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TRANSLATIONAL CANCER THERAPEUTICS

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Our group is developing novel laboratory models that allow us to understand the biological function of key tumour suppressor genes and oncogenes *in vivo* in both normal tissues and tumours.

We aim to identify and characterise the signalling pathways that are deregulated at the early stages of pancreatic cancer, and during the development and progression of the invasive and metastatic phenotype, and that are potential therapeutic targets in advanced disease. Using these models, we will determine how potential anti-cancer agents might best be evaluated in subsequent clinical trials.

Infiltrating ductal carcinoma of the pancreas (PDAC) is the fifth commonest cancer in the UK, and is predicted to become the second commonest cause of cancer-related deaths by the end of this decade. Aggressive invasion and early metastases are characteristic of the disease, such that 90% of patients have surgically unresectable disease at the time of diagnosis. Overall survival remains poor for both resectable and advanced disease using conventional therapies, and has only improved marginally over the last few decades with a preponderance of negative clinical studies using current trial designs.

Our work aims to develop therapeutic interventions for advanced pancreatic cancer by exploiting tumour biology in preclinical models with specific genetic backgrounds, and to optimise therapy of localised disease through inhibition of metastases and local control of inoperable disease.

Optimising molecular targeted therapies

We have developed a number of novel models with a range of genetic backgrounds in collaboration with Owen Sansom's group. Using these models, we are investigating key pathways downstream of mutant KRAS in PDAC, particularly the mTOR pathway. We have previously shown that KC PTEN murine models are dependent on S6K signalling downstream of mTORC1, and blocking mTORC1 signalling with rapamycin extends survival even in late stage disease. In contrast, KPC tumours are resistant to treatment with rapamycin, and thus less dependent on mTORC1 signalling.

However, clinical trials of mTORC1 inhibitors in pancreatic cancer have not demonstrated significant anti-tumour efficacy in unselected patients, raising questions about this therapeutic approach. We employed a genetic approach to delete the obligate mTORC2 subunit *Rictor* and identified the critical times during which tumourigenesis requires mTORC2 signalling and showed that *Rictor* deletion resulted in profoundly delayed tumourigenesis. Whereas previous studies showed that most unselected pancreatic tumours are insensitive to rapamycin, treatment with a dual mTORC1/2 inhibitor strongly suppressed tumourigenesis. In late-stage tumour-bearing models, combined mTORC1/2 and PI3K inhibition significantly increased survival. Thus, targeting mTOR may be a potential therapeutic strategy in advanced pancreatic cancer.

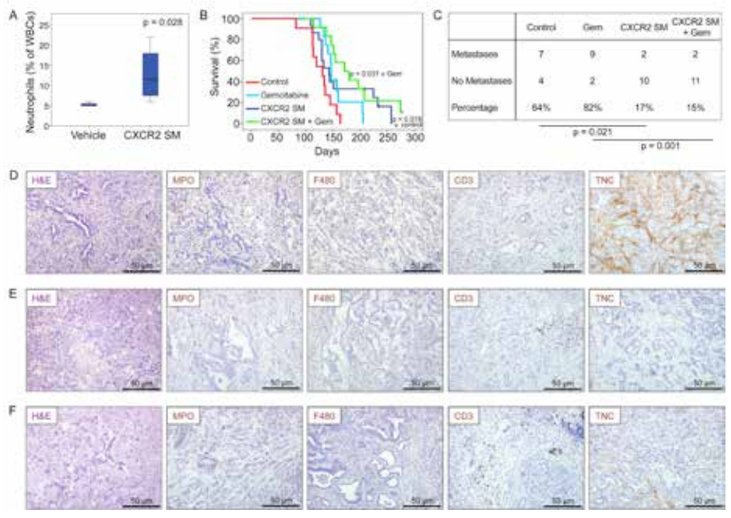

Figure 1
Therapeutic targeting of CXCR2 inhibits metastasis and prolongs survival. A] Boxplot showing circulating neutrophils in models (n = 4) treated with CXCR2 inhibitor. B] Kaplan-Meier survival analysis of KPC models treated from age of 10 weeks with vehicle (n = 11), gemcitabine (n = 14), CXCR2 inhibitor (n = 15) or CXCR2 inhibitor + gemcitabine (n = 12) C] Table comparing incidence of metastases in KPC models treated as indicated. D-F] H&E staining and IHC for MPO, F4/80, CD3 and Tenascin C in tumours in response to vehicle (D), CXCR2 inhibitor (E) and CXCR2 inhibitor + gemcitabine (F).

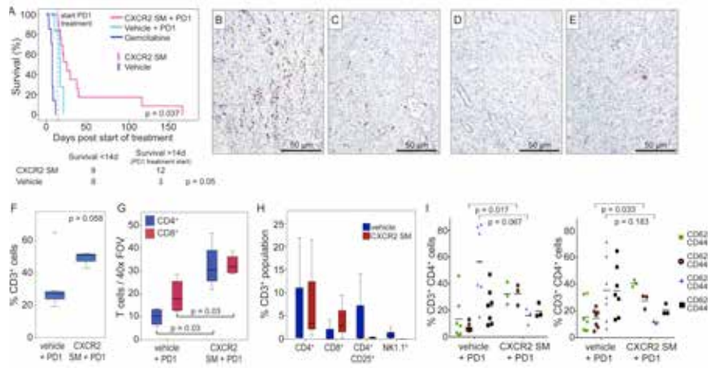

Figure 2
CXCR2 blockade promotes T cell infiltration into tumours and sensitivity to immunotherapy. A] Kaplan-Meier survival analysis of tumour-bearing KPC models treated with either gemcitabine, CXCR2 inhibitor alone for 2 weeks, and then in combination with anti-PD1, vehicle alone for 2 weeks, and then combined with anti-PD1, CXCR2 inhibitor alone or vehicle alone. B-C] IHC for Ki67 in tumours from KPC models treated with vehicle + PD1 (B), or CXCR2 inhibitor + PD1 (C). D-E] IHC for cleaved caspase 3 in tumours from KPC models treated with vehicle + PD1 (D) or CXCR2 inhibitor + PD1 (E). F) FACS analysis of intra-tumoural CD3+ cells in models treated as indicated. (G) Boxplot showing quantification of IHC for CD4+ and CD8+ T cells in tumours from KPC models treated as indicated. (H) FACS analysis of intra-tumoural CD4+, CD8+, CD4+CD25+ and NK1.1+ cells (% of CD3+ cells) in models treated as indicated. (I) FACS profile of CD4+ and CD8+ T cells isolated from tumours in models treated with either vehicle + anti-PD1 or CXCR2 inhibitor+ anti-PD1. staining and IHC for MPO, F4/80, CD3 and Tenascin C in tumours in response to vehicle (D), CXCR2 inhibitor (E) and CXCR2 inhibitor + gemcitabine (F).

Optimising immunotherapy

We are also targeting myeloid cells to enhance immunotherapy in PDAC. One dominant player that can contribute to resistance to immunotherapy in PDAC is the presence of a suppressive immune microenvironment. Key drivers of this immune-suppressive microenvironment include tumour-associated macrophages, and monocyte and granulocyte myeloid-derived suppressor cells (MDSCs). These leucocytes can also promote tumour cell proliferation, confer resistance to cytotoxic stress and facilitate metastatic dissemination. We hypothesise that the efficacy of immunotherapy in PDAC could be improved by overcoming this immune suppression and allowing activated T cells into the tumour.

CXCR2 is a G-protein-coupled receptor for the human CXC chemokines CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7 and CXCL8. The primary immune function of CXCR2 is the regulation of neutrophil migration, as it controls the egress of these cells from the bone marrow and their recruitment to sites of inflammation. CXCR2 also regulates the migration of MDSCs. CXCR2 has been suggested to have both tumour-promoting and tumour-suppressive properties. We have shown that CXCR2 signalling is upregulated in human pancreatic cancer, predominantly in neutrophils/ MDSCs but rarely in tumour cells. Genetic ablation or inhibition of CXCR2 abrogated metastasis, although only inhibition slowed tumourigenesis. Depletion of neutrophils/MDSCs also suppresses metastasis suggesting a key role for CXCR2 in establishing and maintaining the metastatic niche. Importantly, loss or inhibition of CXCR2 improved T-cell entry and combined inhibition of CXCR2 and PD1 in murine models with established disease significantly extended survival. We have also shown that CXCR2 signalling in the myeloid compartment can promote pancreatic tumourigenesis and is required for pancreatic cancer metastasis. Our data also suggest that therapeutic targets that may cause senescence escape will not have deleterious effects in late stage disease in PDAC because tumours have already escaped this checkpoint.

Based on our data, we propose two potential therapeutic opportunities for PDAC: firstly, the addition of CXCR2 inhibitors into the adjuvant setting in patents who have undergone surgical resection of potentially curative disease and who have no visible evidence of metastases; secondly, the combination of CXCR2 inhibition and anti-PD(L)-1 antibodies could be explored in patients with advanced (metastatic) disease.

Clinical trial designs

We will exploit our preclinical studies to inform a UK-wide MAMS (multi-arm molecular stratified) clinical trial that is currently in development in collaboration with Juan Valle (University of Manchester) and the CRUK Clinical Trials Unit, Glasgow. This study will consist of a series of parallel, early phase, efficacy signal-seeking studies in which patients will be recruited into multiple treatment arms of specific agents based on their molecular profile. Critical to these approaches will be identifying potential genotype-specific biomarker signatures in murine models and confirming the clinical relevance of these in human tissue microarrays, and developing robust assays for patient selection to select or enrich the clinical trial population.

Local control of inoperable disease

Optimal local control remains an important clinical issue in patients with non-metastatic disease, particularly in those with 'borderline' operable disease and in whom chemo-radiation is frequently used. Inhibitors of poly (ADP-ribose) polymerase (PARP) may have radio-sensitising effects. We have initiated a phase 1/2 study of the PARP inhibitor, olaparib, in combination with chemo-radiation in locally advanced pancreatic cancer in collaboration with ECMCs in Belfast, Leicester and Guy's & St Thomas'. In the initial phase 1 part of the study, we are determining the optimal dose of olaparib when used in combination with fluoro-pyrimidine-based chemo-radiation therapy in patients with locally advanced inoperable disease. We will then evaluate the recommended doses of this regimen in a cohort of patients with 'borderline' operable disease. The ultimate aim is to improve objective response in the primary tumours to increase the number of patients who are accessible to potentially curative surgery.

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