Project 1
A translational study to assess a novel molecular classification in grade 3 pancreatic neuroendocrine neoplasm

Clinical Background
Neuroendocrine tumours (NETs) are a rare and heterogeneous group of tumours with widely varying morphologies and behaviours. Incidence has risen over the last 20 years to stand at 5.25/100,000/year\(^1\). 5 year overall survival for NETs is in the order of 67.2%\(^2\). Although this prognosis is relatively good in oncological terms it is still life limiting for the majority and significantly worse for many patients. Due to their rarity and heterogeneity, progress in improving the treatment for NETs has been slow. However, in recent years, there have been advances both in the classification of GEP-NETs (2010 WHO classification of Neuroendocrine Neoplasms and ENETS staging system) and treatment options available. New guidelines have been produced taking these changes into account (UKINETs guidelines 2011 / ENETS guidance 2016).

The 2010 WHO Neuroendocrine Neoplasms (NENs) classification divides NENs according to various histopathological features and the tumour’s proliferative index, as assessed by Ki-67 expression or mitotic count. The main division is between well and poorly differentiated tumours, with the former being grouped together as grade 1 and grade 2 NETs and the latter being described as grade 3 Neuroendocrine Carcinomas (NECs). The grades have been shown, in multiple studies, to have prognostic value, with Grade 1 showing the best prognosis, and Grade 3 the worst. Treatment decisions are usually based on grade of disease, as there are no other validated prognostic and/or predictive biomarkers routinely used in clinical practice.

However, there is clear clinical heterogeneity within the grades, as suggested by recent literature and our clinical experience\(^3,4,5\). There is a clear unmet clinical need for markers to complement grade to predict prognosis and guide treatment decisions for GEP-NET patients. For the first time, Dr Sadanandam’s group have defined three molecular subtypes of sporadic pancreatic NETs based on an integrated analysis of gene expression (221 genes), microRNA (miR; 30 miRs) and mutations (targeted mutational profiles of MEN1, DAXX/ATRX, TSC2, PTEN and ATM), collectively named the PanNETassigner signature\(^6\).

The project
Our team has been awarded a flagship BRC grant to conduct a translational study in Gastroenteropancreatic Neuroendocrine tumours (GEP-NETs). In this retrospective tissue collection study we aim to validate and assess the PanNETassigner signature to establish if it can be used to stratify PanNET patients for prognosis and potentially be used to guide treatment. Should this approach prove to be successful the assays validated in this project could be taken forward into the clinic and, combined with current approaches, improve the prediction of prognosis for patients with pancreatic neuroendocrine neoplasms.

The trainee’s project will be to apply the PanNET assigner signature specifically to the grade 3 panNET patient samples. The grade 3 subgroup of panNET covers a wide range of patients with Ki67 ranging from 20-100%. Traditionally such patients have all been treated with up-front aggressive chemotherapy, usually combining a platinum agent with etoposide.
More recently it has been demonstrated that within the grade 3 group, actual level of Ki-67% remains prognostic and that patients with well differentiated grade 3 disease have a better prognosis than those with poorly differentiated disease. Furthermore, it has been suggested that platinum doublet chemotherapy may not be as successful in patients with a Ki 67 of <55% and that these patients should perhaps be considered for some of the treatments usually reserved for grade 1/2 patients.

Based on Dr Sadanandam's preliminary work with the PanNETassigner signature we would expect all grade 3 patients to fall into one of our molecular subtypes, the metastasis like primary (MLP) subtype. However within the MLP samples analysed to date (4 MLP patient samples with grade 3 disease) we observed an increased heterogeneity that may reflect differences within grade 3 PanNETs. We require additional samples from our PanACeA study to investigate this. The trainee will use the molecular pathology techniques outlined below, alongside diagnostic histopathology and our clinical database to establish if applying this novel molecular classification to grade 3 patients can further divide this subgroup and if such a division can provide useful prognostic information.

Within the PanACeA study we will be collecting tissue samples for 77 pancreatic neuroendocrine patients treated at the Royal Marsden over the last 10 years to combine with existing samples from Prof Aldo Scarpa's laboratory in Verona, Italy. Of these samples ~25 are from patients with grade 3 disease and this smaller cohort of patient samples has been selected to enable the trainee to complete this project within the specified 6 months.

Working in collaboration with pathologists from Prof Aldo Scarpa’s group (as a part of the University of Verona, Italy and International Cancer Genome Consortium; ICGC based collaborative funding to Sadanandam) and Prof. Bertram Wiedenmann’s group based in Charite Hospital, Berlin, Germany the trainee will have the opportunity to develop expertise in analysing neuroendocrine tumour samples and in the use of digital pathology. The trainee will also obtain a thorough grounding in molecular pathology working closely alongside the clinical research fellow and scientific officer supported by the main BRC grant. This would include wet-laboratory skills (DNA and RNA isolation, cell culture methods, nCounter (Nanostring), RT-PCR, in situ hybridization methods and next generation sequencing strategies etc.), skills in bioinformatics, data interpretation and integration.

For informal enquiries please contact Dr Kate Young (kate.young@rmh.nhs.uk).

References

