tumours and stratify patients into groups that will benefit from specific therapies. The SUGAR trial, which compared everolimus versus placebo in 109 patients, also did not reach statistical significance,14 but did show that the patients who did respond to therapy had a median progression free survival of 24 months compared to 5 months for the placebo group. This indicates the need for objective markers of response, such as Ki67 and mitotic count, so patients can be enrolled in appropriate trials. In addition, further research into the genetic and molecular heterogeneity of GEPNETs, as exemplified in the above examples, will be important in identifying therapeutic targets.
kinase inhibitor, was trialled in GEPNETs in a phase II trial. This demonstrated response rates of 16.7% in pNETs, but only 2.4% in non-pancreatic NETs. A follow-up placebo-controlled phase III randomised trial confirmed clinical benefit of sunitinib in pNETs, with improvement in progression-free survival from 5.5 to 11.4 months.

Similarly, the RADIANT trials, investigating the use of single-agent everolimus in the treatment of GEPNETS, showed an improvement in progression-free survival in pNETs (RADIANT-3) but not in other GEPNETS (RADIANT-2). This may be due to the differing biology of tumours. While the presence of mutations in the mTOR pathway targeted by everolimus is well established in pNETS (15% demonstrating mutations in TSC, PTEN or PIK3CA), evidence linking it to other GEPNETs is scarce. Despite the progress that has been made in molecular taxonomy, there remains a real need to investigate the presence/absence of molecular differences between pNETs and other GEPNETs in terms of key pathways such as mTOR and VEGF.

For rare diseases with significant heterogeneity, the selection of appropriate subgroups is extremely important. The PROMID study restricted accrual to patients with well-differentiated (Ki67<2%) midgut NETs. This stringent criterion may have led in part to the slow accrual noted above, despite involvement of multiple centres. In addition, while the PROMID trial showed significant improvement in time to progression, its results were difficult to extrapolate to other grades and sites of tumours. The proof of anti-tumour efficacy for somatostatin analogues in other GEPNETs was only determined recently in the CLARINET trial (Lanreotide placebo in grade 1-2 GEPNETs), showing improvement in PFS. Designing trials for rare tumours needs to strike a fine balance between selection of similar patients and clinical feasibility/applicability.

**Nuclear medicine in diagnostics and therapeutics**

The diagnostic and treatment models utilised in GEPNETs can be generalised to other rare tumours. The search for a specific imaging modality can give valuable information regarding the stage of the tumour and gauge response to therapy. The use of fluorodeoxyglucose and gatate positron emission tomography (PET) in GEPNETs has revolutionised the evaluation of GEPNETs. Fluorodeoxyglucose PET is used to detect poorly-differentiated disease, whereas Gatate PET detects well-differentiated disease which overexpresses somatostatin receptors. Gatate PET allows for accurate localisation of disease, but given that the scan reflects sites of octreotide uptake, also predicts for anatomic sites which will take up the administered peptide receptor radionuclide therapy (PRRT), therefore predicting for its effectiveness prior to treatment. Given the linkage of the active radionuclide (such as 177-Lutetium) to octreotide/octreotate, PRRT has the potential to localise treatment to sites of disease showing octreotide uptake. This approach is mirrored in the treatment of other malignancies such as iodine-131 therapy for metastatic papillary and follicular thyroid cancer. If targetable receptors exist in other rare tumours, this would provide valuable investigation and potential treatment modalities.

**Conclusion**

Rare tumours, such as GEPNETs, pose unique challenges in oncology. Issues such as low incidence, molecular and clinical heterogeneity, and optimal trial design, are recurring themes and need to be addressed to facilitate research and progress in the area. Research into other rare cancers would be well served by adopting the above recommendations, hopefully speeding progress towards improved understanding and outcomes from such approaches.

**References:**