CHIEF SCIENTIST OFFICE

CODE:PCL/17/03

RESEARCH PROJECT BRIEFING

Defining the metastatic niche in colorectal cancer

AIMS

To examine colorectal cancer that has spread to the liver (metastases) and determine if characteristics of these tumours assessed by looking down the microscope determine outcome following surgery

To assess the role of neutrophils in the process of colorectal cancer spread to the liver

KEY FINDINGS

- Colorectal cancer metastases do not separate into previously defined subtypes of the disease
- Examination of colorectal cancer histology can provide accurate information on outcome following surgery using a clinically relevant and simple score.
- Patients with low numbers of carry much poorer prognosis than those with high numbers of immune cells present within these metastases.
- Patients with low immune or ‘stromal’ metastases are defined by high levels of infiltrating neutrophils
- Colorectal cancer liver metastases can now be modelled in mice. Drug therapies can be used to reduce neutrophil infiltration and improve T cell infiltration, and decrease the volume of tumour, suggesting an intrinsic dependency of these metastases on neutrophil presence

WHAT DID THE STUDY INVOLVE?

In this study we identified a consecutive group of patients that had synchronous resection of colorectal cancer in the colon and liver to characterise differences.

We subtyped the disease by performing 2 assessments which are clinically relevant and could be performed affordably as part of the post-operative pathology assessment of tissues following surgery. Firstly, we applied a microenvironmental score that grossly assesses stromal (supporting matrix of cancer), and immune cell content at the invasive edge of tumours and metastases, and secondly a molecular subtype based on immunohistochemical analysis. Then we performed detailed immunohistochemical and flow cytometry analysis of immune cell populations within each subtype of disease to distinguish the cells that determine the features of each subtype of metastatic colorectal cancer.

We took findings from human tissues and modelled them in mice. We assessed the role of neutrophils in the disease in mouse models by using inhibitors of neutrophil migration.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

We showed that metastatic colorectal cancer does not adhere to previously published ‘consensus molecular subtypes’ and cannot provide helpful information on outcome following surgery. However, simple assessment of stromal composition and immune cell contexture demonstrates that stromally rich metastases are more likely to recur following resection and patient survival is poor (Figure 1). These stromal metastases had high numbers of neutrophils present relative to other tumours. Interestingly, patients with very high immune cell infiltration to these tumours, particularly CD3 positive T cells, survive longer following surgery. This finding is in-keeping with other authors. Flow cytometry analysis of neutrophils within colorectal cancer in the liver shows them to be activated cells (CD66b activation marker) and more immature (CD10 maturity marker).(Figure 2).

We developed an mouse model that spontaneously develops colorectal cancer that spreads to the liver. These tumours had high numbers of neutrophils and responded to drug therapy targeting neutrophil migration with reduction in tumour burden and in numbers of neutrophils infiltrating the tumours.

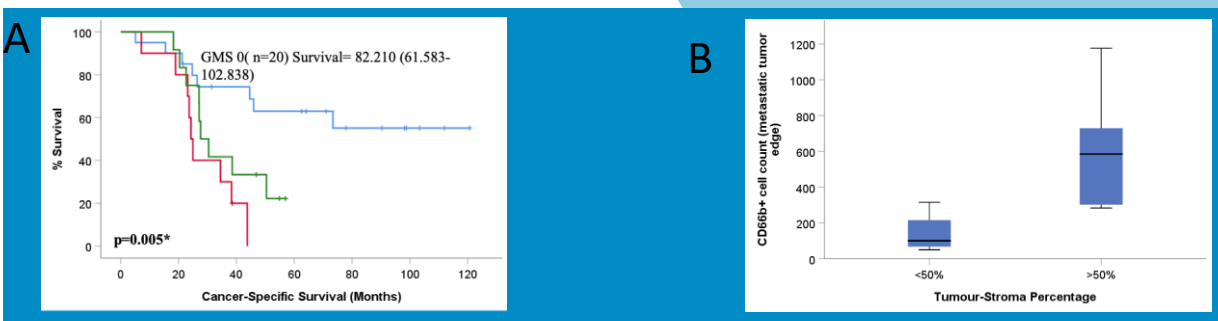

Figure 1: A – Microenvironmental assessment of metastases. Blue: Immune cell rich; Red: Stromal rich; Green: intermediate. B – Neutrophils higher in 'stromal' tumours

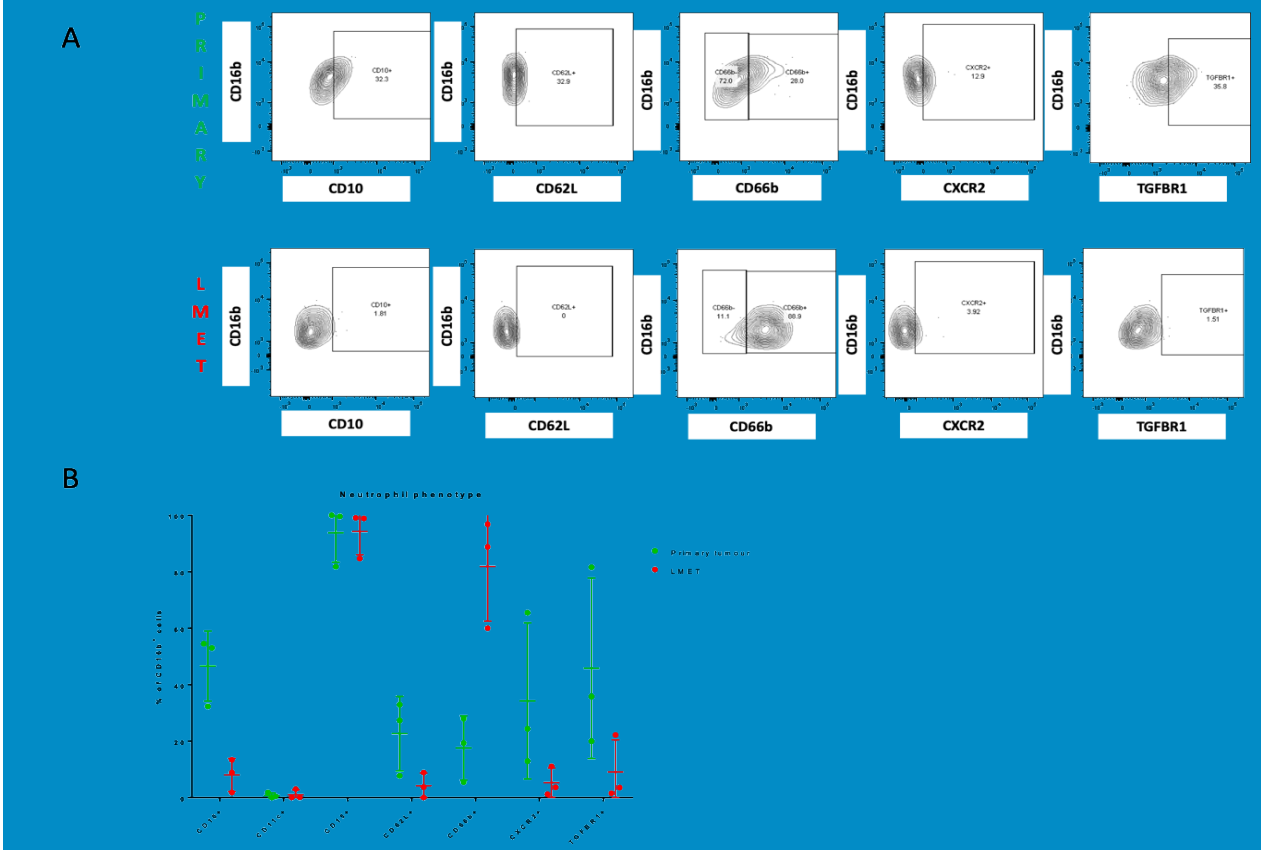

Figure 2: A – Green – Neutrophils from primary cancer in colon, Red – neutrophils from liver tumours. CD16b+ Neutrophil flow cytometry. Primary compared with liver metastasis. CD10 Maturity; CD62L, CD66b Activation, CXCR2, TGFbeta migration. B – Neutrophils in Liver tumours are immature and activated compared with colon tumours

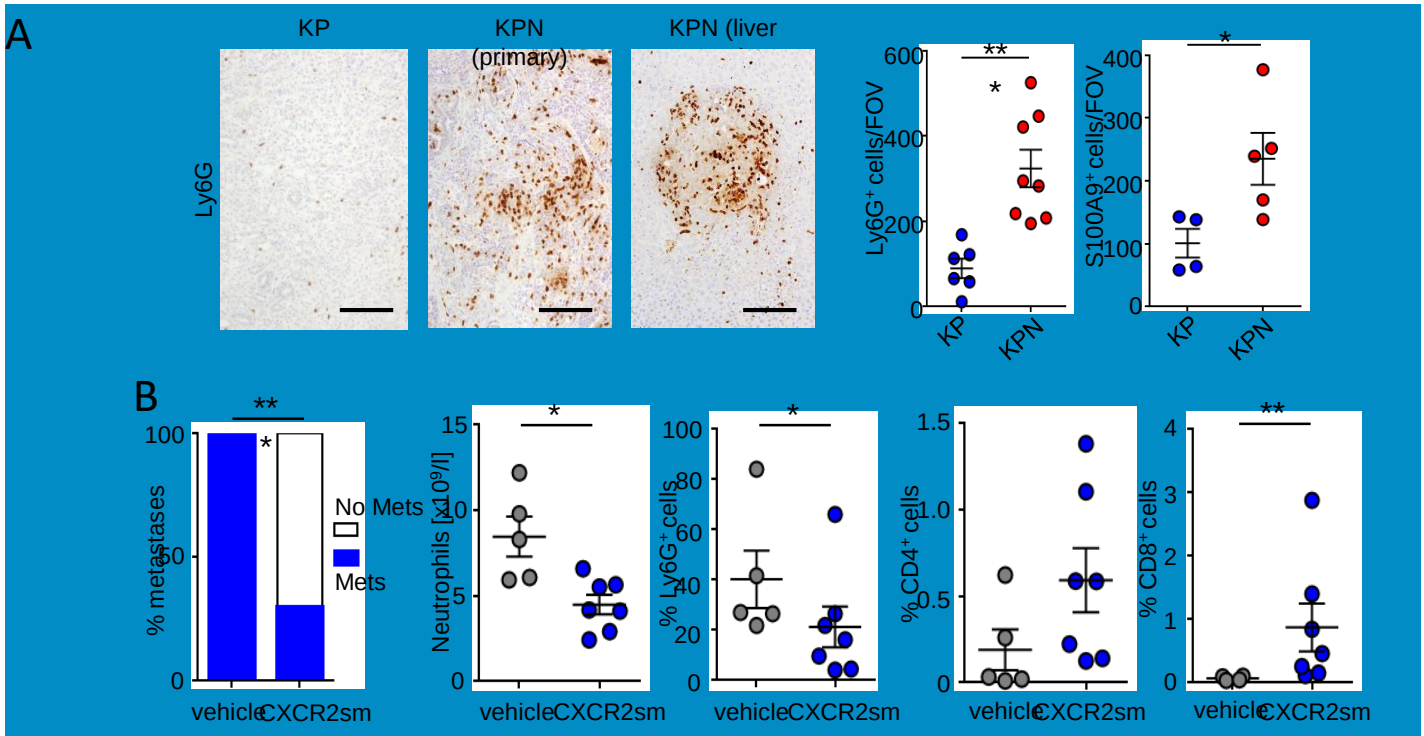

Figure 3. A. Mouse model liver metastases KPN heavily infiltrated with Ly6G+ neutrophils compared with controls KP
B. CXCR2 small molecule decreases neutrophil infiltration and metastases

WHAT IMPACT COULD THE FINDINGS HAVE?

- Data generated from this project has led to a number of avenues of investigation that are now ongoing and are being developed as the subject of further grant applications. Deep sequencing data have been established from these patients to assess the molecular landscape of colorectal cancer that has spread to the liver to help predict those patients that may do poorly following surgery, and secondly may benefit from neutrophil directed drugs.
- Furthermore novel neutrophil specific targets for therapy are likely to be required, as strategies in the clinic to limit migration have not led to significant impact on patient outcomes until now. If neutrophils promoting tumour spread could specifically be targeted this could open up the field to new clinical trials. With this in mind ‘single cell sequencing’ (taking a solution of only these cells and performing analysis of the genes they are expressing) of neutrophils is now being performed in my laboratory to fully characterise these cells and functional studies of these neutrophils are planned to understand how they interact with tumours to help them spread.

HOW WILL THE OUTCOMES BE DISSEMINATED?

The results of this work have been communicated to the NCRI Colorectal subgroup through my position as trainee representative. I have also been invited to present to results at a subgroup translational medicine event in Glasgow. I have presented these data at ASCO GI in San Francisco (January 2020). Modelling work in collaboration with my supervisor Owen Sansom has been published in Cancer Cell 2019, and I will submit clinical data to Clinical Cancer Research.

CONCLUSION

Colorectal cancer that has spread to the liver can be subclassified by looking at slides of the disease. Stroma rich cancer has poor outcomes following surgery. These tumours are often infiltrated by neutrophils with active characteristics. Modelling of this disease reveals neutrophils to be important in disease progression and inhibition of neutrophils with drugs leads to improvement in disease burden. This work has potential to translate to human disease by identification of neutrophil vulnerabilities and targeting via clinical trial.

RESEARCH TEAM & CONTACT

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Additional Information

Project completion December 2019. £25,000 received in support of project