Final Report
Executive Summary

HSC Public Health Agency
Research and Development

HSC R&D Division Final Progress Report

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HSC Public Health Agency
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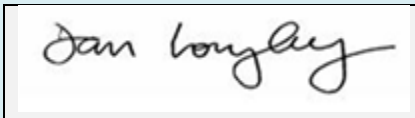
HSC R&D Division Award Details

HSC R&D File Reference	STL/5175/15
HSC R&D Funding Scheme	US-Ireland Partnership Programme
Project Title	Systems Modeling of Tumor Heterogeneity and Therapy Response in Colorectal Cancer
Award Holder Name (Employer)	Professor Daniel Longley (Queen's University Belfast)
Host Research Organisation	Queen's University Belfast
Award Duration	5.5y
Award Start Date	01.12.17
Award End Date	30.06.23
Name of Lead Supervisor: ***(only applicable to training awards)***	

Signature

Award Holder Signature:



Date: 15/6/2023

HSC Public Health Agency
Research and Development

Evidence Brief

(1 page: which may be used for dissemination by HSC R&D Division)

Why did we start? (The need for the research and/or Why the work was commissioned)	Colorectal cancer (CRC) has one of the highest cancer incidences and mortality rates. In stage III, postoperative chemotherapy benefits <20% of patients, while more than 50% will develop distant metastases. In stage II colorectal cancer, the benefits of adjuvant chemotherapy are even more marginal with <5% of unselected patients benefitting. Biomarkers for identification of patients at increased risk of disease recurrence following adjuvant chemotherapy are currently lacking. This programme aimed to address that lack and develop new biomarkers for identifying CRC patients who should receive (and not receive) adjuvant chemotherapy.
What did we do? (Methods)	To explore the levels of key proteins of the apoptosis (cell death) and immune pathways in colorectal cancer (CRC) tissue at the single-cell level, we performed Cell DIVE™ multiplexing to measure expression of pro- and anti-apoptotic proteins and key regulators of anti-tumour immunity in regions of resected primary tumour cores derived from multiple large cohorts of stage II and stage III CRC patients. Disease outcome was correlated with expression of these key markers as well as systems biology predictions of the signalling outcomes from cell death pathways predicted using these protein expression measurements.
What answer did we get? (Findings)	We developed important new methodologies for evaluating protein expression at a single and sub-cellular levels using multiplex immunofluorecent technology. We developed a molecular “atlas” of inter- and intra-tumour heterogeneity of apoptosis competency in colorectal cancer tissue at single-cell resolution. We identified a number of potential immune and apoptosis signalling molecular signatures that have the potential to be developed into risk-stratifying biomarkers to inform clinical decision making (to give or withhold adjuvant chemotherapy) in stage II and III colorectal cancer.
What should be done now? (Practice/Policy Implications and/or Recommendations)	The most promising of the potential risk-stratifying biomarkers will be further evaluated in an independent patient cohort developed in Northern Ireland. Those that look most promising will then be evaluated for their potential for further development into a clinical test with the Precision Medicine Centre of Excellence at the Patrick G Johnston Centre for Cancer Research at Queen’s University Belfast.

Background

Colorectal cancer (CRC) has one of the highest cancer incidences and mortality rates. In stage III, postoperative chemotherapy benefits <20% of patients, while more than 50% will develop distant metastases. In stage II colorectal cancer, the benefits of adjuvant chemotherapy are even more marginal with <5% of unselected patients benefitting. Biomarkers for identification of patients at increased risk of disease recurrence following adjuvant chemotherapy are currently lacking. This programme aimed to address that lack and develop new biomarkers for identifying CRC patients who should receive (and not receive) adjuvant chemotherapy.

Aims and objectives

This programme aimed to address that lack and develop new biomarkers for identifying CRC patients who should receive (and not receive) adjuvant chemotherapy.

Methods

To explore the levels of key proteins of the apoptosis (cell death) and immune pathways in colorectal cancer (CRC) tissue at the single-cell level, we performed Cell DIVE™ multiplexing to measure expression of pro- and anti-apoptotic proteins and key regulators of anti-tumour immunity in regions of resected primary tumour cores derived from multiple large cohorts of stage II and stage III CRC patients. Disease outcome was correlated with expression of these key markers as well as systems biology predictions of the signalling outcomes from cell death pathways predicted using these protein expression measurements.

Personal and Public Involvement (PPI)

At the heart of this programme were colorectal cancer samples (tumour and normal tissues) donated by patients from Northern Ireland, the Republic of Ireland and the USA. Results were disseminated via poster presentations and academic papers. We also met with local stakeholders, including patients and politicians. This included a visit to the Patrick G Johnston Centre for Cancer Research from the then Taoisceach Micheal Martin in 2021 and discussion of the work performed in this programme at the Cancer Knows No Borders event at QUB to celebrate the 25th anniversary of the Good Friday Agreement.

Findings

1) Apoptosis sensitivity is highly heterogeneous and differs between tumor, immune and stromal cells.

We first analyzed 2.4 million apoptosis protein profiles in six different cell types and delivered the first atlas of apoptosis sensitivity in stage III CRC patients (n=168)[2]. Our dynamic systems models (DR_MOMP and APOPTO-CELL) estimated that cancer cells were generally more sensitive to apoptosis signaling than immune or stromal cells, however, with significant heterogeneity between patients (patient and core heterogeneity is demonstrated in **Figure 2B and F**). Specifically, we demonstrated the enhanced ability of cancer cells to activate apoptosis resulted from an enhanced ability to overcome both apoptosis barriers, MOMP and caspase-3 activation downstream of MOMP. Immune cells lacked sensitivity for MOMP due to their relatively high expression of BCL2. Stromal cells showed less sensitivity to caspase-3 activation We predicted that high caspase activity (high APOPTO-CELL model output) was associated with increased DFS when adjusted for either Shannon Entropy or Moran's I (HR 0.6; 95% CI 0.5–0.8; $p < 0.001$).

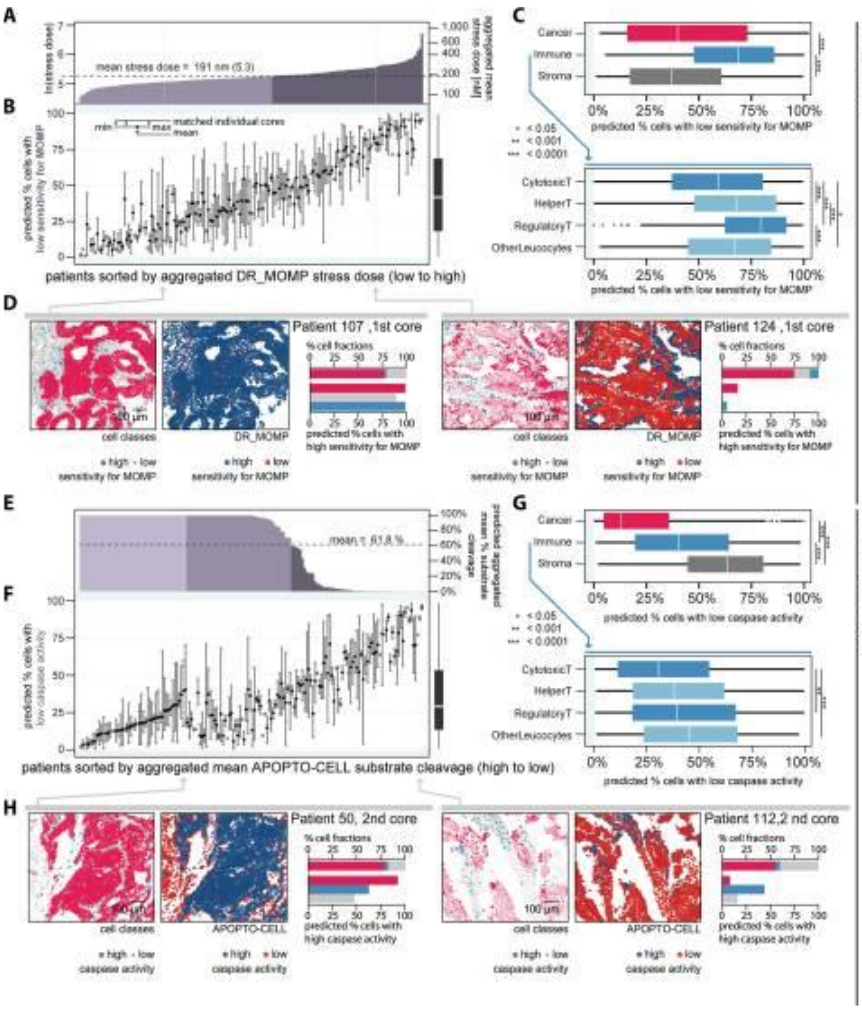


Figure 2: Figure taken from Lindner et al[2] showing: (A-B) distribution of MOMP sensitivity (DR_MOMP model) within and across patients; (C-D) distribution by cell type and MOMP sensitivity tumor cell maps; (F) distribution of low caspase activity (APOPTO-CELL model) within and across patients; (G-H) distribution by cell type and caspase activity tumor cell maps.

2) Stage II CRC patients with apoptosis resistance and 'persister' cell profiles fail to benefit from adjuvant chemotherapy.

Next, to understand the interplay between the individual proteins and whether the balance of apoptosis regulators influences chemotherapy responses and patient outcomes, we investigated (1) whether recurrence in stage II CRC patients can be predicted using single cell analysis of multiple apoptosis and cell death signaling proteins? (2) Is treatment response associated with the abundance of specific apoptotic signatures within the heterogeneous tumor epithelium? Analysis of independent single apoptosis proteins did not reveal significant or consistent

correlations with recurrence risk. However, using K-means clustering of single cells (>400,000 cells), we identified independent cell clusters with distinct expression levels of proteins in the extrinsic and intrinsic pathways (**Figure 3**). Specifically, cell cluster 2, which had higher XIAP (apoptosis inhibition) and lower caspase-3 (pro-apoptosis) expression, was associated with increased risk of recurrence in chemotherapy-treated patients only. This was validated in an independent stage II treated cohort. Using the previously described DR_MOMP and APOPTO-CELL systems models, we demonstrated that cells in cluster 2 showed average MOMP sensitivity but had a reduced sensitivity to apoptosome-dependent caspase activation, suggesting a 'persister' cell profile. This study represents the most comprehensive, integrated analysis to date of apoptosis protein distribution at single-cell level in CRC tumors and identifies a subgroup of stage II patients with an apoptosis resistant, 'persister' cell profile who do not benefit from adjuvant chemotherapy. A manuscript has been submitted to *Cell Death and Differentiation* and is currently under review.

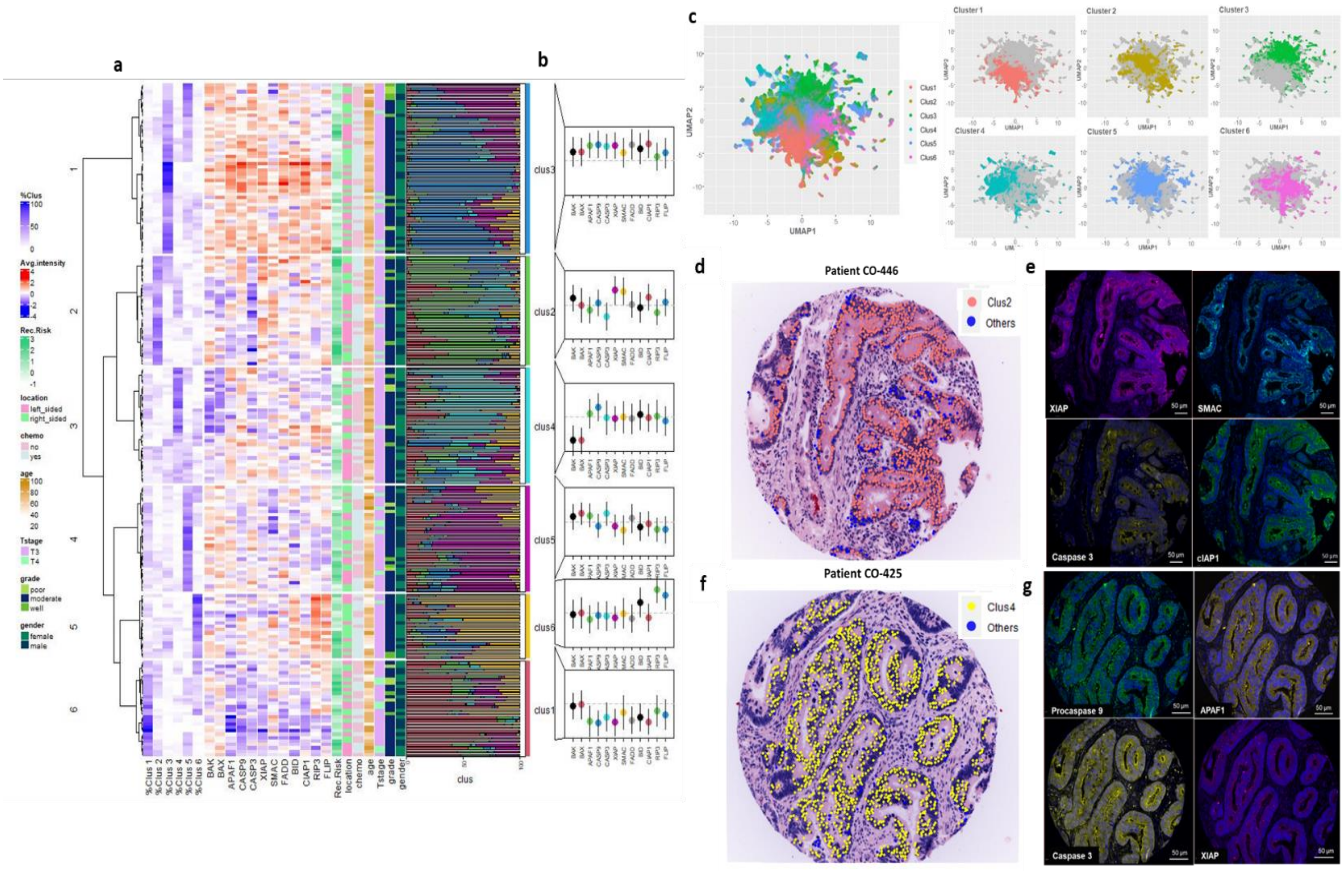


Figure 3: **Proteins from the intrinsic and extrinsic pathway were combined for K-means cluster analysis to quantify protein distributions at a cellular level and contribution to tumor recurrence risk**. **a -** Integrated heat map of all stage II patient clinical and protein data from combined MSK1 and HV cohorts (adjuvant chemotherapy treated and surgery-only); cluster distribution for each patient is shown as a horizontal distribution of all six cluster groups; individual proteins, and other clinical data, and clinical data (age, sex, tumor location, T stage, grade and treatment are included in the heat map for context: **b -** UMAP distributions of clusters illustrating more distinct cell clusters for 1, 3 and 4 with overlaps for cluster 2, 5 and 6; **d -** Tumor with high % cluster 2 cells; **e -** Images show staining features of this high cluster 2 tumor: high XIAP; high SMAC; low caspase-3; high cIAP1. Scale bars 50 µm; **f -** Tumor with high % cluster 4 cells**; g -** Images show staining features of this high cluster 4 tumor: high procaspase 9; high APAF1; average caspase-3; lower XIAP

3) Identification of “normal-like” cancer cell phenotypes associated with reduced recurrence risk.

We compared protein expression between adjacent normal and cancer cells (MSK3 stage 2 and 3 TMAs also included matched adjacent normal regions) and found a number of differentially expressed proteins in normal and tumor cells (Figure 4). Using hierarchical clustering, seven cell clusters were identified, and we evaluated the recurrence rate for each of the cluster cores. Normal cores tended to have a dominant cluster of cells (cluster 2) with higher (as compared to tumor cores) ALDH1, BCL2, CDX2, BAK, BAX, caspase-3, cIAP1 and lower LDHA, Glut1, PKM2, (highlighted in Figure 4). Tumors with cluster 3 (higher in LDHA, Glut1) and 5 (higher in cMyc, Ki67, beta catenin) tended to have higher recurrence rate. Further, the level of MOMP sensitivity (as determined by DR_MOMP model) was relatively similar among all cancer type cores. However, the percentage of cells with substrate cleavage (SC) > 25% tended to be lower in cluster 3 and 5 suggesting a ‘persister’ cell phenotype, as described earlier). For each patient, we also measured the pairwise distance from adjacent normal to cancer cores using 1- correlation of the biomarkers shown in the heatmap. We found a significantly lower recurrence risk in cancers that were more correlated with “normal cell” biomarker phenotypes, highlighting another source of tumor cell heterogeneity. Analysis is ongoing and a manuscript is in preparation.

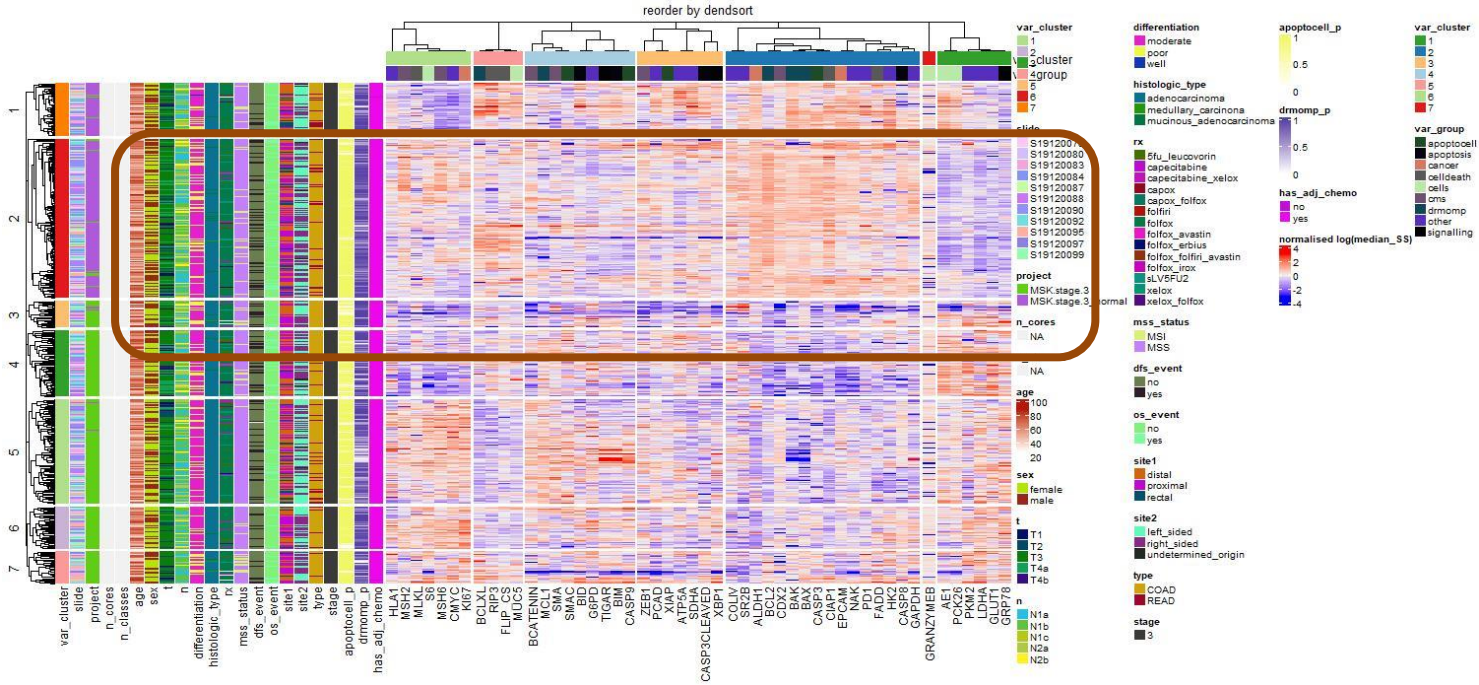


Figure 4: **Cluster analysis of all protein in adjacent normal and tumor cores.** Distinct clusters were found for normal cores (cluster 1 and 2 - highlighted) and cancer cores (cluster 3 and 5 were significantly correlated with recurrence risk).

4) Identification of a stem cell protein signature that separates early vs. late recurrence in stage III patients.

We successfully developed a new pipeline for cell type identification which includes algorithms for the recognition of stem cells, in addition to immune (macrophages, T cells, B cells), endothelial and epithelial cells. First, median protein profile expression in stem cells from each cancer core were compared to adjacent normal cores by means of differential expression analysis (DEA). In paired DEA comparison we found that stem cells in the adjacent normal cores significantly overexpressed BAK, BAX (key regulators of the intrinsic apoptosis pathway) and CDX2 (homeobox transcription factor that is important for intestinal homeostasis) comparison to the paired cancer tissue cores. Furthermore, we analyzed the differences in the individual protein heterogeneity using CV (coefficient of variation) over the population of stem cells within individual cancer cores in comparison to paired normal cores. Interestingly, BAX and BAK showed higher heterogeneity in cancer cores together with the stem cell marker, ALDH1. Second, we analyzed the stem cell protein signature to find a prognostic marker set that may enable separation patients with early recurrence (DFS < 3 years, “Early”) and late recurrence (DFS >= 3, “Late”). For this analysis, stage 3 adjuvant treated patients’ cancer cores without mucinous features were aggregated from MSK3, HV3, RCSI and Taxonomy cohorts (aggregated cohort). The DEA analysis was performed comparing stem cell median protein expression in early vs. late recurrence cores with and without batch correction and the patients’ covariance model. The resulting 14 batch/covariance differentially expressed protein features were selected as a prognostic marker set. Furthermore, a deep neural network was trained to separate the early from late recurrent cores from the pool of cores in the aggregated cohort. We used 80% of randomly assigned cores for training and the remaining 20% were used as the validation cohort. Following an extensive parameter sweep for neural network hyperparameter optimization (type of activation function, number of layers, number of neurons, training period) and random 80/20 training/validation we trained an accurate neural network (**Figure 4**).

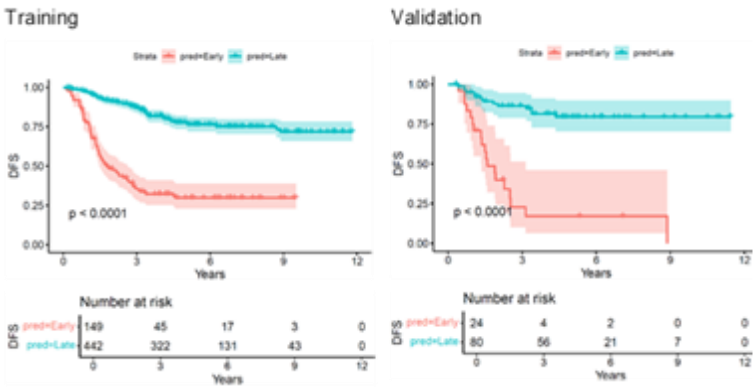


Figure 4: Stem cell protein signature (> median) predicts early recurrence in stage III CRC patients.

5) Tumor cells in close proximity (within 20 microns) to killer T cells have higher expression of caspase-3 protein.

We also investigated whether T cell count and percentage infiltration were significantly correlated with recurrence risk and found that multi-marker classification of regulatory T cells (Tregs: CD3+/CD4+FOXP3+/PD1−) was significantly associated with disease-free survival (DFS) and overall survival (OS) ($p = 0.049$ and 0.032) in treated stage III patients and in an independent validation cohort[3]. This association was not found using single markers, demonstrating the value of multiple markers for cell type

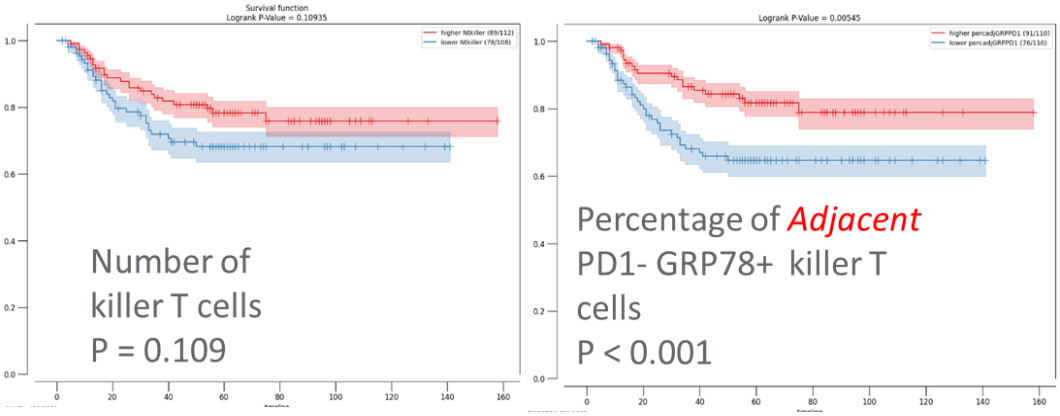


Figure 5: Total count of infiltrating killer T cells was not significantly correlated with recurrence risk, however closer proximity of killer T cells to tumor cells was significantly associated with recurrence risk.

classification. The positive association with PD1-negative regulatory T cells may reflect the presence of an active inflammatory response rather than the establishment of an immunosuppressive TME; this could explain the association which we observed with improved prognosis in this chemotherapy-treated stage III cohort. Interestingly, while there were trends for killer T cell infiltration and reduced recurrence risk across all the CRC cohorts, they were not significant. Investigating this further in the MSK stage III cohort, we found that having a higher number of cytotoxic T cells in proximity (within 20 microns) to cancer cells was significantly associated with increased DFS (**Figure 5**). The effects were strongest for cytotoxic T cells with higher expression of GRP78 (a marker of ER stress) and negative for PD1 (marker of T cell exhaustion). We also found that caspase-3 was significantly higher in these closely adjacent tumor cells, indicating cytotoxicity and induction of apoptosis. Expression of GRP78 may lead to increased expression of cytotoxic molecules, such as perforin and granzymes, which are essential for inducing apoptosis in target cancer cells. In summary, we demonstrate that cancer cell apoptotic response is also dependent on distance of the cytotoxic T cells and their functional status. Additional spatial analysis is underway, and a manuscript is in preparation.

6) **New approach for consensus molecular subtype (CMS) classification based on multiplexed protein expression in tumor cells.**

Currently the gold standard method for CMS classification of CRC is based on RNAseq analysis. Using the RCSI and Taxonomy cohorts for reference (since they both had RNAseq data available and CMS classification) we developed a Gaussian process model to predict CMS based on protein expressions. The model is trained on core-level marker expressions (only on epithelium mask). For the training, we pooled two studies (RCSI and Taxonomy) where RNA-Seq or microarray-based CMS classifications were available. A total number of 668 TMA cores from 226 patients was used. 164 of the CMS classifications were derived from RNA-Seq and 58 of them were classified based on microarrays. Five-fold validations showed >95% accuracies. We compared the clinical features of the predicted molecular subtypes in the MSK cohort. Survival analysis revealed that CMS4 tumors predicted by our model had worse overall. To assess the validity of our model predictions further, we also determined MSI status and tumor locations for predicted subtypes. In the MSK cohort, 40% of the predicted CMS1 samples were identified with mismatch repair deficiency in stage II cancers and 30.4% of the CMS1 samples were classified as MSI in stage III tumors. These were the highest MSI/MMR-d rates among the predicted CMS subtypes. We also found a higher immune cell counts (i.e. CD3+, CD8+ and PD1+ cell frequencies) in CMS1 patients, which is consistent with previous literature. Tumor location showed a similar pattern where 76% and 60.9% of the CMS1 were located on the right side of the colon in stage 2 and stage 3 tumors respectively. Similarly, for CMS2, 57% and 59.6% of them were left-disease-free survival in stage 3 colorectal cancers (p-value = 0.011). This is consistent with previous reports that CMS1 is more associated with right-sided tumors and CMS2 in left-sided tumors. We further calculated the overall expression of the proteins in each tumor core and observed significantly lower CDX2 expression in the epithelial cells CMS1. Moreover, PKM2, SR2B, BCLXL, GRP78, HK2 and KI67 proteins were higher in in the same CMS1 patients. In conclusion, CMS predictions based on our protein expression-based model showed similar clinical characteristics to the original CMS classification study which used RNAseq analysis for classification. Our novel approach opens new opportunities for CMS classification for FFPE samples when RNAseq data is not available, and we provide additional new biological insights into their differences. A manuscript is currently in preparation.

7) Anti-apoptotic protein FLIP is associated with increased recurrence risk in stage III patients and advancements in novel FLIP inhibitors

Stage 3 patients with high epithelial expression of the anti-apoptotic protein FLIP have a poor prognosis when treated adjuvantly with standard-of-care chemotherapy, suggesting that FLIP promotes resistance to chemotherapy (publication in preparation)**.** In support of this, the QUB team has used novel FLIP inhibitors that synergize with chemotherapy to induce apoptosis in *in vitro* and *ex vivo* models of colorectal cancer. This drug discovery program is currently licensed to Ipsen Pharma and is in late lead optimization. An additional component of this work is the link between p53, FLIP and the development of drug-tolerant persister cells (DTPs). In parallel work, the QUB team found that FLIP is a direct target gene of the p53 transcription factor and that this drives resistance to p53-induced apoptosis[4]. Further, colorectal cancer cells with wild-type p53 are less sensitive than those with mutant p53 or to long-term treatment with 5FU-based chemotherapy because they are more able to develop into DTPs. These DTPs have altered expression of FLIP as a result of epigenetic changes in the gene (*CFLAR*) promoter. This correlates with our findings that elevated epithelial FLIP expression correlates with poor prognosis in stage III CRC patients and that tumors which retain wild-type p53 transcriptional activity have a worse prognosis than those that have lost this activity (either p53-MT or p53-WT with suppressed p53 transcriptional activity) in adjuvantly treated stage II and stage III disease. Moreover, our experimental work using *in vitro* and *in vivo* models has shown that the emergence of p53-WT DTPs can be prevented by class-I histone deacetylase inhibitors (HDACi), which we have shown suppress FLIP expression and prevent the epigenetic changes at its promoter that are found in DTPs. This work is being prepared for manuscript submission, and the future plan is to develop these findings into a clinical trial. The QUB team has also used *in vitro, ex vivo* and *in vivo* models to demonstrate that antagonists of inhibitor of apoptosis proteins (IAP inhibitors) enhance sensitivity to standard-of-care chemotherapy[5], and this has led to a phase-1 clinical trial supported by Cancer Research UK (“ASTFOX). They explored the use of a different class of cancer cell death inducing agent, poly(IC) in *in vitro* (including co-cultures) and *in vivo* models of CRC. A manuscript based on this work is in preparation.

Additional key findings:

- **Identification of prognostic biomarkers in mucinous rectal tumors:** Locally advanced rectal cancer is typically managed with multimodal therapy, including neoadjuvant radiotherapy, chemotherapy, and surgery. This approach results in tumor downstaging, improved margin status, and reduced local recurrence, but not for all patients. Immune checkpoint inhibitors (ICI) have been shown to be highly effective as first-line treatment for patients with mismatch repair–deficient (dMMR) metastatic colorectal cancer, as well as for patients with treatment-refractory disease, with clinically significant durability of response, and prolonged overall survival. 14% of rectal cancer have a mucinous subtype. These tumors display a reduced rate of pathological complete response and tumor downstaging following neoadjuvant chemoradiotherapy with an increased rate of positive resection margin after surgery and poorer overall survival following resection. We analysed as part of this study also a subgroup of mucinous rectal cancers. Of note, we showed that mucinous rectal cancer was also associated with an immune-rich tumor microenvironment, not associated with MSI status. We showed that these tumors over-express cytotoxic and regulatory T cells with increased programmed cell death protein 1 expression and T cell immunoglobulin and mucin domain 3 (TIM-3) expression[6]. As a result, there exists significant clinical and biological plausibility that ICI in the neoadjuvant setting may be beneficial in mucinous rectal cancer, and we aim to now initiate a phase II clinical trial to test this hypothesis. Using the multiplex platform, we also demonstrated that mucinous tumors activate necroptotic rather than apoptotic signalling pathways, which was also validated *in vitro*[7]. Supporting the activation of necroptotic signalling pathways may therefore represent a second strategy for these difficult to treat cancers. Supporting the activation of necroptotic signalling pathways may therefore represent a second strategy for these difficult to treat cancers.

- **Positive effects of Fusobacterium nucleatum infection in mucinous rectal tumors:** In the case of mucinous rectal cancers, we also demonstrated high levels of *Fusobacterium nucleatum* infection. This was associated with a pro-inflammatory environment and improved clinical outcome as demonstrated by multiplexing and immune cell profiling[8]. Collectively, these studies suggest that bacterium-host interactions are critically dependent on the respective subtype of colorectal cancer.

Cited publications:

1. Graf, J., Cho, S., McDonough, E., Corwin, A., Sood, A., Lindner, A., Salvucci, M., Stachtea, X., Van Schaeybroeck, S., Dunne, P.D., et al. (2021). FLINO: a new method for immunofluorescence bioimage normalization. Bioinformatics *38*, 520–526. 10.1093/bioinformatics/btab686.

2. Lindner, A.U., Salvucci, M., McDonough, E., Cho, S., Stachtea, X., O'Connell, E.P., Corwin, A.D., Santamaria-Pang, A., Carberry, S., Fichtner, M., et al. (2022). An atlas of inter- and intra-tumor heterogeneity of apoptosis competency in colorectal cancer tissue at single-cell resolution. Cell Death Differ *29*, 806–817. 10.1038/s41418-021-00895-9.

3. Stachtea, X., Loughrey, M.B., Salvucci, M., Lindner, A.U., Cho, S., McDonough, E., Sood, A., Graf, J., Santamaria-Pang, A., Corwin, A., et al. (2022). Stratification of chemotherapy-treated stage III colorectal cancer patients using multiplexed imaging and single-cell analysis of T-cell populations. Mod Pathol *35*, 564–576. 10.1038/s41379-021-00953-0.

4. Lees, A., McIntyre, A.J., Crawford, N.T., Falcone, F., McCann, C., Holohan, C., Quinn, G.P., Roberts, J.Z., Sessler, T., Gallagher, P.F., et al. (2020). The pseudo-caspase FLIP(L) regulates cell fate following p53 activation. Proc Natl Acad Sci U S A *117*, 17808–17819. 10.1073/pnas.2001520117.

5. Crawford, N., Stott, K.J., Sessler, T., McCann, C., McDaid, W., Lees, A., Latimer, C., Fox, J.P., Munck, J.M., Smyth, T., et al. (2021). Clinical Positioning of the IAP Antagonist Tolinapant (ASTX660) in Colorectal Cancer. Mol Cancer Ther *20*, 1627–1639. 10.1158/1535-7163.MCT-20-1050.

6. Duggan, W.P., Kisakol, B., O'Connell, E., Matveeva, A., O'Grady, T., McDonough, E., Lindner, A.U., McNamara, D., Longley, D., Ginty, F., et al. (2023). Multiplexed Immunofluorescence Imaging Reveals an Immune-Rich Tumor Microenvironment in Mucinous Rectal Cancer Characterized by Increased Lymphocyte Infiltration and Enhanced Programmed Cell Death Protein 1 Expression. Dis Colon Rectum *66*, 914–922. 10.1097/DCR.0000000000002624.

7. O'Connell, E., Reynolds, I.S., Lindner, A.U., Salvucci, M., O'Grady, T., Bacon, O., Cho, S., McDonough, E., Longley, D., Ginty, F., et al. (2022). Apoptotic and Necroptotic Mediators are Differentially Expressed in Mucinous and Non-Mucinous Colorectal Cancer. Front Oncol *12*, 815001. 10.3389/fonc.2022.815001.

8. Duggan, W.P., Salvucci, M., Kisakol, B., Lindner, A.U., Reynolds, I.S., Dussmann, H., Fay, J., O'Grady, T., Longley, D.B., Ginty, F., et al. (2023). Increased Fusobacterium tumoural abundance affects immunogenicity in mucinous colorectal cancer and may be associated with improved clinical outcome. J Mol Med. 10.1007/s00109-023-02324-5.

Conclusion

During the course of this work, we developed important new methodologies for evaluating protein expression at a single and sub-cellular levels using multiplex immunofluorecent technology. In addition, we developed the first molecular “atlas” of inter- and intra-tumour heterogeneity of apoptosis competency in colorectal cancer tissue at single-cell resolution. Most importantly, we identified a number of potential immune and apoptosis signalling molecular signatures that have the potential to be developed into risk-stratifying biomarkers to inform clinical decision making (to give or withhold adjuvant chemotherapy) in stage II and III colorectal cancer.

Practice and Policy Implications/Recommendations

Results are not yet confirmed in further patient cohorts (*see below*). Until we have this information, there are no practice-changing recommendations to be made.

Pathway to Impact

The most promising of the potential risk-stratifying biomarkers will be further evaluated in an independent patient cohort developed in Northern Ireland (“EPI700”). Those that look most promising will then be evaluated for their potential for further development into a clinical test with the Precision Medicine Centre of Excellence at the Patrick G Johnston Centre for Cancer Research at Queen’s University Belfast. The development of such a test would have commercial and societal (practice-changing) implications.