Whole Genome Sequencing for Paediatric Oncology Patients in Wales

Comisiwn Bevan Commission

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Project Background:

In 2021 cancer caused ~25% of all UK childhood deaths (Children's Cancers | Cancer Research UK). Current genetic testing for childhood cancers in Wales can include multiple genetic tests and invasive procedures.

Whole Genome Sequencing (WGS) - DNA sequencing method for whole genome analysis. One test can detect a variety of genomic abnormalities including tumour and familial (cancer predisposition genes) abnormalities through paired testing of tumour and germline DNA (Figure 1). (Nakawaga et al., 2015).

There is evidence that WGS provides more accurate diagnosis, prognosis and access to more treatment options for childhood cancer than current standard of care genetic tests (Trotman et al., 2022).

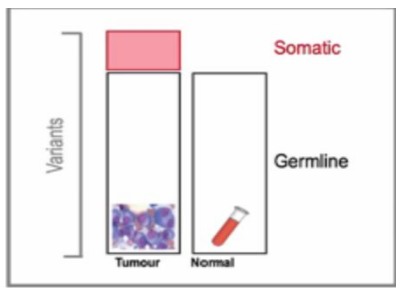

Figure 1: Graphical demonstration of WGS capability to detect tumour (somatic) and germline variants through paired testing of tumour and germline DNA.

Project Aims:

- Validate a whole genome sequencing (WGS) pipeline for paediatric oncology patients at diagnosis, relapse, progression or where all standard of care treatment options are exhausted (<25years)
- Develop a clinical and laboratory pathway that utilises a PCR free WGS testing method for paediatric oncology samples and matched germline samples.

Project Approach:

- All Wales Genomics Laboratory (AWGL) - multi-disciplinary approach with clinical colleagues, industry and other UK genomics laboratories
- Appropriate sample types collected from patients undergoing standard of care (SOC) testing in Wales
- Sample DNA extraction and sequencing carried out in house
- Defined acceptance criteria for technical process evaluation based on acceptance criteria from the validated PCR free WGS pathway for rare disease samples (Table 1)
- Bioinformatics pipeline in development for analysis of sequenced samples (Figure 2)
- Variants detected in commercial cell lines by WGS compared to those detected in same cell lines by TSO500 DNA and RNA NGS in house cancer panels – tests the reliability of the bioinformatics pipeline (Illumina, 2024)
- Variant status of patient samples will be compared to known SOC testing results to ensure concordance for up to 60 patients

17th April - Genomics Partnership Wales, Patient and Public sounding board.

- ✓ Presentation of project and background.
- ✓ Discussion of clinical scenarios (Focus groups)
- ✓ Qualitative data will be thematically analysed.

Project Outcomes:

Procedures in place for sample referral, patient consent, laboratory receipt, sample storage, sample culturing, DNA extraction and sequencing from required samples for WGS for paediatric oncology patients.

- 44/45 sets of patient samples passed DNA extraction quality control parameters for WGS.
- 25/27 sequencing runs met quality parameters (One run repeated twice).
- 10/12 sequencing runs met initial bioinformatics quality parameters (One run repeated twice). (See Table 1).

All SNVs (single nucleotide variants) with greater than 5% VAF (Variant allele frequency) detected in the cancer cell line using TSO500 DNA in house cancer NGS panel were also detected using WGS (Figure 3).

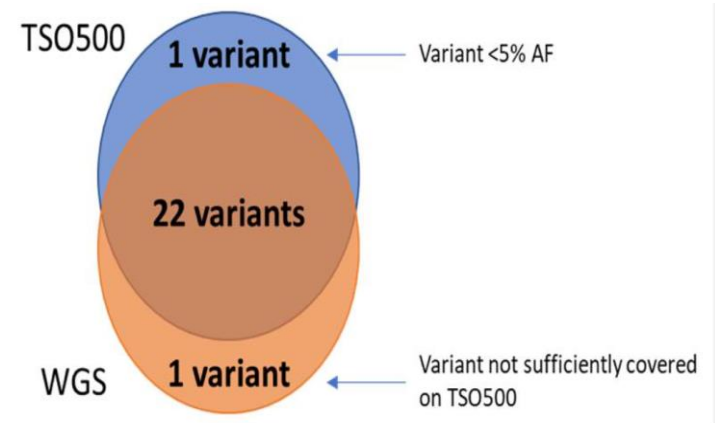

Figure 3: Cancer cell line sample: Venn diagram comparison of variants detected using TSO500 in house cancer DNA NGS panel and WGS.

Project Impact:

An in house WGS service for paediatric oncology patients is possible. DNA extracted from paediatric oncology samples is of sufficient quality for initiation of WGS.

First line WGS testing for paediatric oncology patients could replace the majority of separate standard of care genetic tests and provide greater chance of more accurate diagnosis, treatment and prognosis in the first instance. This could prevent repetitive genetic testing and limit sampling procedures experienced by children.

A paediatric oncology WGS service would be utilised by approximately 80 Welsh patients per year.
Genomics Partnership Wales - Patient and Public sounding board feedback - The sounding board were generally positive about using WGS technology for cancer patients and the patient benefit it could bring.

'Information is power'

"A newborn baby was spared unnecessary chemotherapy when a special blood test revealed a suspected cancerous lump on his leg was actually benign'

Gene test spared baby unnecessary chemotherapy - BBC News

DNA extraction Technical Acceptance Criteria:	
Extraction 260/230 (DNA/contaminants)	>1.7
Extraction 260/280 (DNA/protein)	1.7 to 2.0
Sequencing Technical Acceptance Criteria	
Cluster Passing Filter [2]	≥60%
Q30 [1]	≥75%
NTC contamination [3]	<10%
Bioinformatic Acceptance Criteria:	
Mean sequenced coverage (Tumour samples)* [4]	(minimum) 70X - ~100X (Typical)
Mean sequenced coverage (Germline samples)* [4]	~20X

Table 1: Required acceptance criteria for DNA extraction, sequencing and basic bioinformatic criteria for WGS of somatic and germline samples. 1. Probability of incorrect base call 1 in 1000 2. Percentage of clusters formed during sequencing that give usable data. 3. NTC – No template control – No DNA present in set up 4. average coverage of unique reads across the coding (exonic) regions of the human genome. * Over coding regions as defined in https://github.com/AWGL/DragenWGS/blob/master/config/builds/hg38/hg38_coverage.bed

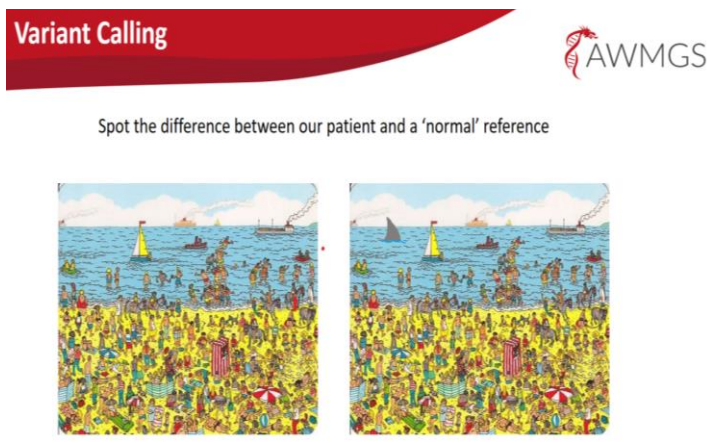

Figure 2: Demonstration of nature of variant calling used in sequencing comparing the query (patient) sequence against a reference (standard) sequence.

Key Conclusions:

- A WGS service for paediatric oncology patients can be delivered through local DNA extraction, sequencing, analysis and reporting carried out at AWGL.
- Project outcomes include the creation of new pathways for transport and storage of fresh frozen tumour pathological specimens.

Next Steps:

Development of the bioinformatics and analysis pipeline continues.

Analyse samples from up to 60 paediatric oncology patients. Identification of variants not detected by SOC testing confirmed using orthogonal methods or confirmation by WGS at another laboratory.

17th April - Genomics Partnership Wales, Patient and Public sounding board.

- ✓ Qualitative data will be thematically analysed.

GTAB/MDT – genomics tumour advisory board discussions started with working group.

Validation used to justify a business plan for implementation of paediatric oncology WGS as an all-Wales service.

Creation of new pathways utilised in future for delivery of all Wales WGS adult cancer services such as sarcoma and brain tumour WGS services.