6. Future Directions

6.1 Single-cell derived splenocyte colonies may help enrich the phylogenetic tree

The phylogenetic reconstruction completed here approximates the early embryonic lineage of the islets. In order to obtain a more precise and complete phylogeny of the early development in this donor, single-cell derived splenocyte colonies are being cultured, in collaboration with Elisa Laurenti at the Wellcome-MRC Stem Cell Institute (Cambridge, UK). In single-cell derived clonal populations, heterozygous variants in the original founding cell take on VAFs of 0.5 in the larger clonal population, irrespective of its original VAF in the tissue. Rare variants can therefore become detectable under current sequencing protocols, at moderate depths, allowing higher-quality phylogenetic trees to be reconstructed using standard phylogenetic approaches (Behjati et al., 2014).

6.2 Immunohistochemistry could play a role in explaining the lineage proportions

Most islets appear to be a polyclonal unit, derived from at least five embryonic founders. Often the islets appeared to have a major lineage accounting for over half of the population, along with several more minor sub-populations (Figure 32). Given that β-cells make up about 60% of the islet, with α-cells being 30% (Ionescu-Tirgoviste et al., 2015), it would be interesting to examine whether the proportions of the islet cell types correlates with the lineages present in the population.

It is unclear whether different lineages could reflect different cell types. It is likely that non-endocrine cells within an islet partially explain the presence of multiple embryonic founders, but this is difficult to assess with the current data. It is also unclear whether different subpopulations of endocrine cells in a given islet may derive from different founder cells, including differences between β-cells and α-cells. With a view of investigating further, immunohistochemistry can be performed on the sections for the markers expressed by each cell type. Key targets for this would include chromogranin, insulin, glucagon, trypsin and CD31 (Campbell-Thompson, Heiple, Montgomery, Zhang, & Schneider, 2012; Lin, Chen, & Wang, 2015; Pusztaszeri, Seelentag, & Bosman, 2006). Their relative fractions could then be compared to those obtained from the whole-genome sequencing data. In preparation for this, 5 μm sections have been obtained; from directly above and below the 16 μm sections cut for LCM. As a

tissue thickness of 5 μm represents less than a single cell layer, the sub-populations of different cell types in the islets excised can be experimentally identified. Building on this, G&T-seq may provide a superior option to identifying cell types within the islet.

6.3 Targeted genotyping of pancreatic tissues may reveal more detailed insights into development and maintenance

One key question is whether transdifferentiation of non-endocrine cells into endocrine cells, particularly β-cells, could be achieved (Bonner-Weir et al., 2008; Kim & Lee, 2016). This possibility has attracted considerable attention for its translational potential, in an age where DM2 has become more widespread, with a growing health burden (World Health Organization, 2016). To investigate transdifferentiation further, as well as more generally assessing the contribution of different lineages to the exocrine pancreas surrounding the islets, LCM has been used to obtain pancreatic ducts and acini from the same patient (290B) (Figure 36).

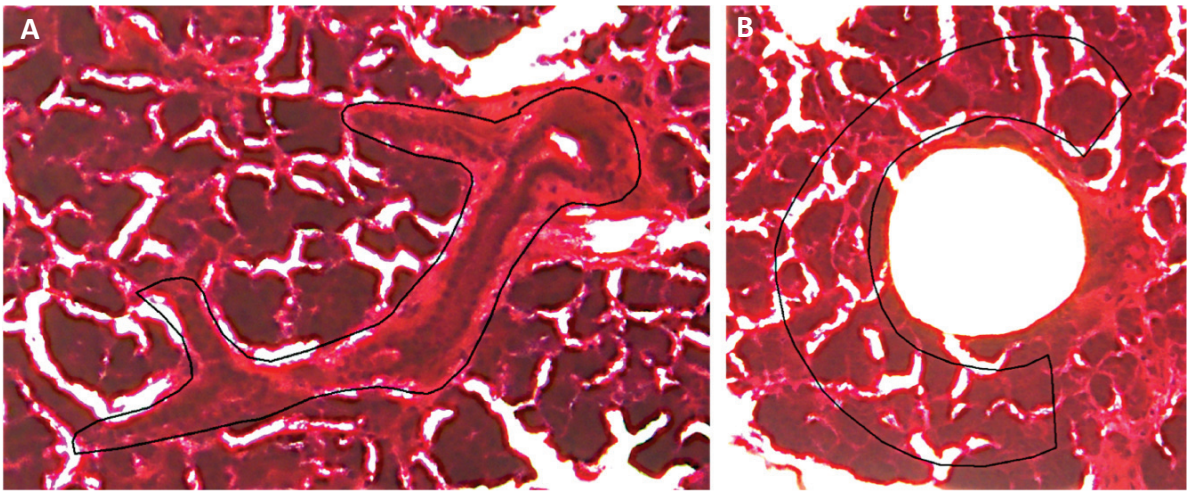

Figure 36 – Further LCM work is focusing on the pancreatic ducts and acini
LCM images from patient 290B. The black lines tracing the borders of the structure indicate the margins of the laser microdissection.

(A) A pancreatic duct demarcated during LCM. The area of tissue obtained was 28,466 μm^2.

(B) A crescentic acinar section surrounding islet PD37726d_lo0030, outlined in black. This has an area of 36,582 μm^2. The islet was previously excised and leaves a white circle in the centre of picture. There is a clear margin of tissue left between the islet and the acinar crescent, to ensure no remnants of the islets are cut with the acinar tissue.

As shown in Figure 36, while the ducts have been taken in the same way as the islets have, a different approach has been used for the acini. Crescents of acinar tissue surrounding islets are being collected with a clear margin of tissue being left between the islets and acinar crescents, to prevent cross-contamination of the cell types. With the whole-genome sequence data from the islets, ultra-deep targeted sequencing of the surrounding acinar crescents, and nearby ducts, can be performed using the mutations identified in the islet whole-genome data as a custom bait. The targeted genotyping of both of these exocrine tissues will then shed light on whether they share the same variants and therefore, ancestry.

To conclude, this body of work serves as a starting point for examining the somatic mutations in the pancreatic islets. Building on this foundation, future work is already under way. Single-cell derived splenocyte colonies are currently being cultured and once sequenced, the phylogenetic tree reconstruction will hopefully reveal an unrivalled insight into the phylogeny of the islets of Langerhans. Immunohistochemistry is a readily available resource to further clarify the lineages seen in each islet and this could frame these results in a more appropriate context. The prospect of transdifferentiation is exciting and if shared ancestry between the endocrine and exocrine pancreas is proven, a remarkable new frontier could open up in regenerative medicine, one that could lead to the development of novel, translational therapies for diabetic patients.