Development of a high-throughput multimodal patient-derived platform integrating autologous fibroblasts and tumour-infiltrating lymphocytes for colorectal cancer drug discovery

RoukenBio

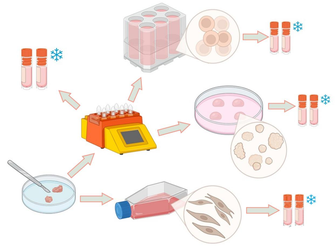
Lewis Campbell[1], Agapitos Patakas[1], Cameron Fyfe[1], Ellen Main[1], Hannah Findlay[1], Athanasios Koulis[1,2] and Daria Paruzina[1]
1. RoukenBio, Rouken Discovery Centre, Newhut Road, Motherwell, ML1 3ST, UK
2. School of Health and Life Sciences, University of the West of Scotland, Lanarkshire, UK

Introduction

Colorectal cancer (CRC) remains one of the leading causes of cancer-related mortality, driven by extensive molecular heterogeneity and the emergence of therapy-resistant subclones. Conventional *in vitro* models fail to reproduce the complexity of the tumour microenvironment (TME). To address this, we developed a high-throughput, multimodal patient-derived tumoroid (PDTO) platform, incorporating autologous tumour-infiltrating lymphocytes (TILs) and/or cancer-associated fibroblasts (CAFs), enabling integrated pharmacologic, genomic and immunologic analyses for translational drug discovery.

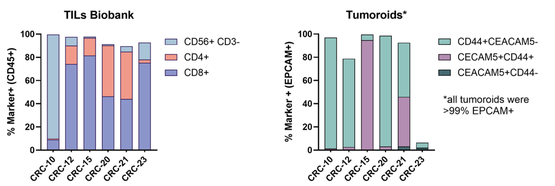
Derivation and Characterisation of CRC Biobanks

Colorectal cancer tissue samples were received and dissociated. A subset of cells were used for tumoroid culture initiation, expansion and biobanking. The remaining cells were used to expand autologous tumour-infiltrating lymphocytes (TILs). A small tissue fraction was also used to establish cancer-associated fibroblasts (CAFs). After expansion, both tumoroids and TILs were subsequently banked and extensively characterised, comparing the banks to the original tissue received.

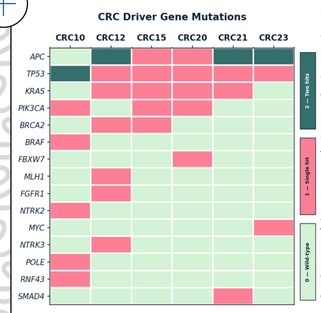


Clinical info
Flow analysis
WES
RNAseq
SOC response

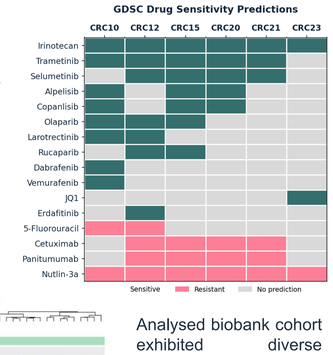
- **Matched tumoroid and TIL biobanks, with more biobanking ongoing**
- **>25 vials of tumoroids per biobank, which can be expanded further**
- **> 30 vials of TILs for each biobank at 5-10x10^6 cells/vial**



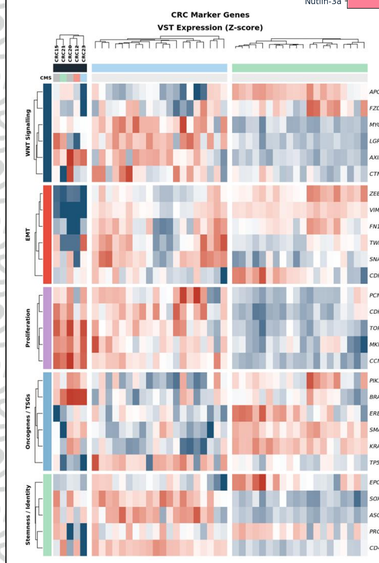
Biobanked tumoroids and TILs were characterised by flow cytometry to define the lymphocyte and epithelial populations derived from the original tissue.



CRC driver gene mutational analysis revealed that TP53 is mutated in all 6 samples - spanning all four mutation types indicating diverse loss-of-function mechanisms. APC is mutated in 5/6 samples, consistent with its role as the canonical CRC initiating event. KRAS is mutated in 4/6 — but notably absent in CRC-10 and CRC-23, which instead carry BRAF V600E and MYC amplification respectively. The mutations in distinct driver genes reflect the genetic diversity of the cohort.

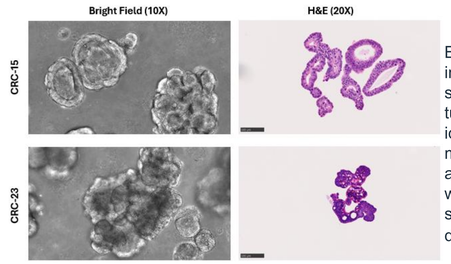


RNA for each tumoroid biobank was analysed for expression of key genetic markers across multiple pathways linked to CRC alongside 20 tumour and 20 healthy TCGA-COAD (The Cancer Genome Atlas Colon Adenocarcinoma Collection) reference samples.



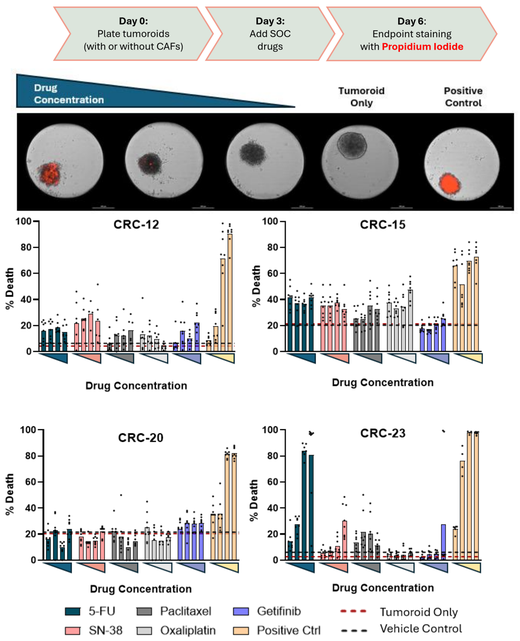
Analysed biobank cohort exhibited diverse expression profiles, with subsets of donors showing elevated expression of stem cell and proliferation markers, or downregulation of WNT pathway genes. This data was also used to run GDSC (Genomics of Drug Sensitivity in Cancer project) drug sensitivity prediction. Predicted resistance to 5-FU for CRC-12 was confirmed in vitro.

Morphological and Histological Characterisation of Tumoroid Biobanks



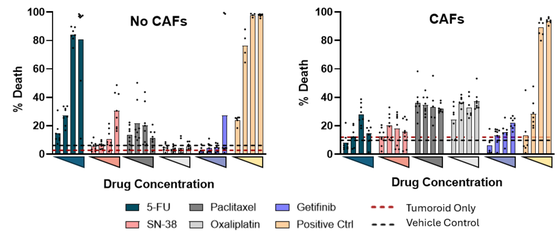
Brightfield imaging and H&E staining of tumoroids identified different morphologies across the cohort, with distinct structural differences.

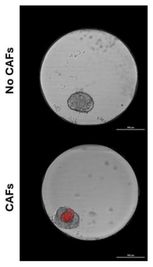
Standard of Care Drug Assessment



Patient-derived CRC tumoroids were treated with standard of care therapeutics across increasing concentrations. Drug-induced cytotoxicity varied between donors, highlighting the diversity of the tumoroid cohort. The positive control treatment consistently induced high levels of tumoroid death, while the test drugs produced variable responses.

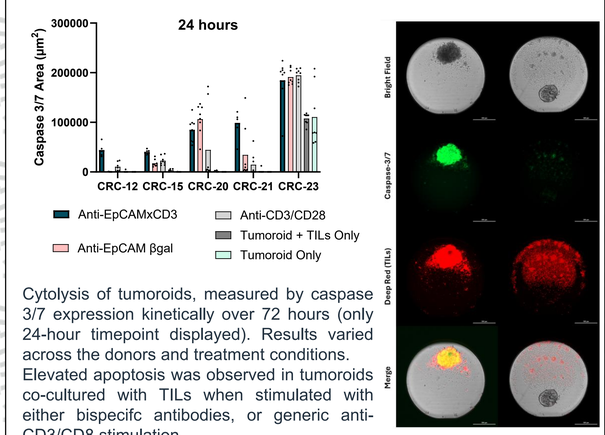
Standard of Care Drug Assessment- Dual Culture





Tumoroid formation was performed using a mixed population of tumour cells and CAFs (stained with CellTracker™ Deep Red) at Day 0 to create complex, multicellular tumoroids (dual cultures). Inclusion of CAFs altered the drug response profile in the initial pilot experiment with a single donor. The findings highlight the modulatory impact of the tumour microenvironment. With the addition of TILs (tricultures), the model can provide a translational platform for drug discovery, predictive biomarker identification, and the rational design of immunotherapy strategies in CRC.

Cytolysis of Tumoroids by Autologous TILs



Cytolysis of tumoroids, measured by caspase 3/7 expression kinetically over 72 hours (only 24-hour timepoint displayed). Results varied across the donors and treatment conditions. Elevated apoptosis was observed in tumoroids co-cultured with TILs when stimulated with either bispecifc antibodies, or generic anti-CD3/CD8 stimulation.

Summary

This multimodal CRC tumoroid platform recapitulates the epithelial, stromal and immune components of the native TME. Its integration of clinical, genomic and gene expression datasets enable high-content functional screening and predictive biomarker discovery. The platform supports translational drug development while reducing the need for large-scale expansion across heterogeneous patient cohorts by capturing heterogeneity of CRC tumours within each culture system.

Human colorectal biopsy samples were obtained from patients under the approval of the Ethical Committee of the National Health Service Greater Glasgow and Clyde (NHSGGC) Biorepository (831). Informed consent has been obtained from all subjects involved.

Scan the code

Download the digital version of this research poster and take all the latest advancements and data home in your pocket.