The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title:	Liquid biopsy to monitor clonal frequency and emergence of resistance mutations in common drivers of paediatric cancers.

SUPERVISORY TEAM	
Primary Supervisor(s):	Dr Mike Hubank
Other supervisory team members:	Dr Andrea Sottoriva Professor Louis Chesler

DIVISIONAL AFFILIATION	
Primary Division:	Molecular Pathology
Primary Team:	Centre for Molecular Pathology and Clinical Studies

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

At ICR and The Royal Marsden Hospital, we have developed and are conducting some of the first clinical trials of precision medicines in relapsed paediatric cancers, using drugs that target kinase domain mutations in ALK and increased expression of the MYC-related transcription factor MYCN. Aligned to these trials, ICR/RM is the designated genomics hub for prospective multi-omic characterisation of blood and tumour tissue from paediatric patients enrolling on these trials (the Stratified Medicine Paediatrics, SMPaeds initiative). In the current project, we are seeking to further develop and implement “liquid biopsy! Using high-sensitivity genomic and digital PCR techniques capable of measuring picogram quantities of circulating (tumour-derived, ctDNA) as a surrogate biomarker of treatment response, and capable of detecting emergence of treatment-resistant tumour clones. Specifically, we will focus on implementing high-throughput, deep-sequencing based quantitation of multiple target mutations (on NextSeq, as compared to digital PCR) combined with digital error suppression (iDES) to efficiently characterise levels of candidate mutations in blood in comparison to paired tumour-tissues and their variance with treatment course. This will be done using the unparalleled expertise present within the Centre for Molecular Pathology, which is the designated prospective clinical testing hub for SMPaeds. There will be a specific focus on monitoring the on-treatment emergence of resistant clones in patients who are undergoing precision treatment using targeted inhibitors of ALK (anaplastic lymphoma kinase) on active clinical trials

PROJECT AIMS

- **Characterise the incidence and spectrum of common oncogene mutations in tumour-tissue and blood from patients treated at The Royal Marsden Hospital, and on the national prospective genomics biomarker testing platform SMPaeds.**
- **Cross-compare the utility of ddPCR and NextSeq-based deep-sequencing for use in liquid-biopsy**

- **Use liquid biopsy of ALK-mutations to monitor treatment-response in patients enrolled on clinical trials of ALK inhibitors.**
- **Monitor the clonal evolution of resistant ALK mutations following targeted ALK inhibition on clinical trials of ALK inhibitors.**

RESEARCH PROPOSAL

Background.

Cancer remains the most common cause of death in children. Of all cancers, solid tumours such as neuroblastoma (nerve), medulloblastoma (brain) and rhabdomyosarcoma (muscle) are the most deadly, the least treatable and the most likely to recur and metastasize (Pizzo and Poplack, 2011). Few novel treatments are on the horizon for the unfortunate group of children diagnosed with these cancers, and our lack of understanding about how common genomic changes drive tumour-development or treatment resistance is a major impediment to development of improved treatments (Moreno et al., 2017). This is particularly sad because in paediatric cancers, the degree of genomic complexity is lower than that of adult cancers, making the potential impact of individual genomic events such as copy number gain (gene amplifications, CNVs), deletions (indels), single nucleotide changes (SNVs) and gene fusion/translocations, on disease development and treatment response much greater than that of adult cancers. By extension, the degree to which children could benefit from “precision medicines” that target individual genomic changes may be much higher.

In children, tumour biopsy is dangerous and requires imaging and anaesthesia, so that it is not routinely performed. Lack of any practical way to precisely determine the genomic changes present in each child’s cancer is a major roadblock that has prevented us from delivering precision treatment to children with cancer. Recently, high-throughput sequencing of tumour-derived nucleic acids has been applied to circulating DNA/RNA present in blood or exosomes (Combaret et al., 2015, Whale et al., 2017), and rapid/reliable testing of common paediatric cancer mutations in blood is underway.

At the Royal Marsden Hospital/ICR we are a major UK site for discovery/targeting of paediatric cancer mutations, and for precision medicine clinical trials using targeted inhibitors of ALK (anaplastic lymphoma kinase, Crizotinib, Ceritinib, Lorlatinib), MYCN, PI3K/mTOR pathway, RAS/MAPK and other major cancer signaling pathways. We are national reference centre for the new paediatric cancer biomarker testing platform (Stratified Medicine Paediatrics, SMPaeds, Chesler, PI), that will use multi-omics to determine the genomic composition of each tumour so that patients can be assigned to targeted therapy in clinical trials. The Centre for Molecular Pathology (CMP, Royal Marsden) is a clinical-grade sequencing facility at the heart of SMPaeds that is deploying novel tumour- and blood-based multi-omic testing to use in paediatric cancer clinical trials.

In this project, aligned to SMPaeds and benefitting from the unique expertise and biopsy tissue in-place within the CMP facility (Mike Hubank, lead), we will implement high-throughput multi-omic approaches to rapidly and sensitively detect the presence of common genomic changes in blood (ctDNA). We will use this information to characterise the frequency of such mutations in paediatric solid tumours, determine whether targetable mutations are clonally expanded at time of relapse, and assess whether resistance to targeted therapy is a result of clonal evolution detectable in blood.

Aims.

A1. Use a cohort of matched tumour- and liquid-biopsy derived DNAs, obtained from paediatric cancer patients treated at The ICR/Royal Marsden Hospital, to determine levels of ctDNA present at time of diagnosis and relapse, and to standardise the optimal methodology suitable for determination of ctDNA changes.

A2. Establish the frequency of common paediatric mutations (gene amplifications/CNVs, point mutation/SNVs, fusions and indels present in tumour tissue from patients with the three major paediatric solid tumours (neuroblastoma, medulloblastoma and rhabdomyosarcoma) present at diagnosis and relapse, using matched tumour-blood samples and paired diagnostic:relapse cohorts.

A3. Establish whether clonal evolution of ALK (anaplastic lymphoma kinase) mutations drives treatment resistance in patients on active treatment with ALK inhibitors (Crizotinib, Lorlatinib, NANT-Lorlatinib and ITCC-CRISP trials, https://clinicaltrials.gov).

Approach.

Robust and reliable determination of point mutations (such as in ALK) and copy number gains (such as in MYCN-amplification) in children has been reported using digital PCR (ddPCR) (Kurihara et al., 2015, Combaret et al., 2015), which we have also established at RMH-CMP. Using the unique cohort of paired tumour:blood DNAs available at RMH, and through SMPaeds we will compare the detection limits and accuracy of ddPCR against newly deployed -omics technologies available within the CMP. This will be done within a clinical grade-facility of the CMP, with access to core expert staff, with the aim of adapting existing commercial deep-sequencing-based and targeted (*Capp-seq*) (Bratman et al., 2015, Newman et al., 2014)approaches to use on paediatric clinical samples. We have access as a testing centre to several Targeted and Expanded ctDNA assay platforms developed in the commercial setting and will adapt these as needed to construct a robust approach that is cost-effective and sensitive, allowing rapid-turnaround clinical reporting on multiple genomic targets. Our initial pilot testing shows comparable sensitivity to ddPCR for a small number of pediatric, GI and Breast cancers, with detection of known variants down to 0.1% VAF. Sequencing of 8 or 16 samples/run will be performed on the Nextseq High using output runs at 4000 – 10,000 X depth of cover. Analysis is done on a dedicated server aligned to the ICR bioinformatics and scientific computing infrastructure. We will use digital error suppression (iDES) to efficiently characterise levels of candidate mutations in blood and lower background (Newman et al., 2016). Focused studies will target: 1) a comparison of allele frequencies in tumour:blood DNA matched tissues, 2) a comparison of allele frequencies and clonal heterogeneity in matched diagnostic:relapse tumour:ctDNA tissue pairs, and 3) serial study of on-treatment ctDNA samples (compared to relapse biopsy tumour tissue) for clonal frequency of ALK mutations.

Expected Outcomes.

- A robust *Capp-Seq* based and iDES-linked assay of ctDNA applicable to high-throughput analysis of genomic changes in paediatric cancer tissues and blood.
- Data and publications describing the levels of ctDNA and frequency of expected genomic events in paediatric solid tumours at time of diagnosis and relapse (neuroblastoma, medulloblastoma, rhabdomyosarcoma), from the RMH and SMPaeds national clinical patient cohorts.

- Focused study of the efficacy of ALK-targeted therapy in patients actively receiving ALK-inhibitor therapy with Crizotinib and Lorlatinib, on the NANT-Lorlatinib and ITCC-CRISP clinical trials.

Impact and Output.

This translational and patient-focused study will contribute biomarker assays that permit direct measurement of the genomic events that drive the development, relapse, and treatment sensitivity of paediatric solid tumours. RMH-CMP and The Institute of Cancer Research are in a leading position in the development of advanced biomarker technology, and in clinical trials of precision medicines for children with cancer. This will have a direct and hopefully measurable impact on survival of children with difficult-to-treat relapsed cancers.

LITERATURE REFERENCES

BRATMAN, S. V., NEWMAN, A. M., ALIZADEH, A. A. & DIEHN, M. 2015. Potential clinical utility of ultrasensitive circulating tumor DNA detection with CAPP-Seq. *Expert Rev Mol Diagn,* 15**,** 715-9.

COMBARET, V., IACONO, I., BELLINI, A., BREJON, S., BERNARD, V., MARABELLE, A., COZE, C., PIERRON, G., LAPOUBLE, E., SCHLEIERMACHER, G. & BLAY, J. Y. 2015. Detection of tumor ALK status in neuroblastoma patients using peripheral blood. *Cancer Med,* 4**,** 540-50.

KURIHARA, S., UEDA, Y., ONITAKE, Y., SUEDA, T., OHTA, E., MORIHARA, N., HIRANO, S., IRISUNA, F. & HIYAMA, E. 2015. Circulating free DNA as non-invasive diagnostic biomarker for childhood solid tumors. *J Pediatr Surg,* 50**,** 2094-7.

MORENO, L., PEARSON, A. D. J., PAOLETTI, X., JIMENEZ, I., GEOERGER, B., KEARNS, P. R., ZWAAN, C. M., DOZ, F., BARUCHEL, A., VORMOOR, J., CASANOVA, M., PFISTER, S. M., MORLAND, B., VASSAL, G. & INNOVATIVE THERAPIES FOR CHILDREN WITH CANCER, C. 2017. Early phase clinical trials of anticancer agents in children and adolescents - an ITCC perspective. *Nat Rev Clin Oncol,* 14**,** 497-507.

NEWMAN, A. M., BRATMAN, S. V., TO, J., WYNNE, J. F., ECLOV, N. C., MODLIN, L. A., LIU, C. L., NEAL, J. W., WAKELEE, H. A., MERRITT, R. E., SHRAGER, J. B., LOO, B. W., JR., ALIZADEH, A. A. & DIEHN, M. 2014. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med,* 20**,** 548-54.

NEWMAN, A. M., LOVEJOY, A. F., KLASS, D. M., KURTZ, D. M., CHABON, J. J., SCHERER, F., STEHR, H., LIU, C. L., BRATMAN, S. V., SAY, C., ZHOU, L., CARTER, J. N., WEST, R. B., SLEDGE, G. W., SHRAGER, J. B., LOO, B. W., JR., NEAL, J. W., WAKELEE, H. A., DIEHN, M. & ALIZADEH, A. A. 2016. Integrated digital error suppression for improved detection of circulating tumor DNA. *Nat Biotechnol,* 34**,** 547-555.

PIZZO, P. A. & POPLACK, D. G. 2011. *Principles and practice of pediatric oncology,* Philadelphia, Wolters Kluwer/Lippincott Williams & Wilkins Health.

WHALE, A. S., DEVONSHIRE, A. S., KARLIN-NEUMANN, G., REGAN, J., JAVIER, L., COWEN, S., FERNANDEZ-GONZALEZ, A., JONES, G. M., REDSHAW, N., BECK, J., BERGER, A. W., COMBARET, V., DAHL KJERSGAARD, N., DAVIS, L., FINA, F., FORSHEW, T., FREDSLUND ANDERSEN, R., GALBIATI, S., GONZALEZ HERNANDEZ, A., HAYNES, C. A., JANKU, F., LACAVE, R., LEE, J., MISTRY, V., PENDER, A., PRADINES, A., PROUDHON, C., SAAL, L. H., STIEGLITZ, E., ULRICH, B., FOY, C. A., PARKES, H., TZONEV, S. & HUGGETT, J. F. 2017. International Interlaboratory Digital PCR Study Demonstrating High Reproducibility for the Measurement of a Rare Sequence Variant. *Anal Chem,* 89**,** 1724-1733.

CANDIDATE PROFILE	
Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)	
Pre-requisite qualifications of applicants: e.g. BSc or equivalent in specific subject area(s)	BSc in biology or tumour biology, exposure to genomic and sequencing technologies, ideally an interest in paediatric cancer, excellent skills in data-analysis and interpretation

Intended learning outcomes:

- Knowledge in handling of biopsy tissues, DNA, RNA to rigorous clinical standard
- Knowledge of modern high-throughput –omics technology and digital PCR
- Basic and advanced techniques of data interpretation and bioinformatics computation
- Understanding of genomic alterations and driver mutations in cancer – techniques in interpretation of clonal frequency and clonal evolution
- Fundamental understanding of principles of precision-medicine, targeted cancer trials and therapy