CRUK Cambridge Centre MRes rotation project

Rotation Project Title	**Overcoming challenges of cell free DNA detection in kidney cancer**
Head of Laboratory (PI) Name	Grant Stewart
Second supervisor if applicable	Nitzan Rosenfeld
Programme	Urological Cancers
Supervisor's Email	Gds35@cam.ac.uk
Laboratory Location	Department of Surgery

Project Outline	**Aims and objectives** Surgery for solid malignancies detected at an early enough stage is the best method of curative treatment for most patients with the majority of cancers. Detection of cancer at an early stage is a challenge as often patients are asymptomatic; this is the case in kidney cancer. Non-invasive approaches would be the ideal method of cancer detection. So called 'liquid biopsies' meet these criteria and are potentially useful for providing patients with a likely prognosis following treatment as well as predicting response to different therapies. Cell free DNA (cfDNA) of tumour origin has been shown to be present in blood (circulating tumour DNA; ctDNA) and urine in certain cancers and has promise for real-time disease monitoring. In kidney cancer our initial work suggests ctDNA is detectable in some, but not all patients, even those with advanced disease (confirming other studies of kidney cancer [Bettegowda et al, 2014]). There are many possible reasons why detection levels are low: too little blood/urine analysed; very low quantities of DNA; DNA not shed into blood/urine; as well as technical limitations (Wan et al, 2017). In this student project we aim to probe the biological and technical reasons for variable detection of cfDNA in kidney cancer using an ex vivo model kidney cancer. In this model a kidney from a patient with kidney cancer is perfused with blood, and urine is produced. We plan to evaluate urine and blood from this model to identify levels of cfDNA present, allowing critical optimisation of this approach as a tool to allow an understanding of the kidney and its mechanisms of ct/cfDNA release into blood and urine. Also the model has enormous potential as a tool for developing/optimising ctDNA analytical methods as well carrying out pharmacogenetic studies. Our data will contribute to efforts to implement the liquid biopsy in the clinic and beyond kidney cancer.
Experimental plan	From consenting patients, following nephrectomy for kidney cancer, the cancerous kidney will be perfused with whole blood. Blood and urine will be sampled directly from the kidney to allow measurement of ctDNA and cfDNA in blood and urine close to the tumour. Various parameters that might influence detection of cf and ctDNA will be tested by this model, e.g. the volume of blood or urine necessary to overcome hard sampling limits. Tissue samples will be taken from the tumour and adjacent normal kidney tissue at the end of the experiment in order to measure representation of tumour lesions in the circulation. This is essential initial experimental work prior to developing further lines of experimentation using the model system.

Main Techniques	• experimental rig set-up and sample collection • ctDNA/cfDNA isolation and sequencing • tissue based DNA isolation and sequencing • bioinformatics
Key References	**Bettegowda et al**, *2014. SciTransMed, 6(224*) **Wan et al**, *2017. NatReviewsCancer, 17(4)*