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PRECLINICAL PANCREATIC CANCER LAB

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Pancreatic cancer is a major healthcare challenge, predicted to become the second most common cause of cancer death in the Western world within the decade. The focus of our research is to better understand the disease and identify and test more effective therapies. We use genetically engineered models that recapitulate human tumours, in terms of both driving mutations and the immuno-suppressive tumour microenvironment and adapt them to mirror heterogeneous subsets of the disease. These models provide a clinically relevant platform in which we trial novel tumour and microenvironment targeting therapies.

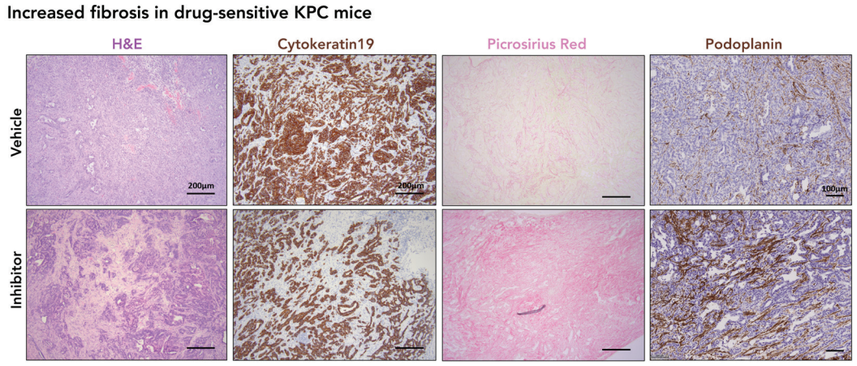
Research has helped improve our understanding of pancreatic cancer evolution, genetic alterations, transcriptional subtypes, and the tumour microenvironment (TME). Activating mutations in *KRAS* are the most prevalent driver mutations, accompanied by loss of function of tumour-suppressor genes. Some mutations found in subsets of patients may confer sensitivity to targeted therapies (Biankin *et al.,* 2012, *Nature*). For that reason, part of our work involves modelling mutations that are found in smaller subsets of human pancreatic cancer, with a view to understanding the biological consequences and therapeutic sensitivities associated with those mutations.

Another feature characteristic of PDAC is the dense fibrotic stroma that surrounds and supports the tumour cells and can account for up to 90% of the tumour volume. This microenvironment consists of fibroblasts and extracellular matrix proteins as well as significant inflammation with prominent myeloid cell infiltration and a dearth of effector T cells. Each component plays an important role in pancreatic cancer progression, influencing tumour cell proliferation and survival, metabolism, migration, immune surveillance, and response to chemotherapy (Candido *et al.,* 2018, *Cell Reports*; Steele e*t al.,* 2016,*Cancer Cell*; Vennin *et al.,*2018, *Gastroenterology*). Therefore, another aim of our work is to understand how stromal signalling impacts on the disease and how we might target it for therapeutic gain. Due to the complex nature of tumour-stromal interactions it is important to study this *in vivo*, in spontaneous tumours with a physiological microenvironment and immune response.

Modelling genetic subsets of patients

We have developed several models to mimic patients with mutations that may be actionable, to identify and to test therapeutic targets. Our collection of models covers many genes/pathways identified in the patient tumours. For example, *RNF43*, the gene encoding ubiquitin E3 ligase ring finger 43, has been shown to be mutated in 10-15% of cases of metastatic pancreatic cancer (Jiang *et al.,* 2013 *PNAS*). Using KPC mice as a backbone (Hingorani *et al.,* 2005, *Cancer Cell*), we have developed a genetically engineered mouse model of *Rnf43* deletion and found that *Rnf43* deletion is a strong driver of pancreatic cancer progression, with loss of even a single copy sufficient to significantly accelerate tumour progression. Mutations in DNA damage repair genes have also been reported in ~15% of pancreatic cancers (Aguirre *et al.,* 2018, *Cancer Discovery*). We have developed models of these patients, by deleting *Atm* or *Brca1* in KPC mice, and found differential sensitivities to DNA damaging agents and notably divergent immune microenvironments. We are now extending these studies to include radiotherapy, as these mutations may render tumours more sensitive to radiation. The use of radiotherapy in pancreatic cancer treatment has been limited thus far, however, this may be due to a lack of understanding of the effects of radiation on the pancreatic TME. Irradiation results in tumour cell death that can elicit a T cell response against the tumour, however, increased inflammation and fibrosis may result in an even more tumour-supporting, immunosuppressive microenvironment. Thus, we are investigating signalling in the TME to identify therapeutic combinations that could promote anti-tumorigenic immune responses while inhibiting pro-tumorigenic immune and fibrotic responses.

Figure 1
Representative Haematoxylin & Eosin (H&E) staining, cytokeratin 19 immunohistochemistry (IHC) for tumour cells, picrosirius red staining for collagen I, and podoplanin IHC for fibroblasts, in vehicle or inhibitor treated pancreatic tumour-bearing KPC (KrasG12D; p53R172H, Pdx1-Cre) mice, demonstrating increased fibrosis in treated tumours.



Therapeutic Resistance

By far the most common event driving pancreatic tumorigenesis is KRAS mutation. Previously believed to be "undruggable", the advent of mutant *KRAS* inhibitors has the potential to be transformative in this disease, particularly now that inhibitors are in development for the most mutated form in pancreatic cancer (Hallin *et al.,* 2022, *Nature Medicine*). We have already observed that inhibition of multiple signalling pathways downstream of Kras can have significant efficacy in tumour-bearing mice (Driscoll *et al.,* 2016, *Cancer Research*). However, our recent data, together with results using KRAS inhibitors in other tumour types, suggested that resistance can develop quickly. In pancreatic cancer, the stroma can drive drug resistance, and we have found that drugs targeting RAS signalling can cause microenvironmental changes associated with acquired resistance. Indeed, most tumours relapsed quickly, and displayed elevated fibrosis, enhanced extracellular matrix deposition, and intriguingly, a re-wiring of signalling in the microenvironment (Figure 1). We are now investigating how signalling within the TME can help tumour cells to adapt to therapeutic intervention and influence the response to treatment. Tumour and stromal compartments display both significant heterogeneity in terms of gene expression and function, for example, discrete populations of cancer-associated fibroblasts with distinct expression profiles can either support or restrict tumour growth (Hutton *et al.,* 2021, *Cancer Cell*). Therefore, to fully understand how best to target different cell types for therapeutic effect, we need to investigate signalling within individual cell types (e.g., Figure 2), but also spatially link molecular changes to therapeutic responses. Building a comprehensive understanding of the relationships between signalling pathways, tumour cells and the TME following therapeutic intervention will allow us to identify the best strategies to overcome resistance.

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Figure 2
Example of Uniform Manifold Approximation and Projection (UMAP) of single cell RNAseq data from control and treated tumour-bearing KPC mice.