End of Grant Report

Dr Sally George. Report at the end of the grant by Christopher's Smile for the 3 year position of Clinical research Fellow in the Paediatric Solid Tumour Biology Team at The Institute of Cancer Research

Christopher's Smile

coz kids get cancer too

Registered Charity 1129906

Contents

1. Position

Title	Clinical Research Fellow in the Paediatric Solid Tumour Biology Team
Role Description	
Start of Grant	2014
Period of Grant	3 Years
Name(s) of person in position	Dr Sally George
Grant funding over period of grant	£240,000
Matched funding over period of grant	£70,320

Overview of role during grant period

For poor outcome paediatric solid tumours such as medulloblastoma, glioma, neuroblastoma and sarcoma survival has not improved in recent decades despite intensification of conventional, often toxic therapies. There is wide consensus in the paediatric oncology community that in order to improve outcomes, stratified medicine initiatives need to be developed, in which novel targeted agents are given according to the genetic alterations identified in an individual's tumour.

In adult oncology practice there are now numerous stratified medicine trials, including in the UK, the lung cancer MATRIX trial, and in other countries similar initiatives for children are showing promising initial results with identification of a high number of 'potentially actionable' mutations. Furthermore as childhood cancers are defined by relatively fewer mutations than adults it is hypothesised that each individual mutation carries a great pathogenic burden, and therefore genetic targeted therapies may be even more effective than in adults. However in the UK there is currently no stratified medicine initiative for children with cancer and limited infrastructure to support widespread NHS-compliant molecular profiling with clinical reporting for children with cancer.

During the same period as this grant, a 72-gene NGS panel which tests for multiple genetic alterations or prognostic and predictive relevance in paediatric solid tumours has been developed by Elisa Izquierdo Delgado, also funded by Christopher's Smile. Sally's work has been closely linked with this and focused on clinical implementation of this panel and other technologies into clinical practice and developing the infrastructure for local, national and international implementation of molecular profiling initiatives for children with cancer in the future.

2. Objectives

Years 1 and 2

- To obtain consensus opinion in the UK for the most important genes to be incorporated into the first version of the NGS panel
- To obtain archival tumour tissue for validation of the paediatric solid tumour NGS Panel
- To develop, write and obtain necessary regulatory and ethical approvals for a protocol offering prospective NGS panel testing for children with solid tumours at the Royal Marsden – the 'tumour profiling study'
- To develop the UK's first molecular tumour board (MTB) for clinical reporting of NGS panel results to the treating clinician
- To open the tumour profiling study and begin recruitment

Year 3

- To continue to recruit patients to the tumour profiling study with clinical reporting of results via MTB
- To broaden availability of NGS panel sequencing across the UK
- To enable multi-institutional participation in the UK in the MTB
- To begin to develop strategies for more widespread collection of alternative, more readily accessible sources of tumour DNA for genetic analysis in the future

3. Work Undertaken

Year 1

Sally initially consulted widely with the paediatric oncology community across the UK including all relevant experts in their field to ensure that the most clinically relevant genetic alterations were incorporated into version 1 of the NGS panel. It was also necessary to obtain a number of representative clinical tumour samples for validation of the panel. This included both formalin fixed and frozen tumour samples, and paired germ line samples including all of the most common groups of poor outcome solid tumours in childhood (e.g. brain tumours, sarcomas, and neuroblastoma). In order to obtain sufficient material, Sally obtained ethical approvals and clinical samples from 3 sources: i) local samples from the Royal Marsden Hospital ii) The Queensland Children's Tumour Bank, Australia and iii) The Children's Cancer and Leukaemia Group (CCLG) Tumour Bank.

During this year Sally also began to write the protocol, patient information sheets, consent forms and standard operating procedures for the 'tumour profiling study' – intended as the initial pilot to test ability to perform the NGS panel test prospectively with clinical reporting of results.

Year 2

In year 2 Sally obtained the relevant local regulatory and National ethical approvals for the tumour profiling study, which opened on 1st March 2016.

The focus of this year was to develop the procedures and practices to ensure successful recruitment into the study, co-ordination of sample flow, pathological processing, and analysis of tissue. The necessary steps are outline in figure 1:

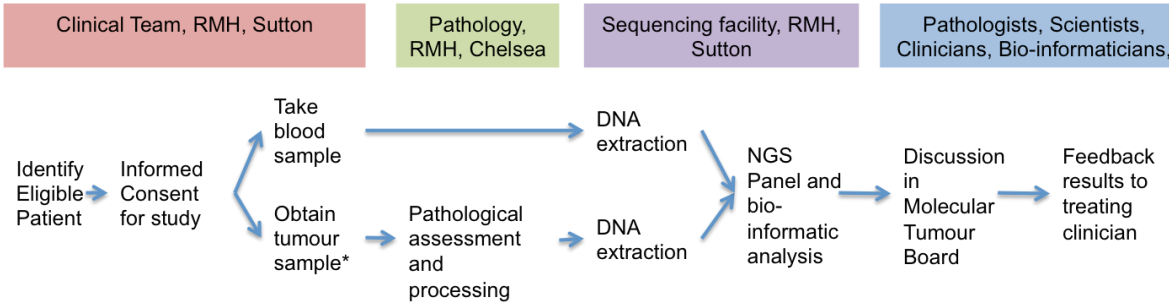

Figure 1: Summary of steps required for NGS panel with clinical reporting of results

Successful implementation of the study into an already overburdened and under-resourced NHS setting was a particular challenge. To successfully implement the study Sally provided education and practical support to the clinical staff to enable recruitment into the study, developed standard operating procedures with Pathology services at Chelsea and set up the multidisciplinary molecular tumour board. Sally also wrote and successfully obtained a National Institute of Health Research (NIHR) grant a grant to employ a tissue collector to assist with the study and to cover associated transport costs of samples across sites.

Sally developed a multidisciplinary molecular tumour board with key defined roles and responsibilities for clinical reporting. The role of the molecular tumour board was to integrate the pathological assessment of the tumour with the clinical features and the sequencing results in order to provide an integrated report to the clinician. The first molecular tumour board was in April 2016.

Year 3

In year 3, recruitment to the tumour profiling study is on going, with 88 Royal Marsden patients recruited and sequenced to date. In line with the objectives, Sally obtained the relevant approvals to roll out NGS panel sequencing across the UK within the CCLG approved biological study METEOR. In brief Sally has worked as part of a national group to develop the infrastructure to role out this technology across the UK. Pathologists from UK primary treatment centres can send tumour samples via 2 routes (a northern hub in Newcastle, and a southern hub at Great Ormond Street hospital). We are working with these hubs to standardise pathological evaluation and processing before the samples are forwarded to RMH for the NGS panel. At the time of writing, 53 patients have been recruited from across the UK thus far. In line with the national role out Sally has further

developed the molecular tumour board which now also includes a core membership of scientists, clinicians and pathologists from Great Ormond Street hospital with the ability of other local centres to 'dial in' as required.
As part of national implementation we continue to monitor sample flow, tracking and turnaround time in order to continuously improve and enable reporting in a clinically relevant time frame. Figure 2 summarised turnaround times since project initiation:

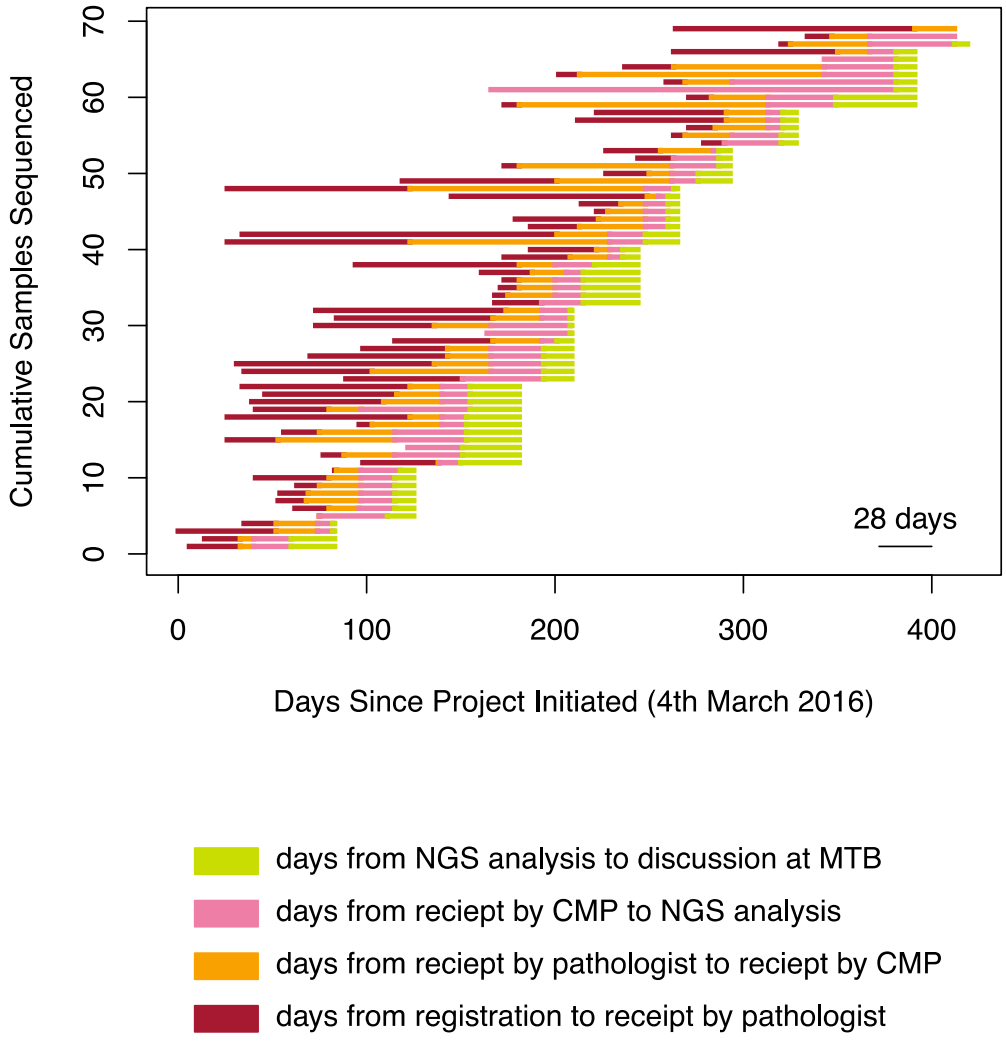

Figure 3: Summary of sample flow

Data analysis is on-going however at the time of the last analysis in early February 2017 over half of enrolled patients had potentially actionable genetic alterations defined as any alteration which affects prognosis, diagnosis or is potentially actionable. A summary of the genetic alterations detected according to tumour type is summarised in figure 2:

figure 2: summary of genetic alterations detected according to tumour type

As an on-going study, clinical data is incomplete, however thus far, we are aware of 4 patients from the Royal Marsden Hospital who have commenced targeted therapies as a direct result of NGS panel sequencing.

Liquid Biopsy

Finally, in both the literature and in our local study it is becoming evident that genetic alterations detected in a tumour at the time of diagnosis may not be an adequate reflection in the genetic alterations detected in the tumour at the time relapse. Biopsy of tumour

tissue at the time or relapse is important for molecular profiling but also may be very invasive and may not always be possible or ethical in children at the time of relapse. For this reason researchers are looking for alternative, more accessible, sources of tumour DNA that can be sequenced and are more easily accessible for serial sampling. One such source of material is circulating free tumour DNA (ctDNA), cell free DNA shed from tumour cells circulating in the blood stream that can be isolated and sequenced.

In parallel to development of NGS panel testing in tumour tissue Sally has also been working to enable sample collection to enable evaluation of circulating free DNA analysis for predictive biomarker identification in the future. Sally has:

1. Integrated the collection of blood for circulating free DNA analysis for research purposes into the tumour profiling study so that the mutational spectrum of ctDNA can be compared with the NGS panel results from the tumour tissue in study patients.
2. As a member of the CCLG biological studies group co-ordinated a research project to decide the best blood collection tube and method for ctDNA analysis and made a recommendation to the national group to commence ctDNA storage in the biobank

4. Key Achievements

Year 1

- Facilitated the design of the v1 NGS Panel and obtained the relevant samples and regulatory approvals for its validation at RMH
- Wrote the Tumour Profiling Study to pilot prospective NGS panel sequencing and clinical reporting at RMH

Year 2

- Obtained ethical approval for, successfully opened and started to recruit patients for the tumour profiling study at RMH and now nationally
- Established the UK's first molecular tumour board for clinical reporting of results at RMH
- Presentations of our NGS panel approach to European (SIOPEN) and National (CCLG) Meetings

Year 3

- On going successful recruitment to tumour profiling study
- RMH became the first Trust to routinely sequence relapsed solid tumour patients as a result of year 1-2
- National Roll out of METEOR – offering NGS panel sequencing across the UK, now increasing recruitment from 5 additional sites (Manchester, Liverpool, Birmingham, Cambridge and Oxford)

- Establishment of joint Royal Marsden/Great Ormond Street Tumour Board
- Collection of ctDNA samples for analysis and national roll out of this procedure through the CCLG biobank for future ctDNA research

5. Objectives not met during grant period

All objectives have been met or will continue to be addressed through ongoing work
Full national implementation or provision to NHS was not a direct goal of this work but was closely linked to the IMPACT and SMPaeds initiatives which will benefit very directly from this initial work.

6. Contribution to published, peer reviewed scientific paper(s)

Integrating Next Generation Sequencing (NGS) into clinical practice: Case studies from the Royal Marsden (RM) paediatric molecular tumour board (MTB). *Sally George, Elisa Izquierdo Delgado, Lina Yuan, Lynley Marshall, Julia Chisholm, Sucheta Vaidya, Stergios Zacharoulis, Giuseppe Barone, Fernando Carceller, Aditi Vedi, Chris Jones, Susanne Gatz, Janet Shipley, Khin Thway, Michael Hubank, David Gonzalez de Castro, Louis Chesler.* Oral presentation CCLG summer meeting, July 2016.

Development of a clinical grade targeted sequencing panel capable of detecting prognostic, predictive and diagnostic markers in patients with Neuroblastoma. Elisa *Izquierdo Delgado, Sally George, Chris Jones, Janet Shipley, Caedyn Stinson, Andrew Moore, Lynley Marshall, Lucas Moreno, Louis Chesler, Andrew Pearson, Lina Yuan, Brian A Walker and David Gonzalez De Castro*. Poster presented at 4th Neuroblastoma Research Symposium 26-27 November 2015

Validation of a Next-Generation Sequencing assay for detecting actionable mutations in Paediatric Solid Tumours. *Elisa Izquierdo Delgado, Lina Yuan, Sally George, Chris Jones, Janet Shipley, Susanne A Gatz, Caedyn Stinson, Andrew Moore, Steven C. Clifford, Debbie Hicks, Janet Lindsey, Rebecca Hill, Thomas Jacques, Jane Chalker, Khin Thway, Lynley Marshall, Lucas Moreno, Andrew Pearson, Louis Chesler, Brian A Walker, David Gonzalez De Castro.* Presented at Childhood Cancer Conference 5-7th September 2016.

7. Overview by Professor Louis Chesler: Team Leader - achievements during grant period

Sally has been tremendously productive and has devoted a huge amount of energy to a logistically very challenging project. While we have not yet achieved routine and efficient clinical reporting of all patients using the NGS panel (now in version 2) nationally, through her efforts we are using the approach clinically at RMH and our practice is fundamentally altered through her work.

We directly alter treatment based on formally reported results, we are leading the first national consultative tumour board to deal with/integrate the information for other centres, and we have two funded prospective implementation projects IMPACT (focusing on provision of testing to all new solid tumour patients nationally) and SMPaeds (focusing on relapsed patients and formal assignment to clinical trials, and to research sequencing).

This is a tremendous achievement for Sally during a short time, and there has already been direct patient impact from her work, and practice-changing data has been generated for NHS as a result of this project.

Our forward goals are to: 1) increase recruitment of both new and relapsed patients nationally, 2) add genomic platforms to increase data generation, 3) build our database of actionable and research mutations for publication, distribution and collaboration internationally, 4) produce an evidence base for NHS to evaluate implementation of the NGS panel as a clinical test within the near future, and 5) continue to roll-out the technology through clinical trials and cooperative groups such as SIOPEN, CCLG, EPSSG.

We would like to thank Christopher's Smile for the funding to support Sally's work over the last three years. Through this and the funding to develop the NGS panel, children are now receiving treatments based on the specific mutations driving their cancer.