MOLECULAR IMAGING

Group Leader
David Lewis

Research Scientists
Robert Bielik[1]
Lisa Duff2
Fraser Edgar[2]
Emma Brown
Irene Caldera Quevedo

Senior Scientific Officers
Abdullah Alyamani[2]

Clinical Research Fellow
Adam Peters[3]

Graduate Students
Emer Curley[4]
Anggraeni Ayu Rengganis

Visiting Scientist
Chrysoula Vraka

1 CRUK Scotland Centre
2 Cancer Grand Challenges
3 CRUK TRACC Programme
4 CRUK Glasgow Centre

Our lab develops new ways to visualise cancer – we create radiolabelled nutrients that image metabolic reprogramming, a hallmark of cancer, and use positron emission tomography (PET) to non-invasively characterise developing tumours. This year, we have focussed on developing technologies to non-invasively stratify colon cancer. Our goal is to classify tumours without the need for invasive biopsies, thereby guiding patient management towards the most effective treatment.

The primary focus of our work is to develop new methods to non-invasively image nutrient usage and then apply these techniques to investigate the causes and consequences of metabolic heterogeneity in mouse models of cancer. Our research has two main themes, first we develop and validate novel technologies such as new metabolic radiotracers and quantitative methods. Second, we exploit PET as a biological imaging modality and investigate the molecular mechanisms and vulnerabilities underlying regional tumour metabolism. The goal of our work is to validate imaging biomarkers for visualising *in vivo* metabolic phenotypes and, by investigating the liabilities of these phenotypes, determine if we can use metabolic imaging to identify susceptibilities that can be used to guide therapy in individual patients.

Stratification of colon cancer using metabolic PET imaging

The current approach for consensus molecular subtyping of colon cancer relies on gene expression profiling tissue, which is invasive and has limited ability to reveal tumour dynamics and spatial heterogeneity. PET imaging presents a non-invasive alternative, however, factors influencing PET imaging phenotype, the suitability of PET radiotracers for differentiating tumour subtypes, and the relationship between PET phenotypes and tumour genotype or gene-expression-based subtyping remain unknown. To address this we conducted a broad PET screening across a spectrum of colon cancer models with four metabolic tracers, [^{18}F]fluorodeoxyglucose ([^{18}F]FDG), [^{18}F]fluoro-ethyl-tyrosine ([^{18}F]FET), 3'-deoxy-3'-[^{18}F]fluorothymidine ([^{18}F]FLT) and [^{11}C]acetate, aiming to identify factors influencing imaging signatures and determine their relationship with genotype, tumour microenvironment and stage.

Our study revealed significant heterogeneity in PET imaging signatures, with distinct radiotracer profiles observed for different cancer models. Notably, oncogenic mutations, such as Kras and Apc loss, showed the most distinctive imaging features, with specific radiotracers like [^{18}F]FLT and [^{18}F]FET be particular effective at stratification of these respectively. Additionally, we found that the tissue microenvironment notably impacted [^{18}F]FDG uptake and higher uptake of [^{18}F]FET was observed in a metastatic model. Overall, this study establishes the feasibility of non-invasive molecular stratification using multiple radiotracer PET (Figure 1; Malviya *et al.*, 2024, *Clin Cancer Res*).

Multi-scale in vivo imaging of tumour development using a germline inducible triple-reporter system

Imaging reporter genes play a crucial role in visualising biological processes *in vivo*, including tumour development, cancer cell dissemination, and treatment response. Historically, incorporating reporter genes into the germline has relied on single imaging modality reporters operating over limited spatial scales. In response, we developed a novel platform technology addressing the challenge of multi-scale imaging of tumour development.

We created and validated a conditional triple-reporter mouse model (Rosa26$^{LSL\text{-}NRL}$), called the "Google-Earth" mouse, integrating imaging reporters for fluorescence, bioluminescence, and PET. This model also features inducible Cre-lox functionality, enabling precise spatiotemporal control of reporter expression. We demonstrated robust reporter inducibility across various tissues in the Rosa26$^{LSL\text{-}NRL}$ mouse, facilitating effective tracking and characterisation of tumours in liver and lung cancer mouse models. Utilising multimodal whole-body imaging, we accurately pinpointed tumour locations, guiding *in situ* lung microscopy to visualise cell-cell interactions within the tumour microenvironment.

This triple-reporter system establishes a robust platform technology for multi-scale investigation of biological processes within whole animals, enabling tissue-specific and sensitive cell tracking from whole-body to cellular scales. This technology is now accessible at the CRUK-Scotland Institute, supporting several new research programs (Dzien et al. 2024).

Publications listed on page 123

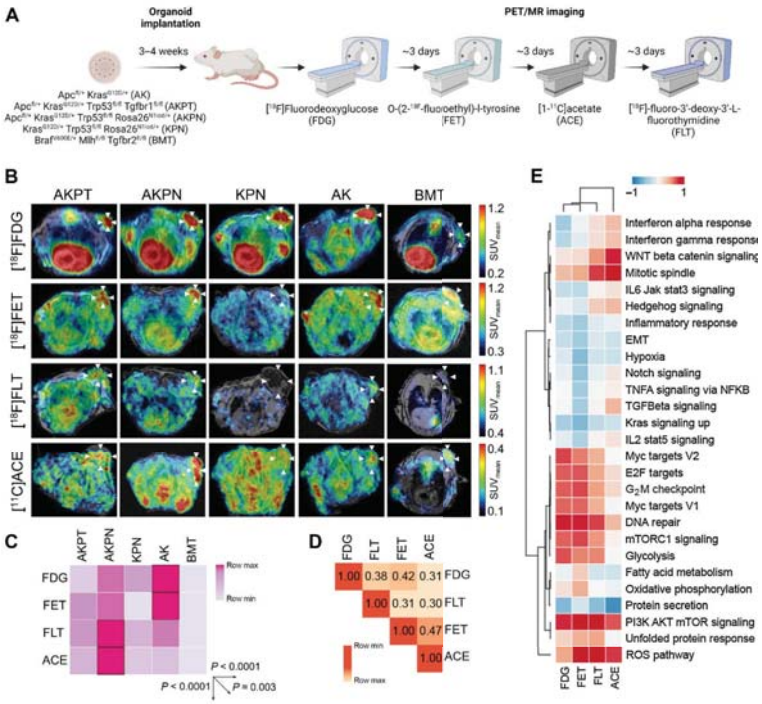

Figure 1. Distinct intermodel heterogeneity in PET imaging signatures. A, In the experimental imaging protocol, five colon cancer organoid models and four PET tracers were used to determine imaging signatures. Details of all mice used in these studies are presented in Supplementary Table S1. **B,** Representative transverse PET images from each model and tracer. The [18F]FDG PET/MR images of the KPN subcutaneous model are reproduced again in Figs. 4B and D and 5B for comparison against other tumors at different sites and stages. **C,** Imaging signature heatmap showing mean tracer uptake, models with highest tracer update highlighted with black outline (representation of the data matrix analyzed with two-way ANOVA). **D,** Correlation matrix of each tracer uptake based on Pearson correlation coefficient. **E,** Heatmap illustrating correlation of PET tracer uptake with gene expression in the Molecular Signatures Database (MSigDB) hallmark dataset, sorted by hierarchal clustering. (**A,** Created with BioRender.com.)