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ADVANCED COLORECTAL CANCER

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Patients die from colorectal cancer due to spread/metastasis to other organs, in particular the liver. The team studies patient tissues accessed at the time of surgery and generates models to better understand the mechanisms underlying colorectal cancer progression in patients with locally advanced rectal cancer and liver metastases with a view to developing and assessing novel targets for therapy.

Colorectal cancer (CRC) is the second most common cause of cancer-related death in the Western world. Disease that is localised to the colon can be treated with surgery. Despite this, 40% of patients will suffer from disease recurrence. Recurrence usually occurs at sites distant from the colon, most commonly liver and lungs and is called metastatic disease. Most patients who die from colorectal cancer do so due to metastatic disease. Unfortunately, treatment options remain limited for these patients, with surgery remaining the best strategy if disease is diagnosed early. The team is focused on understanding why disease recurs following surgery, the patterns of recurrence and whether the disease can be subtyped to permit development of better therapies for patients.

Assessing the heterogeneity of colorectal liver metastases

Assessment of human colorectal liver metastases (CRLM) suggests that different subtypes exist. These can be detected histologically and separated into 'immune', 'stromal' and 'canonical' using transcriptomic analysis (Pitroda *et al.,* 2018, *Nature Comms*). Patients from the immune subgroup do very well following surgical resection and can be cured of their disease. It is likely these patients may also respond to commonly used immunotherapies, however, this is as yet still to be clearly elucidated. We are making efforts to accurately subtype the disease in our patients (Figure 1) and we have partnered with Nanostring to assess the heterogeneity of these subtyped tumours.

Figure 1
Transcriptomic assessment of heterogeneity, primary and metastatic sites in 4 patients.

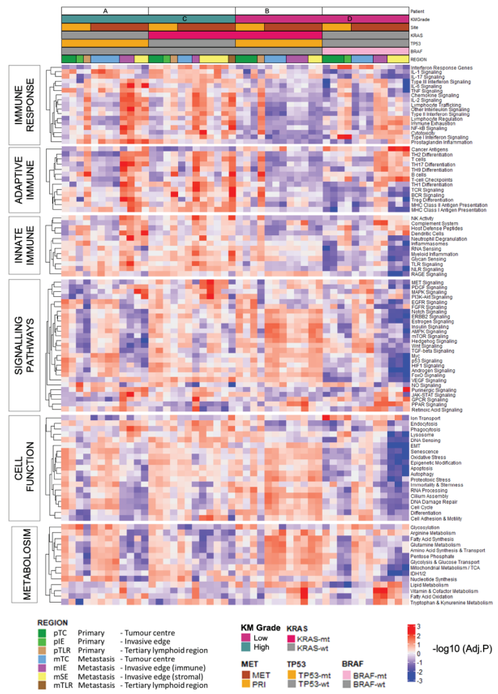


Figure 2
Immunofluorescence. Pink CD45, Blue pan-CK, Yellow alpha-SMA. Immune cell deconvolution representing cellular component derived from transcriptome of each area.

Figure 3
Site specific differential gene expression within neutrophils

Figure 2

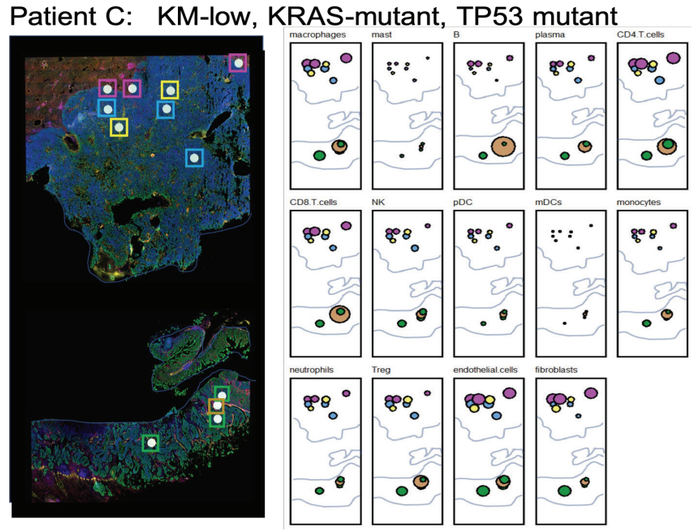
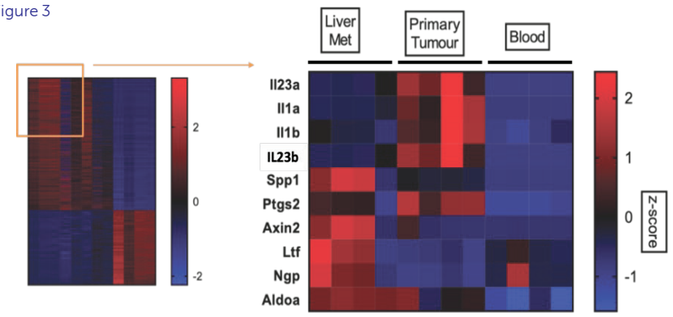


Figure 3



This represents an area for further study with a view to moving these observations into real-time to help guide decision-making for patients in the future. Having established the utility of these technologies, we are now studying neutrophil biology within individual patients. We have performed Nanostring COSMX analysis to provide single cell level data with spatial resolution and are currently mapping neutrophil populations identified using bioinformatic approaches to tissue in attempts to identify pathogenic neutrophils in this context.

Modelling immunosuppressed metastatic CRC and understanding microenvironmental influences for therapeutic gain

We have worked closely with Professor Owen Sansom's laboratory and have been involved in the development of state-of-the-art models of CRLM. Using orthotopic transplantation techniques we can mimic human disease to provide a model of stromal rich metastasis for assessment of anti-metastatic therapies *in vivo*. Our previous work together has revealed that neutrophils were key cellular regulators of the metastatic microenvironment in CRC (Jackstadt *et al.,* 2019, *Cancer Cell*), regulating an immunosuppressed microenvironment as we observed in patients with very poor outcomes. However, the mechanism by which those neutrophils functioned to progress metastatic disease and how to manipulate them *in vivo* remains unknown. We have performed RNA sequencing of neutrophils from sites within our 'KPN' model and found differentially expressed genes within neutrophils associated with metastases (Figure 3). We are currently investigating whether inhibition of specific genes expressed by neutrophils *in vivo* influences their behaviour and progression of metastases. Others have shown: cooperation of gamma delta T cell populations in promoting neutrophil function at metastatic sites (Coffelt *et al.,* 2015, *Nature*); that production of transferrin by neutrophils supports metastatic cells (Liang, Li, & Ferrara, 2018, *PNAS*); the role of neutrophil extracellular traps in awakening dormant tumour cells. (Albrengues *et al.,* 2018, *Science*); and that neutrophils can accompany tumour cells to metastatic sites and help them establish (Szczerba *et al.,* 2019, *Nature*). Modelling these immunosuppressed stromal metastases will allow us to understand immunosuppressive mechanisms using intravital imaging and whether they can be overcome through directly targeting neutrophils in this model. *Ex vivo* study of neutrophil function is being developed to further characterise these cells in this context. T cell-directed therapies are currently being trialled in combination with neutrophil-directed therapies to assess impact on metastatic progression with a view to taking forward for patient benefit in future.

We have identified that CRLM in certain patients were profoundly immunosuppressed with very few activated T cells evident within the microenvironment of these tumours (Figure 1, patient C), while others had significant upregulation of adaptive immune responses particularly at the edges of metastases (Patient B). We observed higher numbers of myeloid cell populations within the microenvironment of immunosuppressed and stromal tumours including neutrophils and macrophages, using immune cell deconvolution techniques (Figure 2) and confirmed using IHC. These patients had contrasting survival based on their immune response, with patients able to obtain long term survival following surgery for liver metastases if they displayed a strong adaptive immune response (*Submitted Cancer Research*), while patients with neutrophils surrounding metastatic disease had very poor survival following surgery.

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