www.crukscotlandinstitute.ac.uk/jen_morton

PRECLINICAL PANCREATIC CANCER

Group Leader
Jen Morton

Research Scientists
Abigail Elliot[1]
Mónica Fernández[2]
Karen Pickering
Mathias Tesson[3]

Scientific Officers
Saadia Karim
Kay Kong
Lynsey McKenzie[4]

Graduate Student
David MacLean

[1]MRC
[2]CRUK EDD
[3]Pancreatic Cancer UK
[4]CRUK Scotland Centre

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 mouse (GEM) 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.

Pancreatic cancer kills over 430,000 people every year. It is one of the deadliest epithelial malignancies, and both incidence and mortality are rising. Indeed, it is predicted to be the second most common cause of cancer death within the next decade. In the UK alone, there are around 30 new cases every day. Less than 8% of those patients will survive their disease for five years, and only 1% are likely to survive beyond ten years. Despite improvements in surgical management and significant investment in clinical trials, cure rates have only minimally increased over the last 50 years, and current therapies are largely ineffective.

Research has helped improve our understanding of disease evolution, genetic alterations, and the tumour microenvironment. 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 gene 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 pancreatic cancer 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 (ECM) proteins as well as significant inflammation but a dearth of effector T cells. Each component plays an important role in pancreatic cancer progression, influencing tumour cell proliferation and survival, metabolism, migration, and immune surveillance (Candido *et al.*, 2018, *Cell Reports*; Steele *et al.*, 2016, *Cancer Cell*; Vennin *et al.*, 2018, *Gastroenterology*). Therefore, another aim of work in our lab is to investigate 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.

Radiotherapy in Pancreatic Cancer

The stroma can have profound effects on therapeutic response; however, therapeutic interventions may also have significant effects on the stroma. For example, radiotherapy can cause remodelling of the tumour microenvironment (TME) which may favour tumour growth and treatment resistance. Using our small animal radiotherapy research platform we have developed a protocol for tumour-targeted radiotherapy in GEM models of pancreatic cancer (Figure 1A). 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 effect of radiation on the pancreatic TME. Irradiation results in tumour cell death and release of tumour-associated antigens that can elicit a cytotoxic T cell response against the tumour. However, it can also drive the release of inflammatory cytokines and chemokines which can result in altered fibroblast secretory output, ECM remodelling, macrophage polarisation and a more immunosuppressive microenvironment. We have found that radiation alone can provide some survival benefit in pancreatic tumour-bearing mice (Figure 1B), however, this is accompanied by distinct cellular changes in the TME (Figure 1C).

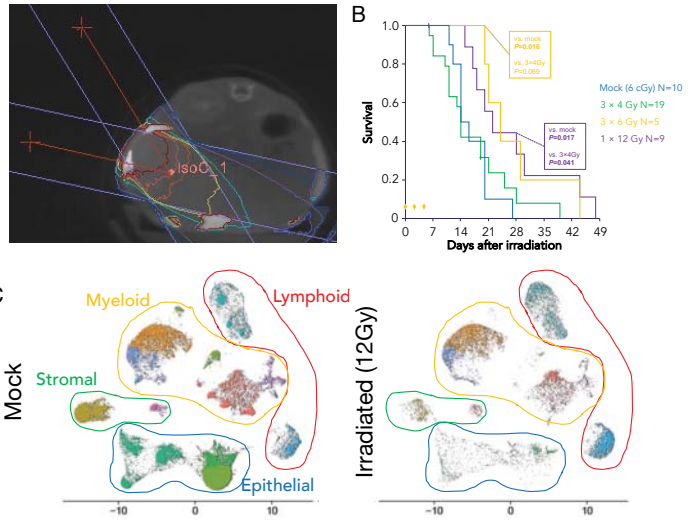

Figure 1. A Radiotherapy delivery plan using the small animal radiotherapy research platform. **B** Kaplan-Meier curve showing improved survival of pancreatic tumour-bearing mice in response to radiotherapy. **C** scRNAseq analyses show changes in cell populations post-radiotherapy in treated mice.

Thus, we are using our models to investigate responses in individual cells in the TME to determine the mechanisms controlling pro-tumourigenic immune and fibrotic responses with the aim of identifying rationale therapeutic combinations to promote anti-tumourigenic immune responses while inhibiting pro-tumourigenic immune and fibrotic responses. We are also using models lacking certain tumour suppressor genes to investigate whether certain mutations can render tumour cells more sensitive to therapy.

Targeting KRAS

By far the most common event driving pancreatic tumourigenesis is KRAS mutation. Previously believed to be "undruggable", the advent of mutant KRAS inhibitors could be transformative in this disease, particularly now that inhibitors have been developed 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, resistance can develop quickly, concomitant with deregulation of signalling in both tumour and stromal cells. We are now investigating how these pathways can help tumour cells to adapt to therapeutic intervention and influence the response to treatment. For example, we have recently been investigating a protein called Gremlin1, an antagonist of bone morphogenic protein (BMP) signalling which is overexpressed in cancer-associated stromal cells and reported to be a driver of fibrosis in chronic pancreatitis (Figure 2A). Overexpression of the gene also correlates with decreased survival in pancreatic cancer patients. We found that treating pancreatic tumour-bearing mice with a therapeutic antibody against Grem1, in combination with an inhibitor of the KRAS target MEK, resulted in significantly slower tumour growth and tumour stasis in some mice, and a significant increase in survival (Figure 2B). We are currently investigating the mechanisms behind this synergistic efficacy using the Nanostring GeoMx spatial transcriptomic platform (Figure 2C). Our data have also led to a clinical trial to test this promising combination in patients with pancreatic cancer, which we hope will demonstrate the potential impact of this work for patient benefit.

Publications listed on page 124

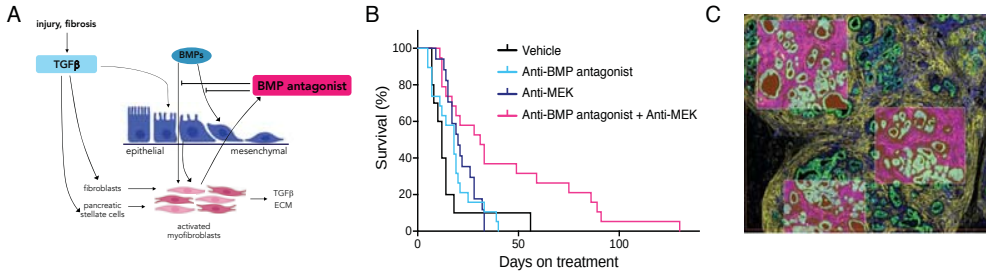

Figure 2. A Schematic of the role of BMPs in pancreatic cancer. **B** Kaplan-Meier curve showing improved survival of pancreatic tumour-bearing mice in response to combination therapy. **C** Region selection and cell type masking for GeoMx analysis. Mouse pancreatic tissue stained for CK19 (green, tumour cells), PDPN (yellow, fibroblasts) and DAPI (blue, CK19-PDPN- cells).