Three distinct genomic landscapes define clinical outcome of pancreatic neuroendocrine tumours (pNETs)

Ben Lawrence[1], Cherie Blenkiron[2], Kate Parker[1], Peter Tsai[2], Sandra Fitzgerald[2], Paula Shields[2], Tamsin Robb[2], Mee Ling Yeong[3], Nicole Kramer[3], Sarah James[2], Mik Black[4], Vicky Fan[2], Nooriyah Poonawala[2], Patrick Yap[3], Esther Coats[1], Braden Woodhouse[1], Reena Ramsaroop[5], Masato Yozu[6], Bridget Robinson[7], Kimiora Henare[8], Jonathan Koea[5], Peter Johnston[3], Richard Carroll[9], Saxon Connor[7], Helen Morrin[7], Marianne Elston[10], Christopher Jackson[11], Papaarangi Reid[12], John Windsor[13], Andrew MacCormick[6], Richard Babor[6], Adam Bartlett[13], Dragan Damianovich[3], Nicholas Knowlton[2], Sean Grimmond[14], Michael Findlay[15], Cristin Print[2].

1 Discipline of Oncology, University of Auckland, NZ; 2 Department of Molecular Medicine and Pathology, University of Auckland, NZ; 3 Auckland District Health Board, NZ; 4 Department of Biochemistry, University of Otago, Dunedin, NZ; 5 Waitemata District Health Board, Auckland, NZ; 6 Counties Manukau District Health Board, Auckland, NZ; 7 Canterbury District Health Board, Christchurch, NZ; 8 ACSRC, University of Auckland, NZ; 9 Capital and Coast District Health Board, Wellington, NZ; 10 Waikato District Health Board, Hamilton, NZ; 11 Southern District Health Board, Otago, NZ; 12 Te Kupenga Hauora Maori, University of Auckland, NZ; 13 Department of Surgery, University of Auckland, NZ; 14 Centre for Cancer Research, University of Melbourne, Australia; 15 Cancer Trials New Zealand, The University of Auckland, Auckland, NZ.

Background:

- pNETs are a poorly understood cancer with a highly variable clinical outcome.
- Genomic analysis of pNETs may provide biological insights that guide therapy.

Methods:

69 sporadic well-differentiated pNETs from 60 individuals along with matched normal tissues underwent deep hybridization capture DNA sequencing of 638 genes and Affymetrix RNA microarrays. More in-depth genomic analysis was undertaken for 12 pNETs including low coverage whole genome sequencing, RNAseq analysis, methylation microarray analysis and microRNA expression microarray analysis.

Careful clinical annotation was conducted for each case, then cases de-identified prior to linking with genomic findings. Clinically relevant findings were returned to the patient's physician if deemed appropriate by an incidental findings committee, for patients who consented.

Results

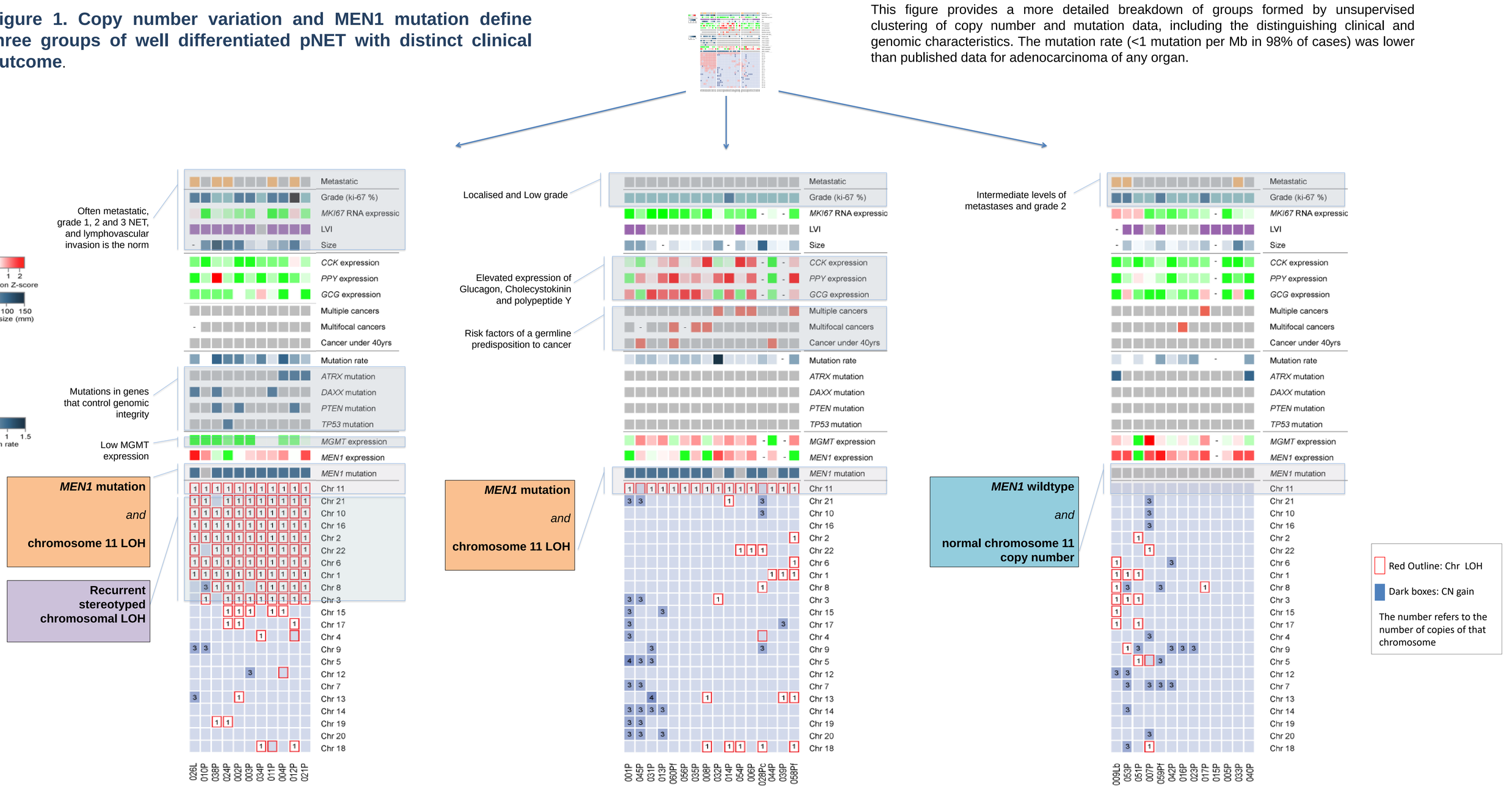
Unsupervised clustering of copy number changes defined three groups of pNETs with differing clinical characteristics and outcomes.

pNETs in group 1 (n=11) showed a recurrent pattern of LoH affecting the same 10 chromosomes, usually in the context of somatic *MEN1* mutation, and often coupled with mutations in genes affecting genome integrity (*ATRX*, *DAXX PTEN*, *MSH2* and *TP53*). Outcomes were unfavourable in this group; 5 of the 11 tumours metastasized, three patients progressed during the study, and 10 had lymphovascular invasion.

pNETs in group 2 (n=16) also showed chromosome 11 LoH, usually in the context of MEN1 mutation, but few other chromosomal copy number changes or mutations. This group had favourable outcomes; no patients metastasized, 15 were low grade (Ki-67 <2%), all had low expression of proliferation-associated RNAs and only three had LVI.

By contrast, group 3 (n=13) was characterized by absence of *MEN1* gene mutation, contained tumours with variable patterns of aneuploidy (ranging from none to extensive) and normal Chromosome 11 copy number. pNETs in this group had intermediate outcomes.

Figure 1. Copy number variation and MEN1 mutation define three groups of well differentiated pNET with distinct clinical outcome.



pNET group 1 (n=11)

Poor Outcome. Patients in this group often develop metastatic disease. The low MGMT expression may make temozolomide-based therapy an appropriate systemic therapy. Low MGMT expression may be caused by MGMT haploinsufficiency (loss of 1 copy of chromosome 10), as there was no evidence of MGMT promoter hypermethylation. The role of targeted therapies will be discussed in the accompanying oral presentation.

pNET group 2 (n=16)

Good outcome. Patients in this group have low grade pNETs that do not appear to metastasise. This group undergo surgical resection without relapse. There is a suggestion that these patients have a germline tendency to cancer, as they are more likely to develop second cancer, have multifocal NETs, and develop NETs at a young age. Systemic therapy is not required for these patients. The relevant clinical question is whether surgical resection is required, in light of the lack of metastasis.

pNET group 3 (n=13)

Intermediate outcome. Patients in this group have mixed clinical features, perhaps because the group remains heterogenous outside the defining wild-type *MEN1* and normal copy number of chromosome 11. Approximately half of this tumours in this group were aneuploid, and half euploid.

Conclusions:

- The clinical outcome of pNETs is related to a combination of somatic *MEN1* mutation, changes in copy number at a chromosomal level, and mutations in genes related to genome integrity.
- Group 2 pNETs appear to be cured by surgical resection. Given the morbidity of surgery to the head of the pancreas, pNETs in this group might be suitable for a clinical trial that tests the role of of observation vs resection.
- Group 1 pNETs will often require systemic therapy. Low MGMT expression may favour the use of temozolomide in this group. A project involving retrospective testing of chromosome 11 LOH and MGMT expression in pNETs treated with temozolomide is underway.