2020 CRUK KHP Centre PhD Project Catalogue

Contents

Applying artificial intelligence techniques to digital pathology images and molecular data for cancer early detection

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Background: The presence of lymph node (LN) metastasis is one of the most important prognostic factors in breast and many other cancers, and the overall survival decreases as the number of cancerous (involved) LNs increases. Despite, a subset of these patients responds well to treatment and achieve long-term survival.
Most commonly, regional LNs are excised, histopathologically processed and examined by a pathologist, to determine if LNs harbour cancerous cells. Although tumour cell metastasis is often preceded by alterations in the microenvironment of the metastatic organ in preparation for the arrival of and an effective colonisation by malignant cells, little attention is given to cancer-free (uninvolved) LNs and the progression from an uninvolved LN to an involved LN. We were the first to report on morphological changes in the uninvolved LNs, being risk predictive of developing distant metastasis and are now elucidating the underlying biological and translational relevance of the pre-metastatic LNs.

Scientific Hypothesis: We believe that the pre-metastatic microenvironment of cancer-free (uninvolved) LNs offers an unexplored avenue for innovative therapeutic approaches to distinguish breast cancer patients with aggressive disease progression and those who we could spare unnecessary treatment. Our aims are to explore the different LN stages, starting from the normal tissue via the low-risk pre-malignant, then the high-risk pre-malignant towards the fully cancerous (involved) LN. By implementing machine learning / artificial intelligence (AI) methods for digital pathology image analyses, integrated with transcriptomics, multiplexed IHC, and FLIM-FRET of transcription factors, we aim to identify early indication of transitions indicative of potential cancerous spread to these small immune organs.

Experimental Plan:
1st year: Improve the implementation of AI and machine learning methods for image segmentation and feature identification. Developed methods will be compared with available software (e.g.: Visiopharm, ilastik) and the with manual ground truth, i.e. human pathologist signing off a diagnosis made by AI.

2nd year: Having access to LNs and primary tumour images of hundreds of breast cancers, developed methods will be applied to quantify morphological features within the LN, further refinement of the methods, and through association with disease progression will identify key features for prediction.

3rd and 4th years: For a subset of LNs, other data modalities, including RNA-seq, multiplexed IHC makers, and FLIM-FRET for transcription factors involved in B cell development will be studied. These results will be integrated with digital image analyses to shed light on the local immune responses, support AI interpretation and the determination of robust markers for early detection of LN metastasis.

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Integrative personalised modelling for patient stratification in myeloid malignancies

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Background: Myeloid malignancies such as MDS, MPN and AML are heterogeneous diseases with multifactorial pathogenesis. Response to therapy is significantly varied within these patients and a robust prediction model for patient stratification and response to (immune) therapies is an immediate clinical need. The aim of this project is to use a combination of clinical data, diagnostic tests, OMICS data and systems integrative analysis to create robust and comprehensive predictive models for response to therapy and disease progression in these diseases.
This PhD project benefits from interdisciplinary support (clinical, basic science and computational biology) and the student will have the opportunity to gain a variety of techniques in both wet-lab and dry-lab settings.
Moreover, the TOUR team of Dr Van Hemelrijck has extensive experience in management and analyses of big data so that the student will be working in a multidisciplinary translational research setting where clinical epidemiology and biostatistics will be applied.

Scientific hypothesis: Sufficient evidence exists to support the role of the 'immunome' as an important and independent factor in MDS and AML patient stratification. Nonetheless, immune responses against malignant clones require coordination between cell types and across tissues, and a systems-immunity screening approach is necessary to evaluate the overall 'immune fitness' in cancer, as previously shown[1].
We hypothesise that a collection of comprehensive omics datasets will leverage the development of a computational pipeline specific to myeloid malignancies, in particular MDS, MPN and AML, that will help to identify key features at various biological levels, their interconnectivity, and to better predict patient outcomes.

Experimental plan: We have access to peripheral blood and bone marrow samples from patients with MDS and AML at Guy's hospital for the prospective part of this research. Through our EU collaborators, we have also access to more samples which are already stored as part of past and current clinical trials.
Identify the Predictive immune signature(s): We will use a two-step approach, combining CyTOF and single cell RNA sequencing (scRNAseq) to identify immune signatures which predict response to therapy and/or disease progression. We will delineate relevant cell clusters, using our in-house pipeline for cell clustering (CytoClustr) which can be used for both CyTOF as well as scRNAseq data (https://github.com/kordastilab/cytoClustR)[2,3].
Data integration and modelling: Through working with the TOUR team, the student will learn database management/analyses to ensure that the diverse data are captured, protected, integrated, classified and analysed using the latest techniques and statistical methods (e.g. advanced survival analyses, latent class analyses)4.

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Tissue-resident macrophages and regulation of the stem cell niche

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Background

Macrophages are found in all tissues of the body where they play important roles in tissue remodelling and homeostasis. Macrophages were also among the first immune cells shown to modulate stem cell function. For example, mammary gland stem cells require signals from tissue macrophages during ductal morphogenesis, which was shown to be mediated by Wnt ligands produced by macrophages in response to Notch signalling between macrophages and mammary gland stem cells. We recently identified a subset of tissue-resident macrophages that co-express Tim4 and CD163. Upon specific depletion of these macrophages in a mouse model or ovarian cancer we observed a significant reduction in the frequency of tumour cells with cancer stem cell (CSC)-characteristics and the metastatic spread of disease.

Scientific hypothesis: This project will test the hypothesis that Tim4$^+$ CD163$^+$ tissue-resident macrophages represent an important component of the cancer stem cell (CSC) niche in mouse models of ovarian cancer and mammary gland carcinogenesis. We predict that specific axes of cross-talk between tissue-resident macrophages and cancer cells leads to the acquisition of stem cell characteristics that aid the metastatic spread of disease.

Experimental plan: This project will address 5 main research aims:

1. Fate-map resident Tim4$^+$ CD163$^+$ macrophages in mouse models of mammary and ovarian carcinomas and their association with CSCs.
2. Specific depletion of Tim4$^+$ CD163$^+$ macrophages and determine the effects of tumour progression and acquisition of CSC-characteristics.
3. Identify the molecular axes of cross-talk between resident tissue macrophages and cancer cells that promote stem cell characteristics.
4. Establish human iPSC-derived macrophages that reflect tissue-resident cells and assess their impact on stem cell functions in organotypic culture systems.

These studies will utilise new and unique genetic tools that have recently been developed in our laboratories to dissect the roles of Tim4$^+$ CD163$^+$ tissue resident macrophages and iPSC-derived human macrophages.

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Imaging replication stress and DNA damage response in cancer

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Background: The majority of cancer deaths result from ineffective treatment of metastatic disease. Currently, there is no satisfactory way to identify patients that will not respond to treatment. The identification of cancer patients that are refractory to the standard of care will allow the selection of alternative therapies that have the potential to improve patient response and survival.

One approach we have taken to visualise therapy resistance is through the imaging of the tumour antioxidant response, which acts to prevent treatment-induced oxidative stress. We are currently evaluating the positron emission tomography imaging agent, [^{18}F]FSPG (**Fig. 1a**), as a non-invasive marker of the tumour redox microenvironment (**Fig. 1b**). Acting as a surrogate for *de novo* glutathione biosynthesis (**Fig. 1c**), [^{18}F]FSPG tumour uptake is altered following oxidising chemotherapy prior to tumour shrinkage (**Fig. 1d**) [1]. Moreover, we have shown baseline [^{18}F]FSPG tumour uptake is predictive of response to standard chemotherapies [2].

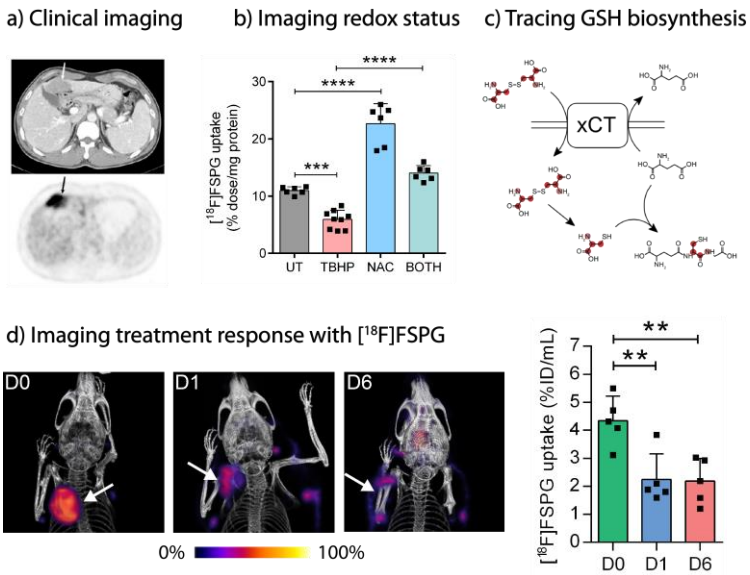

Figure 1. [^{18}F]FSPG is a sensitive marker of tumour redox status. a. [^{18}F]FSPG has been used for the clinical imaging of numerous malignancies. b. Treatment with either oxidising agents (TBHP) or reducing agents (NAC) results in marked changes in [^{18}F]FSPG tumour cell uptake, which were rescued by their combined treatment (BOTH). c. Redox-sensitive changes in [^{18}F]FSPG uptake is a result of altered rates of de novo glutathione biosynthesis mediated through the transporter xCT. d. [^{18}F]FSPG is an early marker of treatment response in vivo. Arrows indicate the tumour.

Scientific hypothesis: In highly proliferating tumour cells, a common consequence is the amplification of DNA damage and DNA replication errors. To ensure faithful inheritance of their genomes, mammalian cells have evolved a DNA damage response (DDR) to repair these defects. Inhibition of DDR pathways in tumours with DDR defects has a synthetic lethal effect [3]. Despite the success of these clinically-translated DDR inhibitors [4], there is no method to accurately determine which patients will respond to these therapies. Here, we will evaluate [18F]FSPG PET as a sensitive marker of the tumour redox environment and investigate its relationship to drug-induced replication stress.Using the refined markers identified from scRNA-seq data will perform more in-depth sequencing and further analysis TAM subsets, this data will be used for pathway analysis to reveal molecular targets and ultimately resolve the function of these subsets through functional studies using our genetic tools.

Experimental plan: As part of a multidisciplinary project, encompassing cancer biology, radiochemistry and the imaging sciences, the PhD student will: 1) Compare baseline [18F]FSPG uptake in vivo in lung tumours harbouring NRF2 mutations following treatment with the PARP inhibitor olaparib; 2) assess tumour response with [18F]FSPG to the DDR inhibitor VX-970, administered as a single agent and in combination with platinum in genetically-modified mouse models of lung cancer; 3) Understand the mechanisms that underpin differential [18F]FSPG uptake through the measurement of ROS, GSH utilisation and oxidative DNA damage, and determine whether baseline [18F]FSPG uptake is predictive of response; 4) use peripheral blood exosome and immune-modifying microRNA quantification to assess the unfolded protein response as a companion prognostic marker [5]. Together, this programme of research will provide an early non-invasive marker of DDR treatment efficacy in vivo. Identifying DDR drug resistance will inform patient management and second-line therapy selection, thereby improving patient outcome.

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