Validation of therapeutic targets using human pancreatic cancer organoids

Supervisory team: Axel Behrens (primary supervisor, Crick) and Debasish Sarker (King’s College London)

Pancreatic ductal adenocarcinoma (PDAC) is one of the most challenging cancers to treat, with unchanged 5 year overall survival of <5% for the last 30 years. Identifying new and targetable pathways in human tumours is an important goal in order to develop more effective therapies. Rspandin-based 3D tumour organoids derived from patient biopsies closely recapitulate several properties of the original tumour (Boj et al., 2015). Human pancreatic cancer tissue will be obtained from patients undergoing curative resection for PDAC at King's College Hospital, UK's largest pancreatic surgical centre. Here, we propose to use human pancreatic tumour organoids to develop and assess the efficacy of novel therapies.

Several potential therapeutic targets will be investigated, one of which will be the deubiquitinase USP28. The transcription factor c-Myc plays an essential role in pancreatic tumours, with amplification of c-Myc recently reported and associated with poor prognosis (Saborowski et al., 2014; Witkiewicz et al., 2015). Although c-Myc activity is difficult to inhibit directly, c-Myc protein is stabilized by the deubiquitinase USP28. We have previously shown that inactivation of USP28 in murine colorectal tumours reduced c-Myc protein levels and caused tumour regression (Diefenbacher et al., 2014). However, the role of USP28 in human pancreatic cancer is unknown.

We will use the CRISPR/Cas system to either inactivate the USP28 gene, or mutate the active site cysteine to mimic the effects of a pharmacological USP28 inhibitor. The effects of inactivating USP28 on PDAC will be quantified in vitro using tumour sphere growth, and in vivo by intrapancreatic xenografts of PDAC cells. The molecular function of USP28 in PDAC will be characterised using biochemical and genome-wide transcriptional analysis. As deubiquitinases are druggable enzymes, this work aims to validate inhibition of USP28 enzymatic activity as a strategy for PDAC therapy.

A similar combination of genetic and biochemical approaches will be used to investigate the function of other therapeutic targets.

The project will involve training in all aspects of cancer organoid biology and a wide range of modern molecular biology techniques.

References:

Characterisation of mesenchymal stroma cells in acute myeloid leukaemia patients

Supervisory team: Dominique Bonnet (primary supervisor, Crick), Tariq Enver (UCL) and John Gribben (Barts Cancer Institute, QMUL)

Acute Myeloid Leukaemia (AML) is a heterogeneous myeloid disease, which has long been considered a haematopoietic-cell autonomous disorder in which disease initiation, and progression is driven by haematopoietic cell intrinsic genetic events. Recent experimental findings in diverse model systems have challenged this view, implicating multiple stromal cells of the bone marrow (BM) in disease pathogenesis (1-5). Thus, deregulation of the BM microenvironment has emerged to be an important factor in the development of myeloid malignancies. Leukaemic cells can turn BM niches into “leukaemic niches”, which support malignant clones and impair the maintenance of normal Haematopoietic stem cells (HSCs). Thus the stem cell niche has recently emerged as an oncogenic unit and an important element in regulating cancer stem cells, including HSCs. Nevertheless, relatively little data come from directly analysing AML patients’ stroma.

This project aims at identifying candidates, clinically applicable, biomarkers of mesenchymal stromal cells perturbation in patients with AML.

Aim 1: Characterize the molecular and epigenetic profile of MSC in AML using single cell analysis. RNAseq and epigenetic profiling techniques for small to single cells available in supervisor 1 and 2's teams.

We will use for this two approaches:

1- establishment of mesenchymal stroma cells (MSCs) from the bone marrow of AML patients.
2- Sorting strategy to enriched in phenotypically defined MSC from the bone marrow of AML samples.

Advantages: The first approach will allow the evaluation of the proliferation kinetic, differentiation potential and the capacity of the MSC derived from AML patients to support both normal and malignant haematopoiesis ex vivo.

Pitfalls: Ex vivo culture and expansion of MSC might modulate the molecular profile of MSCs. We will thus use approach 2 to directly sort MSC without culture. We will compare results obtained by the two approaches.

Aim 2: Provide experimental proof of principle that MSC’s perturbation is implicated in the pathogenesis of AML and test whether therapeutic targeting of MSC could impede AML development.

We will test whether activation or down-regulation of specific pathways define in Aim 1 results in an increase proliferative advantage of leukaemia at the expense of residual normal haematopoiesis, using both ex vivo and in vivo 3D scaffold model available in supervisor 1’s team. Cooperation between a “modified” niche and aberrant leukaemic cells will be interrogered in transplant experiments. Lastly, we will also test whether therapeutically targeting of some of these “perturbed” pathways in AML-MSC can impede leukaemic development and restore normal haematopoiesis.

The over all goal of this project is to better define how malignant AML cells impede their stroma microenvironment to their own advantage and define new therapeutic tools to reverse these alterations in order to prevent the evolution of the disease and improve the survival and quality of life of the patients. Overall, this project will generate an invaluable resource of well-characterised mechanisms of niche alteration(s) paving the way for future development of predictive and preventive measures as well as potential innovative therapies.

References

Molecular mechanisms of lymphoma resistance to therapy

Supervisory team: Dinis Calado (primary supervisor, Crick), Jude Fitzgibbon (Barts Cancer Institute, QMUL) and Paul Fields (Guy's and St Thomas's NHS Foundation Trust)

Despite the success in Lymphoma treatment, a significant proportion of patients (~30-40%) are refractory to the current standard treatment (Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone; R-CHOP) or relapse at a later date (ref 1). Knowledge of mechanisms of resistance to R-CHOP is poor, impairing the medical decision to treat patients with alternative or combination therapies. To address this unmet need, the aim of this project is to develop an informative lymphoma mouse model system, which allows identifying and testing mechanisms of refractoriness and relapse to R-CHOP therapy.

1. PhD Year 1: Develop a robust caspase-based therapeutic model in lymphoma. The project to the appropriate intensity of first line of treatment.

2. PhD Year 2: Using an already established mouse model of lymphoma (Diffuse Large B cell Lymphoma, ref 2 and 3) generated in laboratory, the aim is to identify a therapeutic window, perform treatment of the lymphoma using the standard treatment, and investigate the genomic alterations present in refractory or relapsed disease versus non-treated controls using high-throughput sequencing (ref 3 and 4).

3. PhD Year 2-3: Investigate candidate alterations for R-CHOP treatment resistance by performing interspecies (human/mouse) lymphoma oncogenic and gene expression comparisons to identify candidate genetic and signalling pathway alterations for R-CHOP treatment resistance. To test candidate alterations, we will use lymphoma cell lines derived from tumours of our mouse model system under non R-CHOP treated conditions and modify them using CRISPR-CAS technology. Modified and un-modified lymphoma cells will be compared for their ability to form tumours in syngeneic animals, upon treatment or not with R-CHOP.

This project aims to identify and study molecular resistance mechanisms to R-CHOP in the presence of a functional immune system, allowing future testing of novel therapies such as immune-checkpoint blockade. We expect in addition to identify alterations that function as biomarkers to risk-stratify patients to the appropriate intensity of first line of treatment.

The project involves training skills in high throughput sequencing; bioinformatic analysis of high content data content; pathological characterisations of affected organs using immunohistochemistry and fluorescence in-situ hybridization; analysis of tumour cells by multicolor flow-cytometry; whole mouse body imaging techniques including PET and ultrasounds; genetic manipulation using CRISPR-CAS technology; and advanced knowledge of mouse models of disease.

References:
Understanding the influence of stromal fibroblasts on the immune-phenotype and therapeutic responses of head and neck cancer

Supervisory team: Erik Sahai (primary supervisor, Crick), Kevin Harrington (ICR) and Alan Melcher (ICR)

Background
The Sahai lab has a long-standing interest in how stromal fibroblasts modulate cancer phenotypes. We have previously shown how they promote the collective cancer cell invasion and modulate responses to targeted therapies (Gaggioli et al Nature Cell Biology 2007, Calvo et al Nature Cell Biology 2013, Chaudhry et al Oncogene 2013, Hirata et al Cancer Cell 2015, Derzsi et al under review). There is also growing interest in their ability to modulate the immune system and their ablation improves αCTLA-4 responses in a pre-clinical model of pancreatic ductal adenocarcinoma (Özdemir et al Cancer Cell 2014). Related to this, we have recently observed that direct contact between squamous cell carcinoma cells and cancer-associated fibroblasts triggers a dramatic increase in the expression of inflammatory cytokines and anti-viral genes. Thus the type and activity of the leukocytic infiltrate into tumours can be significantly influenced by stromal fibroblasts.

Project outline
In the proposed project we would like to explore the connections between stromal fibroblasts and leukocytic infiltrate in squamous cell carcinoma of the head and neck. In particular, we will perform detailed characterisation of stromal fibroblasts and the immune infiltrate in both treatment-naive disease and following therapeutic intervention. We will obtain serial samples from the same patient prior to and following conventional concomitant platin-based chemoradiotherapy and also new agents being trialled in window of opportunity studies being led by Prof. Kevin Harrington at the RMH. The ethical approval for the former is already in place and can be written into the latter trials as the protocols are developed. These analyses will then be used to generate specific molecular hypotheses that will be tested in experimental models.

The Sahai laboratory, in collaboration with Kevin Harrington at the ICR/RMH, has established cell culture models of HNSCC fibroblasts and developed methods to analyse their ability to modulate different leukocyte populations within PBMCs and are now developing ways to study tumour-infiltrating leukocytes. These experiments will combine therapeutic agents with molecular manipulations such as siRNA or Crispr to identify the mediators of cross-talk between different cell populations. Further, we are currently establishing syngeneic C57/BL6 oral SCC cell lines that can be grafted into either wild-type mice or mice with genetic manipulation of stromal fibroblasts (using Fsp-Cre or αSMA-Cre drivers) or leukocyte subsets. The growth, spread and therapy response of tumours would be monitored as endpoints. Taken together these approaches will lead to an enhanced understanding of how fibroblasts influence the immune-microenvironment in both treatment-naive and treated squamous cell carcinoma. This information will then feed into ongoing efforts to optimise therapies that exploit the immune system, including checkpoint inhibitors, TLR and other innate immune agonists (eg STING agonist, MK-1454), and oncolytic viral immunotherapies (eg T-VEC, Coxsackievirus A21, Newcastle disease virus - Donnelly et al J R Soc Med 2013).

References
2. Hirata et al - Cancer Cell 2015
3. Özdemir et al - Cancer Cell 2014
Modelling APOBEC3B mediated nucleic acid deamination with a view to cancer prevention and treatment

Supervisory team: Willie Taylor (joint primary supervisor, Crick), Kate Bishop (joint primary supervisor, Crick) and James Scott (Imperial)

Background
James Scott's group discovered that cytosine to uracil base modification can change the coding of nucleic acid. In collaboration with Willie Taylor's group they demonstrating that this nucleic acid editing activity was mediated by specific nucleic binding cytidine deaminases, which they traced back to a prokaryotic cytosine deaminase in E.coli through homology and molecular modelling. The enzyme was designated apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1, APOBEC1, exclusively after their reported findings.

This work led directly to the identification and characterisation of a family of enzymes that mediate cytosine to uracil modification in nucleic acid, and the alteration of its coding ability. These enzymes have been shown to have a canonical role in physiology and pathology; including blood lipoprotein metabolism, adaptive immunity by antibody formation, innate immunity against viruses, and a major cause of malignant transformation. They suggested and it was subsequently confirmed that the APOBEC family members concerned with antibody formation and viral inhibition operate, at least for the main part, by DNA deamination.

Kate Bishop has approached the APOBECs through studying their role in HIV restriction which involves several members of the APOBEC3 subfamily (first identified by Scott and colleagues), including APOBEC3B, and has accumulated considerable expertise that can now be redirected towards their role in cancer. She has extensive knowledge of the mutational profiles of APOBECs and has developed cell based and bacterial assays to study hypermutation.

Further studies by other scientists, including Harris, Neuberger and Stratton, now implicate the APOBEC3 mediated mutagenesis, including hyper-mutation in multiple common tumours, and “one of the most significant human carcinogens with prevalence superseding that of tobacco smoking and exposure to UV light” (Alexandrov & Stratton, 2014).

Project
Research on the APOBEC family of enzymes has expanded greatly from its original identification as an RNA editing enzyme. There are currently four major types, with the largest family (APOBEC3) having eight subtypes in humans, designated APOBEC3-A to H. Their activity has been identified with generating variation in the immune system, viral restriction and most recently, as an important factor in raising susceptibility to several, and perhaps most, types of cancer (Swanton et al., 2015).

In all these areas, and the latter in particular, a wealth of data is being generated (often using high-through-put sequencing methods) that has revealed much about the function and specificity of these enzymes. However, these data from different fields have not been integrated under a common model of APOBEC action. Given the clear similarity between the members of the family, there is good reason to assume that each member of the family will retain a core mode of action. Nevertheless, different members of the family have different combinations of domains, and the substrate can be either RNA or DNA. One possibility is that APOBECs bind nucleic acids in the context of nucleic acid polymerisation, for example at a transcription fork or during reverse transcription of retroviral RNA. Although structures are available for both amino and carboxy terminal domains, there is no known structure for any intact native enzyme and little is known about how the nucleotides are bound, beyond small co-crystalised fragments.

For this project, we propose to marshal all the available structural and sequence data across all the areas of APOBEC activity with a view to developing a common mode of action that may provide a broad therapeutic target. Our approach will be based on molecular modelling, including coarse-grained modelling of possible nucleic acid complexes, guided by existing mutational studies and an analysis of amino acids co-variation signals in multiply aligned sequences. Alternative models generated by this approach will be proposed to collaborators for testing using appropriate biological assays.
A potential outcome of this research may be the development of vaccination or drug strategies to prevent cancer or cancer recurrence, or limit the mutational diversity within tumours.

References

1. Ludmil B Alexandrov and Michael R Stratton
2. Charles Swanton, Nicholas McGranahan, Gabriel J. Starrett, and Reuben S. Harris
   APOBEC Enzymes: Mutagenic Fuel for Cancer Evolution and Heterogeneity. Cancer Discov; 5:704-12, 2015
4. Kate N. Bishop, Rebecca K. Holmes, Ann M. Sheehy, Nicholas O. Davidson, Soo-Jin Cho and Michael H. Malim
5. Adam Jarmuz, Ann Chester, Jayne Bayliss, Jane Gisbourne, Ian Dunham, James Scott and Naveenan Navaratnam.
The role of arginine and serine in tumorigenesis and its therapeutic exploitation

Supervisory team: Karen Vousden (primary supervisor, Crick) and Peter Szlosarek (Barts Cancer Institute, QMUL)

Metabolic deregulation is a key hallmark of tumorigenesis. Based on the successful paradigm of asparagine deprivation in the treatment of leukemia, additional amino acids are under the translational spotlight. The Szlosarek Lab has focused on epigenetic loss of the arginine biosynthetic enzyme, argininosuccinate synthetase 1 (ASS1) which has been described in various chemorefractory cancers. While loss of ASS1 in the liver leads to urea cycle disorders, loss of ASS1 in cancer cells can support tumour cell growth by promoting diversion of the precursor aspartate for nucleotide synthesis and enhanced mTOR activity [1,2]. Loss of ASS1 is seen in several tumour types, including half of all malignant pleural mesothelioma, a rare but highly aggressive tumour [3].

Recently, pharmacological depletion of arginine with the enzyme pegylated arginine deiminase (ADI-PEG20) has proven effective in the first randomized cancer trial in patients with ASS1-deficient mesothelioma [3]. Initial studies to understand resistance mechanisms have revealed a role for re-expression of ASS1 following demethylation of the ASS1 promoter and alternate metabolic pathways, involving polyamines and delivery of argininosuccinate by stromal cells. Given the role of increased nucleotide synthesis in cancers with depleted ASS1 expression, further clinical studies have been initiated using ADI-PEG20 in combination with antifolates, with the goal of more efficiently eliminating the tumour cells and avoiding the development of resistance [4].

Nucleotide synthesis depends on the one-carbon cycle, which is supported by serine, a focus of research in the Vousden group [5,6]. Like arginine, serine is a non-essential amino acid that can be synthesised de novo or taken up from exogenous sources. However, cancers showing up-regulation of the endogenous serine synthesis pathway (SSP) tend to show a dependence on this route of serine production, regardless of the availability of exogenous serine [7]. Intriguingly, we detected a reciprocal relationship between tumoural ASS1 deficiency and increase in expression of the SSP enzymes PHGDH and PSAT1 following ADI-PEG20 treatment. Conversely, some cancers have lost the ability to synthesize serine, making them highly dependent on exogenous serine.

We propose to explore the relationship between ASS1 depletion and serine synthesis, and to test the potential synergy between arginine limitation and PHGDH inhibition (using small molecule inhibitors) or serine starvation. Our end goal is to translate this into the clinic.

The project will involve:

1. Assessing the activity and importance of the de novo SSP in ASS1 deficient mesothelioma cells following arginine depletion
2. Determine whether ASS1 deficient tumour cells that do not increase SSP enzyme expression become more sensitive to withdrawal of exogenous serine
3. Understand changes in metabolism in response to ASS1 inhibition and/or arginine depletion with SSP inhibition and/or serine depletion
4. Develop preclinical mouse models to test the efficacy of combination therapies limiting arginine and serine availability.
5. Aim to develop clinical trials to test promising combinations.

References

1. Szlosarek. PNAS 2014
Metabolic stratification of tumours for chemotherapy

Supervisory team: Mariia Yuneva (primary supervisor, Crick), Robert Stein (UCL) and Gyorgy Szabadkai (UCL)

The overarching aim of our research programme is to understand the correlation between transcriptional regulation of nuclear encoded mitochondrial genes, cancer cell bioenergetics and tumour chemosensitivity. We use (i) unbiased approaches, including bioinformatic analysis of genome sequencing and gene expression data as well as (ii) high content functional imaging of metabolic function on cellular and patient derived organotypic cultures and xenograft mouse models. Overall we aim (i) to establish gene expression based biomarkers for metabolic stratification of tumours to predict tumour chemosensitivity, and (ii) to identify therapeutic targets in metabolic pathways to develop novel treatment strategies.

The PhD studentship will address one of the following two specific projects to be decided upon consultation with the supervisors.


Here we will validate a novel, predictive biomarker tool, which reveals fundamental biology of tumours thus can more effectively predict chemosensitivity and clinical outcome. We have developed a novel algorithm for a mitochondrial gene expression pattern (mGEP)-based scoring system informing on fundamental biological properties of luminal breast cancers. It effectively predicts the metabolic phenotype of breast tumours, which in turn informs on their pharmacological sensitivity. The method has been proved effective in breast cancer cell-line models. Accordingly, the principal objective of the project is to perform validation on human tumours. We aim to stratify breast cancers using mGEPs as biomarkers, and to develop novel mitochondria/metabolism targeting therapeutic protocols for the treatment of these tumours. We will:

a. Classify breast cancers according to their mitochondrial biogenesis patterns driven by specific transcription factors/nuclear receptors and co-regulators.
b. Verify the functional mitochondrial and metabolic phenotype in in vivo human tumour xenograft models using the biochemical and functional imaging platform (UCL) and metabolomics (Crick) facilities.
c. Identify the correlation between mGEP patterns and chemosensitivity using cellular and in vivo models.

II. Define the mitochondrial and metabolic contribution to the development of chemoresistance to platinum based chemotherapy of ovarian cancer.

We have previously shown that positive selection of mtDNA mutations and accompanying changes in mitochondrial-nuclear signalling and cellular metabolism contribute to the development of platinum resistant cancer. To develop further these findings and to identify therapeutic targets and biomarkers we will:

a. Describe the mutational landscape of mtDNA and nuclear genes encoding mitochondrial respiratory chain (RC) components in patient samples before and after development of chemoresistance in ovarian cancer.
b. Establish cellular and in vivo xenograft models of human chemoresistant cancers.
c. Analyse metabolic fluxes in the cellular and in vivo models.

We expect that these approaches will identify the target metabolic pathways to revert chemoresistance. In addition, we will establish the metabolic and genetic findings as biomarkers predicting the risk of developing chemoresistance

References
mediates adaptive chemoresistance associated with mitochondrial DNA mutations. Oncogene 2013, 32:2592-600
