THERAPY

The role of liquid biopsies to manage and predict PRRT for NETs

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Patients with neuroendocrine tumours are increasingly treated with peptide receptor radionuclide therapy. However, tumour somatostatin receptor expression evaluation cannot accurately predict who will respond to therapy. Additional criteria to identify which patients are most likely to respond and those who will develop radiation-associated sequelae are critical requirements.

Refers to Strosberg, J. *et al.* Phase 3 trial of ^{177}Lu-Dotatate for midgut neuroendocrine tumors. *N. Engl. J. Med.* **376**, 125–135 (2017)

The management of neuroendocrine tumours (NETs) has been characterized by slow progress in the development of useful diagnostics and adequate treatments. This poor progress reflects a paucity of biological knowledge and the reliance on oncological strategies that are neither NET-specific nor particularly effective. The NETTER-1 study of peptide receptor radionuclide therapy (PRRT; ^{177}Lu-Dotatate) versus a somatostatin analogue[1] represents a substantial advance as — for the first time in 20 years — the discipline of nuclear medicine has undertaken a large, multicentre, prospective, randomized phase III trial. PRRT was substantially more effective than a somatostatin analogue, drugs that have long been touted as effective anti-proliferative approaches despite only two prospective studies demonstrating marginal evidence of efficacy.

A key concern in these studies is the use of the artificial surrogate (progression-free survival, PFS) based upon imaging as an endpoint[2]. As imaging (standard CT or MRI) and RECIST criteria are acknowledged as limited and have not been validated for assessing NET treatment efficacy, basing PFS on them seems paradoxical; no study of non-surgical treatment has demonstrated improvement in overall survival for NETs.

Despite its efficacy, adverse effects occur with PRRT, including bone marrow and renal damage[3]. Pretreatment or intra-treatment prediction of such toxic events are critical to maximize the patient benefit. Complementary diagnostics, such as circulating gene expression assays, are available to identify tumours likely to respond to therapy and individuals susceptible to radiation-associated sequelae. Thus, the NETTER study, in moving the field forwards, raises two important issues: the need to determine the likelihood of efficacy before treatment and to define individuals who will exhibit major adverse radiation-related events.

NET treatment has progressed from generalized, non-targeted approaches (such as chemotherapy) to agents that affect known signalling pathways (for example, mTOR inhibitors such as everolimus)[4]. Expression of therapeutic drug targets are not determined in tumours before therapy despite knowledge that only 10–15% of tumours are probably susceptible[2]. The latest advance is the delivery of anti-proliferative or cytotoxic agents directly to the tumour, which is best exemplified by PRRT that exploits the overexpression of somatostatin receptors (especially type 2) to enable delivery of radiation[3,5]. The optimal isotope is ^{177}Lutetium (a beta-emitter), which is therapeutically delivered conjoined to octreotate (a synthetic somatostatin analogue) by peripheral intravenous infusion and accesses the tumour cell by endocytosis after membrane receptor-mediated binding. Radiation internalization cleaves DNA, inducing apoptosis and tumour destruction. Companion imaging agents (^{68}Gallium-octreotate or ^{111}Indium-octreotide) that identify tumour somatostatin receptors are currently used as study inclusion criteria and to predict the likelihood of therapy effectiveness[6]. Somatostatin receptor scintigraphy defines somatostatin receptor expression (SSRE) from "none" (grade 0) to "intense" (grade 4), or quantifies it as the normalized maximum standardized isotope uptake, SUV_{max}. However, SSRE is heterogeneous within a tumour, between individual tumours and between different individuals, and has limited capacity to predict response or identify susceptibility to isotope-induced toxicity[2,6]. Other factors related to PRRT efficacy include tumour cell type and biology — response rates range from 22% (small bowel carcinoids) to 60% (for insulinoma: pancreas)[3] — suggesting cell-to-cell variations in susceptibility. The grade of the tumour can also be relevant[5], as can tumour metabolic activity.

The NETTER-1 study evaluated PRRT efficacy ($n = 116$) compared with a separate group ($n = 113$) that received a somatostatin analogue (60 mg every 4 weeks) alone[1]. Tumours were well-differentiated, low grade (grade I or II, Ki67 <20%), metastatic midgut NETs. By 20 months, the median PFS was not reached in the radiation arm; in the non-radiation arm it was 8.4 months. Significantly more patients died in the non-PRRT treated group (26 versus 14 with PRRT). The overall response rate was 18% for PRRT; however, adverse events occurred in 86% of patients versus 31% in those treatd only with somatostatin analogues. The PRRT-adverse events were predominantly nausea and vomiting; haematological disorders occurred in 5–25% of patients in the PRRT group. These efficacy metrics compared favourably with the FDA-approved targeted drug therapies everolimus[4] and sunitinib[7].

In a sub-analysis, the predictive utility of clinical factors for PRRT response were evaluated. All patients in the NETTER-1 trial were SSRE-positive, and SSRE was not associated with efficacy. The hazard ratio for PFS in a grade 4 NET (intense uptake) was 0.18, whereas tumours with less uptake (grades 2–3) responded just as well (HR, 0.23). Evidently, SSRE, although useful in identifying targetable tumours, has minimal value as a PRRT companion diagnostic. Tumour grade itself was not predictive. Differences in pretreatment levels of either urinary 5-hydroxyindoleacetic acid (a marker of serotonin overproduction) or chromogranin A (CgA, a marker of tumour secretion) were also not linked to outcome. Tumour metabolic activity was not measured. The authors noted that treatment benefits

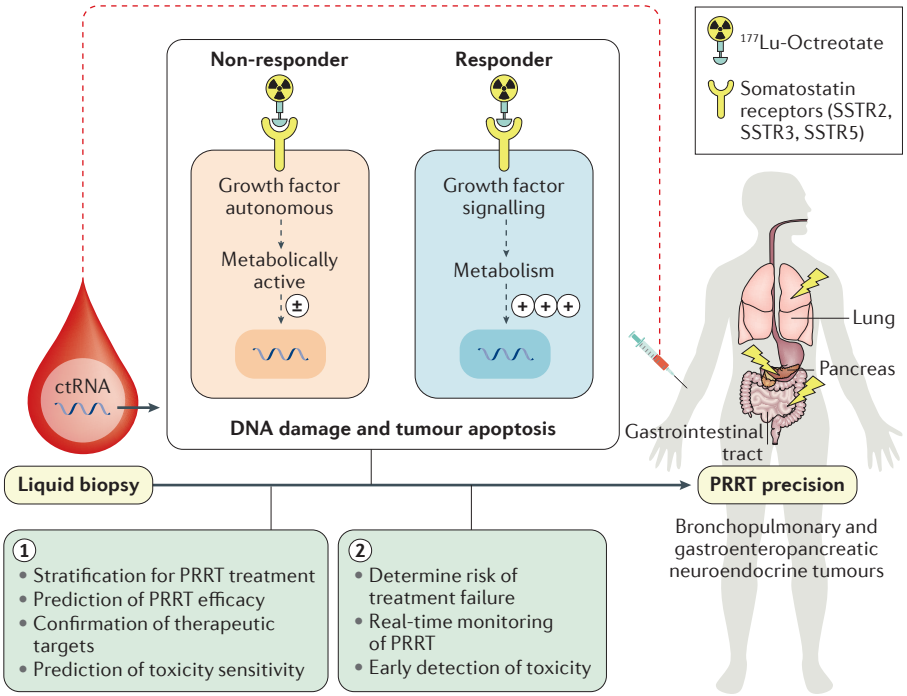

Figure 1 | **Towards precision PRRT for neuroendocrine tumours.** Liquid biopsies could help tailor peptide receptor radionuclide therapy (PRRT) for neuroendocrine tumours. The schematic shows tumour cell response to ^{177}Lu-Octreotate therapy. Tumours (blue) that exhibit a circulating gastroenteropancreatic gene fingerprint with intact, regulated growth factor signalling pathways and well-differentiated metabolic pathways are responsive to PRRT and undergo substantial DNA damage and tumour apoptosis. Tumours (orange) that are autonomous of growth factor modulation and highly metabolically active (+++) exhibit variable responses to PRRT. ctRNA, circulating tumour RNA.

were observed irrespective of stratification and prognostic factors (such as tumour grade and tumour marker levels)[1]. The conclusion must be that standard clinical (including imaging) and laboratory assays are not clinically viable diagnostic markers for predicting PRRT efficacy or the potential for adverse events.

A companion or complementary diagnostic, per FDA definition, is any medical device that can provide information regarding the safety and effectiveness of a corresponding therapeutic agent. These tests typically measure by DNA sequencing, or immunohistochemistry, the status of a drug target. To date, all approved tests are for the measurement of single analytes; however, multidimensional information is advantageous for capturing tumour behaviour and assessing real-time responses during treatment. Developments in oncology have focused on the application of novel biomarkers utilizing circulating genetic information such as circulating tumour DNA or RNA with actionable mutations or other relevant genetic information. These so-called liquid biopsies represent management adjuncts with substantial clinical utility and can provide tangible information at any treatment time point. For example, treatment responses can be monitored through emergence of *de novo* mutations, or minimal residual disease after surgery or recurrence detected by measuring circulating tumour DNA.

A strategy using circulating mRNA measurements as a complementary diagnostic is available for assessment of PRRT in NETs[8] (FIG. 1). NET genes can be detected in blood[9] using a sensitive (>90%) and specific (>90%) gene expression assay[10], which can define the efficacy of clinical interventions (surgery or somatostatin analogues)[2]. Circulating levels of these genes are decreased after surgery, and elevated expression can predict tumour progression and provide a real-time assessment of tumour biology (such as capture metabolism, epigenetic regulation and SSRE)[10]. These NET blood gene expression assays can also be used as a complementary diagnostic for ^{177}Lu-PRRT[8]. When integrated with tumour grade, growth factor signalling pathway and metabolism gene expression enabled development of a prediction quotient index that accurately predicted PRRT response better than SSRE and abnormal CgA levels. Moreover, the median PFS was markedly different, not reached in those predicted to respond by the PQI versus 17 months in those predicted not to respond. Delineation of the biological nature of the tumour is, therefore, important in identifying individuals that will respond to PRRT.

Defining the specific pathways that regulate NET pathobiology and using this information to predict responses to PRRT is an important step forward in amplifying the benefit of personalized therapy for NET disease. To further optimize the risk:benefit ratio of PRRT, it is necessary to predict which patients might develop renal or haematological toxicities. Thus, although the NETTER study represents an advance — albeit 20 years in the making — the nuclear medicine and oncology community should optimize the effect on patient care by not simply adding another treatment but by complementing therapy with stratification of management and prediction of adverse effects using state-of-the-art-molecular platforms.

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Competing interests statement
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