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COLORECTAL CANCER AND WNT SIGNALLING

Group Leader
Owen Sansom
FRSE FMedSci
FRCPS(Glasg)

Research Team Leader
Andrew Campbell

Group Research Manager
Rachel Ridgway

Project Coordinator
Fiona Paulin-Ali

Scientific Writer
Nathalie Sphyris

Bioinformaticians
Kathryn Gilroy[1]
Rosalin Simpson[2]

Translational Scientists
Ainsley Antao
Hannah Tovell[3]

Research Scientists
Patricia Centeno[4,5]
Nuray Gunduz
Andrew Hartley[6]
Georgios Kanellos[7]
Nadia Nasreddin[1,4]
Alexander Raven[5]
Susanti Susanti[8]
Ayse Yazgili[9,10]

Scientific Officers
Catriona Ford[3]
Thomas Jamieson
Tamsin Lannagan[3]

Clinical Research Fellows
Narthana Ilenkovan[11]
Kyrillus Shohdy[11]
Mark White[12]

Graduate Students
Krittaya Aksonnam[1]
Jasmine Ho
Megan Mills
Laura Millett[13]
Teena Thakur[14]
Sarah Williams

[1]University of Glasgow
[2]CRUK/Newcastle University
[3]AstraZeneca
[4]Cancer Grand Challenges "SpecifiCancer"
[5]Novartis
[6]Boehringer Ingelheim
[7]CRUK Therapeutic Catalyst Award
[8]CRUK/University of Oxford
[9]Beatson Cancer Charity
[10]The Mark Foundation for Cancer Research
[11]CRUK TRACC Programme
[12]CRUK Clinical Academic Training Programme
[13]CRUK Scotland Centre
[14]McNab Centre/University of Glasgow

Colorectal cancer (CRC) is a heterogeneous disease comprising distinct molecular subgroups that differ in histopathological features, prognosis, metastatic propensity, and response to therapy. Leveraging omics and spatial biology technologies in state-of-the-art preclinical models harbouring key driver mutations, we are interrogating the molecular underpinnings of CRC initiation and progression to identify early-stage diagnostic biomarkers and develop stage- and subtype-specific targeted therapies.

To investigate the molecular pathogenesis and evolutionary trajectory of CRC, we have generated a suite of genetically engineered mouse models (GEMMs) that develop early-stage adenomas through to treatment-refractory, advanced CRCs, with GEMM- and patient-derived organoid cultures providing a tractable means to observe epithelial cell plasticity and adaptive therapeutic responses in real-time *ex vivo*. Through an extensive network of scientific and clinical collaborators, we have also expanded our disease modelling into other tissues, such as the pancreas, liver, and skin, to gain insights into the pathogenetic and molecular mechanisms underlying different disease domains and to evaluate potentially actionable therapeutic targets across tumour types. These disease-positioned mouse and organoid models are enabling the development and preclinical testing of prospective therapeutic approaches, the characterisation of patient-relevant biomarkers, and the identification of drug resistance mechanisms, and are integral to our overarching goal of translating promising preclinical findings into a tangible clinical benefit.

Preclinical evaluation of combinatorial therapeutic strategies in *Kras*-mutant tumour models

The RAS signalling pathway is commonly dysregulated in CRC and pancreatic ductal adenocarcinoma (PDAC), owing to the high prevalence of activating mutations in *KRAS*, as well as in melanoma, which typically harbours activating *BRAF* mutations. Such tumours thrive on the constitutive hyperactivation of RAS/RAF/MEK/ERK downstream signalling, which augments tumorigenic capacity by driving cell proliferation, survival, and invasiveness. Whilst KRAS has long been considered "undruggable," recently developed mutant-selective KRAS inhibitors have shown promising efficacy in certain disease contexts. Nevertheless, *KRAS*-mutant CRCs have proven refractory to conventional chemotherapies and single-agent kinase inhibitors, such as MEK- and EGFR-targeted monotherapies, lack efficacy in this setting. Thus, there is an urgent unmet need for the development of combinatorial therapeutic approaches for *KRAS*-mutant CRCs and other KRAS-driven tumours.

Recent studies have combined mutant-selective KRAS inhibitors with immunotherapies or other targeted treatments, and examined how combination strategies could overcome emergent resistance mechanisms. In collaboration with Karen Cichowski (Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA), we showed that concurrent inhibition of KRAS and the histone methyltransferase EZH2—an epigenetic silencer of key genes involved in differentiation—elicits robust and durable tumour regression in multiple human and mouse CRC models (Loi *et al.*, 2024, *Cancer Discov*). Notably, the EZH2-inhibitor tazemetostat potentiated the efficacy of various RAS-pathway inhibitors (the phospho-MEK inhibitor trametinib as well as mutant-selective KRAS inhibitors) only in tumours driven by mutations in RAS-pathway components, underscoring the selectivity of this drug synergy. Mechanistically, these drug combinations synergistically suppress Wnt-driven transcription and drive colorectal tumours into a Groucho/TLE4-mediated differentiated cell state, underpinned by the elevated expression of Wnt-pathway inhibitors, the induction of key markers of the secretory lineage, and the downregulation of stem cell–associated genes. This induction of differentiation, by the Wnt-pathway repressor TLE4, sensitizes cells to apoptosis via the expression of the proapoptotic protein BMF, eliciting clearance of tumour cells and ensuring the durability of the response. Importantly, these drug combinations are well tolerated with no toxicity towards the normal intestinal epithelium. Collectively, these studies suggest that combining epigenetic and oncogenic inhibitors targeting EZH2 and KRAS may hold therapeutic promise for the treatment of *KRAS*-mutant CRCs.

In metastatic melanoma, we explored how to overcome the emergent resistance to BRAF-targeted therapies, which commonly arises through the acquisition of activating hotspot mutations in *RAC1*. Such mutations sustain activation of the MAPK signalling pathway and, consequently, circumvent the effects of BRAF inhibition. Using GEMMs and patient-derived $BRAF^{V600E}$–mutant melanoma cell lines, we found that whilst PREX2 activity is dispensable for melanoma initiation and progression, targeting the PREX2/RAC1/PI3Kβ signalling axis sensitizes tumour cells to MAPK pathway–targeted therapies. These findings suggest that PREX2 or PI3Kβ inhibition could be combined with MAPK-targeted drugs to improve therapeutic outcomes in *BRAF*-mutant metastatic melanoma (Ford *et al.*, 2024, *Cancer Res*).

Overall, these studies are evaluating combinatorial therapeutic strategies and yielding multifaceted biological and molecular insights that have the potential to inform the development of clinical trials for *KRAS*-mutant tumours.

Kras allelic imbalance drives tumour initiation but suppresses metastasis and sensitizes to MEK inhibition in CRC and PDAC models

Whilst a plethora of studies have addressed how oncogenic *Kras* mutations drive tumorigenesis, our recent studies in GEMMs recapitulating CRC (Najumudeen *et al.*, 2024, *Nat Commun*) and PDAC (Fey *et al.*, 2024, *Cancer Res*) have revealed that the retention or loss of the wild-type *Kras* allele influences the function of its oncogenic counterpart, profoundly impacting disease trajectory and therapeutic outcome. Mechanistically, loss of the remaining wild-type *Kras* allele promotes the initiation of oncogenic KRAS-driven small-intestinal (Figure 1A) and pancreatic tumours, by increasing pro-proliferative downstream MAPK signalling (Figures 2A and 2B), suggesting that wild-type KRAS restrains the oncogenic effects of mutant KRAS.

Deletion of wild-type *Kras* in oncogenic $KRAS^{G12D}$–driven, aggressive tumour models accelerates tumorigenesis and shortens survival (Figure 1B),

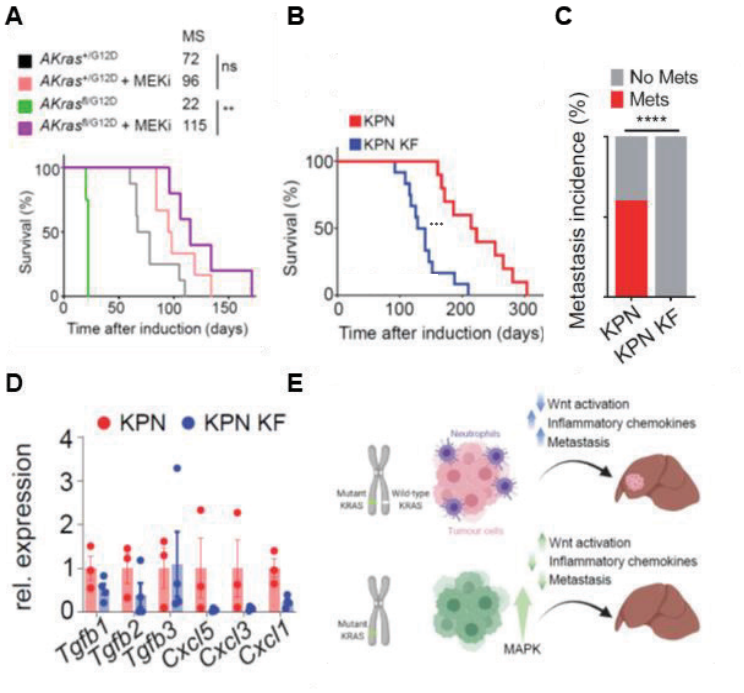

Figure 1: Loss of wild-type *Kras* sensitises to MEK inhibition and suppresses metastasis of $Kras^{G12D}$–mutant intestinal tumours. **A)** Kaplan–Meier survival curves for VilCre^{ER}*Apc*$^{fl/+}$*Kras*$^{+/G12D}$ (*AKras*$^{+/G12D}$) and VilCre^{ER}*Apc*$^{fl/+}$*Kras*$^{fl/G12D}$ (*AKras*$^{fl/G12D}$) mice, treated with MEK-inhibitor (MEKi) one day post tamoxifen-induction and aged until clinical endpoint. *AKras*$^{+/G12D}$, n=8 (2M, 6F); *AKras*$^{+/G12D}$ + MEKi, n=6 (2M, 4F); *AKras*$^{fl/G12D}$, n=4 (2M, 2F); *AKras*$^{fl/G12D}$ + MEKi, n=5 (4M, 1F). $^{**}P$=0.0050; ns, not significant; log-rank (Mantel–Cox) test. **B)** Kaplan–Meier survival curves for VilCre^{ER}*Kras*$^{+/G12D}$*Trp53*$^{fl/fl}$*Rosa26*$^{Nlicd/+}$ (KPN) and VilCre^{ER}*Kras*$^{fl/G12D}$*Trp53*$^{fl/fl}$*Rosa26*$^{Nlicd/+}$ (KPN KF) mice aged until clinical endpoint. KPN, n=10 (5M, 5F); KPN KF, n=12 (6M, 6F). $^{***}P$=2×10^{-4}; log-rank (Mantel–Cox) test. C) Incidence of metastasis in KPN and KPN KF mice aged until clinical endpoint. KPN, n=11 (6M, 5F); KPN KF, n = 11 (5M, 6F). $^{****}P$=1×10^{-15}; two-tailed chi-square test. D) Relative expression of transcripts encoding TGFβ ligands and chemokines in organoids derived from KPN and KPN KF tumours. KPN, n=3 (3M); KPN KF, n=4 (1M, 3F). Data, mean ± s.e.m. E) Schematic depicting the mechanisms whereby loss of wild-type KRAS activates Wnt signalling and reduces neutrophil recruitment, compromising the metastatic competence of KPN KF tumours. MS, median survival; M, males; F, females; s.e.m., standard error of the mean.

Figure 2: Loss of wild-type *Kras* in KPC *Kras*$^{G12D/fl}$ pancreatic tumours potentiates oncogenic KRAS-driven MAPK signalling and sensitizes to MEK1/2 inhibition.
A) Immunoblotting for indicated RAS-pathway components in KPC *Kras*$^{G12D/fl}$ and KPC *Kras*$^{G12D/+}$ tumours at endpoint. β-actin, loading control. Each lane represents PDAC tissue from an individual mouse of the indicated genotype. **B)** Transcript levels of indicated MAPK-pathway downstream target genes in KPC *Kras*$^{G12D/fl}$ and KPC *Kras*$^{G12D/+}$ tumours. Transcript levels were normalized to *Gapdh*. KPC *Kras*$^{G12D/+}$, n=5; KPC *Kras*$^{G12D/fl}$, n=6. Data, mean ± s.e.m. *P*=0.2 (*Etv4*), **P*=0.0087 (*Etv5*), *P*=0.4 (*Dusp4*), **P*=0.0087 (*Dusp6*), **P*=0.0152 (*Spry1*), **P*=0.0411 (*Spry2*); one-way Mann–Whitney U test. **C)** Kaplan–Meier survival curves for KPC *Kras*$^{G12D/+}$ and KPC *Kras*$^{G12D/fl}$ mice, treated with vehicle or MEK1/2 inhibitor (AZD6244), until clinical endpoint. KPC *Kras*$^{G12D/fl}$ + vehicle, n=5; KPC *Kras*$^{G12D/fl}$ + AZD6244, n=8; KPC *Kras*$^{G12D/+}$ + vehicle, n=6; KPC *Kras*$^{G12D/+}$ + AZD6244, n=5. MS, median survival; s.e.m., standard error of the mean.

yet remarkably the corresponding small-intestinal (Figure 1C) and pancreatic tumours are less likely to metastasize. In KPN KF (VilCre^{ER}*Kras*$^{fl/G12D}$*Trp53*$^{fl/fl}$*Rosa26*$^{Nlicd/+}$) small-intestinal tumours, loss of wild-type *Kras* activates Wnt signalling and reduces neutrophil recruitment to the premetastatic niche (Figure 1D) blunting metastasis formation, compared with KPN lesions retaining the wild-type *Kras* allele (VilCre^{ER}*Kras*$^{+/G12D}$*Trp53*$^{fl/fl}$*Rosa26*$^{Nlicd/+}$; Figures 1C and 1E). In the *Kras*$^{G12D/fl}$*Trp53*$^{R172H/+}$Pdx1-Cre (KPC *Kras*$^{G12D/fl}$) mouse model of PDAC, loss of wild-type *Kras* accelerates tumour initiation, giving rise to slow-growing, poorly metastatic tumours that exhibit a desmoplastic stroma with increased immune-cell infiltration, in contrast to the highly immune-excluded KPC *Kras*$^{G12D/+}$ tumours.

Notably, loss of the wild-type *Kras* allele in our CRC and PDAC models increases MAPK signalling (Figures 2A and 2B), which sensitizes them to MEK1/2 inhibition (Figures 1A and 2C). However, these findings unveil only a short window of therapeutic vulnerability, as pancreatic tumours eventually become resistant and progress rapidly. Conversely, retention of wild-type *Kras* dampens tumour dependence on MAPK signalling and confers resistance to MEK inhibition *ab initio* (Figures 1A and 2C), limiting treatment options for this group.

Together, these two studies advocate for patient stratification by *KRAS* allelic status in addition to screening for *KRAS* mutation status. Such patient selection strategies will discern those patients (whose tumours have lost the wild-type *KRAS* allele) that are most likely to benefit from targeted inhibition of downstream effector signalling.

Disease positioning of preclinical models and patient tumours

In collaboration with Philip Dunne's group (Queen's University Belfast and CRUK Scotland Institute), we integrated human and mouse multi-omics data to align our GEMMs to the consensus molecular subtypes (CMS) of human CRC, affirming that they recapitulate key features of disease progression and response to therapy (Amirkhah *et al.*, 2023, *Br J Cancer*; Malla *et al.*, 2024, *Nat Genet*). We employed a biological pathway–level approach and gene ontology—rather than relying on individual gene-centric biomarkers—to identify three biologically distinct pathway-derived subtypes (PDS1–3) of CRC (Malla *et al.*, 2024, *Nat Genet*). We found that PDS1 tumours are enriched for canonical *LGR5*$^{+}$ stem-like signatures and MYC downstream targets, whereas PDS2 CRCs are characterised by the expression of regenerative markers and elevated stromal and inflammatory signatures. This analysis also recognised the hitherto unknown PDS3 class: a slow-cycling, highly differentiated subset of canonical/CMS2 CRCs, which lacks discernible stem-like populations but is instead enriched for enterocyte and enteroendocrine lineages. Molecularly, PDS3 tumours exhibit reduced levels of components of the polycomb repressive complex (PRC), which results in the upregulation of PRC target genes that promote cellular differentiation. Given that none of our existing mouse models align with human PDS3 biology, we are developing tractable GEMMs to gain insights into the biological complexity of this newly recognised group of CRCs with the worst prognosis.

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