Background

- Neuroendocrine tumours (NETs) are a rare and mixed group of tumours and progress in improving their treatment has been slow.
- Decisions about treatment are currently based on the “Grade” of a patient’s disease. Patients may have Grade 1, 2 or 3 disease.
- Grade is an assessment of how quickly the tumour cells are multiplying and therefore how quickly a tumour is growing. Grade is scored looking at tumour samples under a microscope using special stains like Ki-67.
- Grade 3 disease is the most aggressive and patients are usually treated with immediate chemotherapy. Patients with Grade 1 and 2 disease are often monitored without treatment as their disease is often more stable.
- However, within these Grades the behaviour of patients’ disease may vary considerably. In the clinic some patients have Grade 2 tumours which behave more like Grade 3 tumours, and perhaps should be treated more aggressively upfront.
- Our study aims to find out if using new molecular technologies, for example looking at changes in DNA and RNA seen in tumour samples, we can divide NET patients into molecular subtypes. Dr Sadanandam’s laboratory has already started this work in a small number of pancreatic NET patients. We will continue this work in pancreatic NET patients and investigate other NET patients whose disease is in the gastrointestinal tract. We will then see if these subtypes can give us useful information about how aggressively a patient’s disease will behave.
- If successful the tests assessed in this project could be taken forward into the clinic and, combined with current approaches, improve the prediction of prognosis for GEP-NET patients and help guide treatment decisions.

PaNACeA Study Workflow

1. Pancreatic NETs
   - 200 RNA samples with matched clinical data from patients
   - Apply PanNETSigner algorithm and reference using panNETscores, then validate with additional RNA samples
   - Test refined panel in FFPE formalin-fixed paraffin embedded (FFPE) samples with matched clinical data from RMH
   - Correlate molecular panel results with matched clinical data and establish, if possible, association according to molecular subgroup
   - Develop immunohistochemistry (IHC) markers for each subgroup for subsequent testing in clinic

2. Small Bowel NETs
   - 50 RNA samples with matched clinical data from Charite Hospital, Berlin
   - Apply PanNETSigner algorithm and reference using panNETscores, then validate with additional RNA samples
   - Test refined panel in FFPE formalin-fixed paraffin embedded (FFPE) samples with matched clinical data from ICR
   - Correlate molecular panel results with matched clinical data and establish, if possible, association according to molecular subgroup
   - Develop immunohistochemistry (IHC) markers for each subgroup for subsequent testing in clinic

Grades of Disease (using Ki-67 staining as a marker for cell growth)

- Grade 1 (Ki-67 <2%) Less Aggressive
- Grade 2 (Ki-67 ~10%) Intermediate
- Grade 3 (Ki-67 >90%) More Aggressive

Work conducted to date

- Dr Sadanandam’s laboratory have previously identified 3 molecular subtypes in pancreatic NET:
  1. Metastases-like primary tumours (Tumours enriched for distant metastasis and associated with DAXX/ATRX, TSC2, PTEN and ATM mutations)
  2. MEN1-like/intermediate tumours (primarily enriched for multiple endocrine neoplasia- MEN1mutations)
  3. Insulinoma-like tumours (enriched for insulinomas with mutations in TSC2, PTEN and ATM)
- We have created a clinical database of patients treated at the Royal Marsden for GEP-NETs over the last 10 years and have combined this with similar databases from the University of Verona, Italy and the Charite Hospital, Berlin
- With patient and public involvement we have written and obtained regulatory approval for a translational study protocol for this work and have started collecting tissue samples from RMH, Verona and Berlin
- We have selected a 228 gene panel from the work conducted by Dr Sadanandam’s team and will shortly start work using nanostring (a digital technique used to count messenger RNA, mRNA, present in a sample) to further refine and shrink this panel. We will confirm the results with real time quantitative polymerase chain reaction (qPCR) which is another technique used to measure mRNA.

Gene expression Profile

Pancreatic NET subtypes, clinical features and gene signature associated with them (Work previously conducted by Dr Sadanandam’s team)

References