The Westmead health precinct hosts a cluster of important institutions of medical science – major teaching hospitals and medical research institutes – affiliated with The University of Sydney, including:

Institute of Dental Research (IDR) is the oldest named dental institute in the world. Research at the IDR embraces innovative research through broad themes of chronic diseases combined with a deeper understanding of the impact of oral health on the whole body. The IDR’s research strengths include microbial pathogenicity, bioengineering and mucosal pathologies.

Kids Research Institute (KRI) at The Children’s Hospital at Westmead (CHW) KRI and the CHW Clinical School has approximately 300 medical researchers and support staff involved in research that covers a very wide spectrum of childhood diseases, divided into seven major research themes: neuroscience and mental health, cancer, infectious diseases & immunity, population health & health services research, genomic rare diseases, chronic diseases of childhood and clinical sciences.

The Children’s Medical Research Institute (CMRI) pioneered microsurgery, immunisations against lethal childhood illnesses and care for premature babies, all of which has improved the lives of countless Australian children over the last 50 years. Today, CMRI is the site of world-leading basic and translational research in the areas of cancer, neurobiology, embryology, genetics and gene therapy.

The Westmead Institute for Medical Research (WIMR) is one of Australia’s largest and most productive medical research centres, internationally recognised for its ground breaking work into many of the most significant diseases affecting humankind. WIMR’s more than 300 researchers investigate infectious and immune diseases, cancer and leukaemia, liver and metabolic diseases, eye and brain-related disorders and heart and respiratory diseases.

Westmead Hospital (WH) is a major teaching hospital of The University of Sydney for allied health, dental, medical and nursing students, and is one of Australia’s largest centres for postgraduate training at specialist level in all health fields. Westmead Hospital conducts biomedical, clinical and public health research in hospital-based centres and departments. As such, the research covers a broad spectrum including, but not limited to, laboratory, drug and device trials, and epidemiological studies. Each of these institutions hosts a critical mass of specialised scientists working on diverse, ground breaking research, and all increasingly are offering opportunities for Talented Student Program projects.

For more information on TSP projects at Westmead, contact: Dr Wendy Gold

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Identifying the misuse of nucleotide sequence reagents within biomedical research publications (2nd or 3rd year project).

Incorrect published research results waste resources directed towards scientific research, slow research translation, and reduce broader trust in science and use of the scientific method. This project investigates the hypothesis that incorrect use of nucleotide sequence reagents represents an underestimated source of error in the biomedical research literature. Our work has previously described a cohort of highly similar pre-clinical cancer research publications characterized by the frequent incorrect description and experimental use of nucleotide sequence reagents (such as gene knock down targeting sequences and PCR primers). To reliably and efficiently identify publications that include incorrect descriptions and use of nucleotide sequence reagents, this project will test and optimise a semi-automated tool to identify misused reagents within published papers. The project involves manual analysis of cohorts of publications that describe the use of nucleotide sequence reagents, and then cross-checking these results with those from automated screening. This will lead to suggested improvements in the automated method which can be engineered by collaborators in France. Publications that describe misidentified nucleotide sequence reagents can then be analysed to identify common features that may help to explain their origins.

Dr Caroline Royle (caroline.royle@sydney.edu.au), Centre for Virus Research, WIMR (3rd year, 12CP). Investigating the Interaction of HIV with the Interferon System

Dendritic cells and macrophages are one of the first cells to encounter HIV during sexual transmission. Normally during a viral infection these cells will secrete large amounts of IFN-γ, which helps to limit viral spread as well as activate other arms of the immune system. This lab however has previously shown that HIV directly inhibits the production of IFN-γ in both dendritic cells and macrophages by binding to a protein (TBK1) within the IFN signalling pathway. This project will further examine this interaction. We will be establishing and troubleshooting a co-immunoprecipitation assay to verify which HIV accessory proteins directly interact with TBK1; a protein involved in the IFN signalling pathway. Successful set up of this assay will then allow us to delve further into the specific domains of the TBK1 protein involved in this binding. There will also be the possibility of optimising a protocol for the generation of lentivirus expressing the TBK1 protein, which will be used in a future study to determine if we can reverse the ability of HIV to inhibit IFN-γ production in dendritic cells and macrophages.

You will learn: Cell culture techniques, DNA transfection, co-immunoprecipitation, SDS-PAGE and western blotting
Deciphering early cell fate commitment by 3D computational image analysis

This project will aim at deciphering early commitment of embryonic stem cells into endoderm. Several growth factors are generally used to direct the cells toward definitive endoderm such as Nodal and Activin. We want to compare the differences between these two members of the TGFβ superfamily by analyzing molecular events at the single cell level during cell specialization.

To achieve this goal, we will use embryonic stem cell reporter lines for endodermal genes (Foxa2 and Sox17) that we will differentiate toward the endoderm. In brief, the student will also generate CRISPR/Cas9 plasmids to edit genes of interest, then differentiate the stem cells into definitive endoderm to finally perform 3D image analysis by using newly developed microscopy (lightsheet technology).

The student will work closely with a PostDoc researcher and a Bioinformatician for the statistical analysis.

The research questions to be asked will be:
- Establishing exact timing of stem cell commitment into early cell lineages
- Assessing heterogeneity during specialization by statistical analysis at the single cell level
- Reconstructing temporal and spatial pattern of expression of lineage markers

Targeting structured DNA at telomeres to kill cancer cells

The continuous proliferation of cancer cells requires activation of a telomere lengthening mechanism to compensate for normal telomere shortening. In many cancers, telomeres are lengthened by the enzyme telomerase, while the remainder use a homologous recombination-dependent DNA synthesis mechanism, Alternative Lengthening of Telomeres (ALT). To develop treatments for ALT cancers, we aim to identify small-molecule drugs that exploit vulnerabilities in cells that use ALT. Loss of expression of the chromatin-remodelling protein ATRX is a hallmark of ALT cancers and cell lines. ATRX facilitates DNA replication and has been hypothesised to localise to four-stranded DNA structures known as G-quadruplexes (G4). Using a recently developed G4-specific antibody, we have made the exciting discovery that G4-stabilising small molecules cause a dramatic increase in the occurrence of these structures at telomeres and across the genome of ALT cells. In this project, the student will carry out experiments to determine the role of G4 DNA in the genome instability that is characteristic of cancers with ALT. The data will contribute to a body of work that we are planning to publish soon. Specific questions to be addressed are:

1) Do ALT cells show greater stabilisation of G4 across their genomes than non-ALT cells?
2) Do the G4 in ALT cell genomes involve RNA:DNA hybrids?
3) Do G4 ligands preferentially kill cancer cells using ALT?

Methods used:
1) Culture of human cancer cells
2) Fluorescence microscopy techniques: immunofluorescence and fluorescence in situ hybridisation (FISH)
3) Quantitation of images from fluorescence microscopy
4) Cell viability assays
Economic evaluation of gene panel approach versus traditional investigations in children with epilepsy (2nd or 3rd year project)

Routine diagnostic evaluation of refractory epilepsy in infants and children is extremely challenging in clinical practice as it involves performing a large number of metabolic and imaging investigations to identify the cause. Many of these tests require hospital admission and general anesthetics, which are costly and have health risks. Genetic causes are important cause of epilepsy and identification of genetic causes using early use of gene panel testing avoids diagnostic odyssey thereby reducing need for investigations and costs associated with it. Several studies on targeted gene panels of 35–265 genes have been reported in the literature with diagnostic yields varying between 10% and 48.5%. There is limited information available on cost benefits of doing the gene panel approach versus traditional evaluation in children with epilepsy. Identification of cost benefits would be helpful for planning diagnostic approach in children with epilepsy thereby facilitating economic use of limited resources. **Research Aim:** To compare the cost of investigations in children with epilepsy who underwent early gene panel testing with those evaluated with traditional investigations.

**Methodology:** Using epileptic encephalopathy database, we identified 105 children who underwent gene panel testing using MPS testing between January 2014 and September 2016 at The Children's Hospital at Westmead (CHW). The yield of genetic testing was 28.5%. We performed preliminary analysis in 19 patients and cost of evaluation was cheaper using gene panel approach compared to traditional evaluation. We are planning to extend the study to analyse all patients who underwent gene panel testing. All patients underwent range of diagnostic evaluation prior to enrolling in gene panel testing. A retrospective review of medical records will be performed to identify diagnostic tests and the procedures patients underwent and will be grouped into 4 categories as discussed below: 1) metabolic tests including CSF studies (first line and second line), 2) neuroimaging, 3) admissions and procedures, 4) presurgical evaluation including video telemetry monitoring for surgical indications, PET and SPECT and 5) other genetic testing. The costing information on investigations will be accessed through the clinical costing centre in Management Support Analysis Unit at our institute as done before with preliminary analysis. The cost of investigations will be compared in the patients that were investigated with gene panel approach with those that underwent traditional investigation. We hope that this study will identify the cost effective approach to investigation of patients with epilepsy.
Correlation of cell height with cell stiffness by atomic force microscopy

There is increasing interest in the relationship between mechanical cell stiffness and cellular functions. We recently published ‘stiffness fingerprints’ as a nuanced Atomic Force Microscopy method for cell stiffness characterization. This project will use stiffness fingerprints at the single cell level, to examine the relationship between individual cell height and cell stiffness. Cell stiffness appears to play a role in malignant cell metastasis and invasion. We recently published a novel approach to characterization of cell stiffness by atomic force microscopy, that generates ‘stiffness fingerprints’. Our purpose in developing this nuanced method for stiffness evaluation, was to examine the possible role of cell stiffness in inter-cellular exchange of cytoplasm, a previously unrecognized biological process. Our more recent data, suggests that cells with low stiffness are more likely to receive cytoplasm from exchange partners. In addition, we have found that exchange is correlated with increased cell height. The question that emerges from our current data, is if cultured cells that have greater cell height, also have inherently lower cell stiffness, or if the exchange process is responsible for the increased cell height seen. The current study will examine this question, by determining stiffness fingerprints for a range of cultured cells, correlating cell height with stiffness at the single cell level.

Establishing a bi-national registry/database for Biliary Atresia

The aim of this project will be to help design and construct and online database to monitor the outcomes of Biliary Atresia in both New Zealand and Australia. Biliary atresia is a condition in which the bile ducts fail to form, leading to progressive liver dysfunction and ultimately liver failure. Biliary atresia is still the most common reason for liver transplantation in children. Surgery to correct this condition is possible if done within the first 100 days of life. The success is still quite variable depending on the age of the patient and the experience of the treating surgeon and hospital. In the United Kingdom the treatment of Biliary atresia has been regionalised to 3 centres due to evidence linking volume of cases performed and surgical expertise to successful treatment. National registries have been established in the UK, and Canada. These databases enable monitoring of outcomes and improvement in the treatment of Biliary atresia in those countries.

The aim of this project will be to help design and construct and online database to monitor the outcomes of Biliary Atresia in both New Zealand and Australia. It will be the first multicentre paediatric surgical database to be established in Australasia. The data base will be designed using REDCap, and online database research tool. The project will also entail retrospectively populating this database with cases from the Children’s hospital (the highest volume centre in Australia and New Zealand for paediatric liver transplants) over the last 10 years.
Three dimensional characterization of canalicular fragmentation in apoptotic endothelium

We earlier discovered that apoptosis in vascular endothelial cells is unique, in that the plasma membrane undergoes extreme invagination to form an extensive network of canaliculi. The current project is to firstly fully characterize canalicular fragmentation by serial section scanning electron microscopy, and to then explore the effects of canalicular fragmentation on cell stiffness by atomic force microscopy. When we first observed ‘canalicular fragmentation’, we felt it likely that this was a mechanism for reducing micro-emobolism and micro-thrombosis. We have published data supporting this idea, but despite this, little remains known of this process or it’s true biological function. Recent developments in three dimensional reconstruction by serial section scanning electron microscopy, as well as in atomic force microscopy, raise the possibility for more complete characterization of canalicular fragmentation, both with regard to structural features and the functionality of this process. Human endothelium will be cultured, and apoptosis induced by combined serum starvation and deprivation of adhesion. Cells will be harvested and embedded for serial sectioning with scanning electron microscopy, and in-silico three dimensional reconstruction used to properly define the morphology of canalicular fragmentation in individual cells. Dependent on time, atomic force microscopy will be used to correlate canalicular fragmentation in individual cells with cell stiffness.

Cross-species analysis of serum albumin with reference to the possible anti-apoptotic domain for endothelial cells (2nd or 3rd year project)

We earlier published that serum albumin, the major blood protein, contains a cryptic domain that mediates anti-apoptotic activity in vascular endothelial cells. The current project is to perform a structural analysis across species to help identify the likely active protein domain. Endothelial apoptosis is important for microvascular remodeling during growth, development, wound healing and cancer, and probably plays an important role in atherosclerosis and both diabetic and hypertensive vasculopathy. Our earlier work demonstrated a potent anti-apoptotic activity in serum albumin for vascular endothelium, and data indicates a likely putative active anti-apoptotic protein domain. The current project is to further explore the possible location of this protein domain by structural analysis across species, on the assumption that more highly conserved domains are more likely to responsible for the activity under study. This will involve comparisons of serum albumin protein sequence data, as well as structural three-dimensional analysis using SPDB protein viewer software. In this way, amino acids will be considered with regard to their type, as well as with reference to their surface topology. This will form the basis for further work using molecular expression systems to explore the mechanisms for the anti-apoptotic activity.
**A/Prof Ky-Anh Nguyen (Ky-anh.nguyen@sydney.edu.au), Faculty of Dentistry, Institute of Dental Research, Westmead (2nd or 3rd year project)**

**Characterisation of Porphyromonas gingivalis RgpB CTD-junction mutants**

RgpB is an important virulence protease from the periodontal pathogen Porphyromonas gingivalis. Maturation of RgpB is dependent on correct processing of its C-terminal domain (CTD). A series of site-directed mutants of the CTD-cleavage site have been created to define critical motif for processing. This project will characterise these mutants for their RgpB phenotype. The project requires learning to culture the RgpB mutants in an anaerobic chamber using microbiological techniques and to harvest bacteria for analysis. Analysis will involve subcellular fractionation of each mutant to define the partitioning of RgpB protease by Western blot and through enzymatic assays.

**A/Prof Ky-Anh Nguyen (Ky-anh.nguyen@sydney.edu.au), Faculty of Dentistry, Institute of Dental Research, Westmead (2nd or 3rd year project)**

**Recombinant production of PorT from Porphyromonas gingivalis**

PorT is an essential outer membrane protein for the secretion of virulence factors from the periodontal pathogen Porphyromonas gingivalis. Its structure and function is currently unknown. This project will determine the optimal condition for recombinant production of this protein for further structural and/or functional studies. The gene for PorT has been cloned into an expression plasmid pET20b(+) in E. coli. Expression is controlled by the T7 promoter system inducible by the lac operon. Initial analysis suggests that this protein is toxic in E. coli. To minimize effect of toxicity during heterologous expression in E. coli, a defined medium will need to be used to minimize the presence of lactose which can induce leaky expression of the protein. This project will aim to optimize the growth condition in the defined medium and to determine the optimal condition for expression and affinity chromatography purification of the protein.

**Dr Samantha Ginn (sginn@cmri.org.au), CMRI, Gene Therapy Research Unit (2nd or 3rd year project, 12 CP)**

**Evaluation of genome editing reagents for gene therapy targeting primary hepatocytes in vivo**

Momentum surrounding the use of vectors based on adeno-associated virus (AAV) for gene therapy applications in the liver has increased dramatically, with efficacy demonstrated in patients with haemophilia making hepatocytes a compelling therapeutic target. Our group is therefore exploring genome editing strategies that target primary hepatocytes in vivo using AAV-mediated delivery of CRISPR/Cas9 to enhance homology-directed repair of human liver loci. The project will explore strategies for inhibiting the non-homologous end joining (NHEJ) pathway to increase the efficiency of CRISPR/Cas9-mediated genome editing by homology-directed template repair. Potential strategies include drug treatment or the use of small-molecule inhibitors at the time of treatment. This project will screen genomic DNA extracted from treated mice for evidence of a single nucleotide replacement and compare this to the rate observed in untreated animals. This will be achieved by PCR amplification across the modified locus, cloning of PCR amplicons and restriction digestion followed by confirmation using Sanger sequencing. Depending on time, there is the potential to learn the techniques of molecular cloning, DNA transfection, AAV vector production and titration by quantitative PCR.
Dr Kerrie Sandgren (kerrie.sandgren@sydney.edu.au), Centre for Virus Research, The Westmead Institute

Profiling non-human primate skin for the development of an in vivo model for skin vaccination. (3rd year project, 12 CP)

Most vaccines today are injected into the muscle, however the skin is now being considered as an ideal site for vaccination due to its far denser network of the immune cells that are stimulated by vaccines. This project will focus on profiling the anatomy of macaque skin in order to build a foundation for the development of an in vivo non-human primate (NHP) model for skin vaccination. We will examine the anatomy and populations of resident immune cells in macaque skin and compare this to human skin. This information is essential for the adaptation of vaccine delivery devices for the skin, such as microneedle patches, for use in NHPs, which may be critical for accurate preclinical trials of future vaccines. There are two main parts to this project: 1) examination of macaque skin biopsies that we already have in the lab by fluorescence microscopy and quantitative analysis of this data; 2) analyzing skin by flow cytometry to look at the phenotype and quantity of immune cell subsets. The latter will be optimised on human skin samples, which we receive regularly from plastic surgery, for later application to macaque skin, which we receive intermittently and unpredictably.

You will learn: microscopy techniques – staining, microscope operation, image analysis; how to process tissue into single cell suspensions for flow cytometry; flow cytometry techniques – staining, data acquisition and analysis.

Dr Naisana Seyedasli (Naisana.seyedasli@sydney.edu.au), Discipline of Life Sciences, Faculty of Dentistry, Westmead Hospital (2nd or 3rd year, 12 CP).

Study of epithelial mesenchymal plasticity in human epithelial carcinoma

This project uses human head and neck cancer cell-derived spheroids as a model to understand the cellular and molecular dynamics of EMT during tumour homeostasis and in response to radiation therapy. The concept of tumour cell heterogeneity has attracted a large degree of attention as a source of differential tumour behaviour in response to stimulants and drugs. One aspect of cellular heterogeneity is adoption of a range of phenotypes along the epithelial-mesenchymal axis. In this project, human oral squamous carcinoma cells will be used to generate tumour spheroids. The spheroids will then be used to dissect the molecular pathways involved in tumour homeostasis, and in response to radiation as a mode of therapy.

Dr Naisana Seyedasli (Naisana.seyedasli@sydney.edu.au), Discipline of Life Sciences, Faculty of Dentistry, Westmead Hospital (2nd or 3rd year, 12 CP).

Understanding the molecular players of chemotherapy resistance in human epithelial carcinoma

This project uses chemotherapy-resistant human epithelial carcinoma cells to study the pathways that act upstream of chemo-resistance and will look at the molecular signatures of resistant and naïve sub clones within tumour cells. Resistance to chemotherapy is a major prognostic challenge to majority of cancer types, including epithelial carcinoma. In this project, platinum-resistant human ovarian carcinoma cells will be used as a model to dissect the molecular pathways that act upstream the chemo-resistance compartment. Tweaking these pathways will provided means to generate more homogenous responses to chemotherapy, hence potentiating better post-treatment prognosis.
A/Prof Julie Redfern (julie.redfern@sydney.edu.au) Westmead Adults hospital (2nd or 3rd year project)

Investigating the appropriateness and effectiveness of eConsent forms in low risk clinical trials
This project has a couple of components: clinical, ethical and practical. The student will be required to perform a background review on eConsent, identify barriers and facilitators, liaise with members of the ethics team in regards to elements of the project and look at the various options of obtaining eConsent. eConsent (Electronic-Consent) is an evolving platform for consenting patients using a computer based consent form rather than traditional paper documentation. For this study the student would collaborate with an expert group with experience in creating PISCFs and ethics requirements. The student will compile a background review at the start of the project to provide a description of current research on eConsent, possible applications and the design for a small RCT testing the effectiveness and appropriateness of eConsent using a current PISCF of a low risk clinical trial. The student will be required to look into various options of obtaining eConsent, including RedCap data management, PDF file maker and other options. Finally, they will also be involved in the design of a consumer feedback survey aiming to measure suitable outcome measures such as user-friendliness, participant understanding and time spend to complete the consent process.

Prof Clara Chow (cchow@georgeinstitute.org.au) The George Institute for Global Health/University of Sydney (2nd or 3rd year project)

Customising Text Messaging Program for participants from non-English background
This project is part of a RCT evaluating a text messaging program on lifestyle in people with chronic disease. The student will be required to familiarize themselves with the protocol and identify potential ways of implementing the program in different languages. Ideally this student is fluent in Mandarin or Arabic. Text messaging has been shown to be effective and simple and our group has developed several text message programs that promote lifestyle change. All our programs to date have been delivered in English, however we are keen to explore the option of delivering the messages in other languages, including Mandarin and Arabic. The student will be required to familiarise themselves with the protocol and identify possible ways and barriers of including a text message program in a different language. We aim to implement the program in approximately 25 people and at the completion of the trial we will organise focus groups and in-depth interviews to evaluate the program. The student will collaborate with the team in the design of a small study to test the acceptability of the text message program in a language other than English and assist with the ethics application, writing of study documents and final analysis.