

Clinical Impact of Ultra-Fast Whole Genome Sequencing in Paediatric Haematology-Oncology Practice

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Abstract

Whole genome sequencing (WGS) improves childhood cancer diagnosis, enabling precision treatment. However, timely clinical decisions are made within days, while WGS clinical reports using current technologies and workflows, can exceed 6 weeks. Here we report an 'Ultra-fast WGS (UF-WGS)' workflow, providing rapid and accurate molecular profiling of childhood malignancies. In 54 children with suspected or confirmed cancer, UF-WGS reduced turnaround time from 37 to 3 days. It captured 95% of clinically actionable variants found by conventional methods, and identified 19 additional actionable variants. UF-WGS led to demonstrable improvements in care for 51% of prospective patients, including avoidance of over-medicalisation and support for timely precision therapy. UF-WGS was feasible across diverse tumour types and sample sources, with additional technical advantages in variant detection and workflow simplicity. These findings indicate UF-WGS can significantly improve the management of paediatric haematology/oncology patients, specifically through precision diagnosis, risk stratification and molecularly informed treatment escalation/de-escalation.

Introduction

Whole genome sequencing (WGS) has improved the molecular diagnosis and management of paediatric cancers in recent years, enabling detection of prognostic and therapeutically actionable molecular variants beyond what is possible with standard molecular assays¹⁻⁴. The clinical utility of WGS has been demonstrated in childhood leukaemia⁵ and solid cancers^{1,6-9}. The Genomic Medicine Service (GMS) of NHS England currently offers WGS as part of routine diagnostic care for all children with suspected or relapsed cancer, yet the turnaround time (TAT) for clinically interpretable results can exceed six weeks¹⁰. This delay is particularly significant for aggressive malignancies, rare cancer subtypes, and presentations where rapid molecular insights could facilitate life-saving or toxicity-sparing interventions. Here, we evaluated the feasibility, accuracy, and clinical impact of Ultra-Fast WGS (UF-WGS) using Illumina's Constellation mapped read technology, implemented in a tertiary UK paediatric haematology-oncology centre.

Children with suspected or confirmed cancer were prospectively recruited between April 2023 and March 2025, with a further retrospective cohort selected based on variant complexity and archival tissue availability. All cases underwent UF-WGS; their tumour, bone marrow (BM), or peripheral blood (PB) samples were analysed in parallel with standard GMS-WGS as a benchmark for clinically-actionable variant detection. Overall, 104 samples from 54 children were analysed, including 35 recruited prospectively and 19 retrospectively (Supp Fig 1A). Mean age at diagnosis was 6.9 years (range 0–17.5 years), and diagnoses represented 37 distinct paediatric haematological and solid tumour entities. The study included children with leukaemia, lymphoma, neuroblastoma, Wilms tumour, and rarer entities such as mixed phenotype acute leukaemia and pancreatic acinic cell carcinoma (Supp Fig 1B, Supp Table 1).

The UF-WGS workflow yielded equivalent or enhanced performance in somatic and germline variant detection, tumour mutational burden estimation, and mutational signature analysis. Sequencing depth and quality were robust, with UF-WGS delivering a median coverage of 137x for tumour samples and 84x for germline samples, compared with 97x and 42x, respectively, for GMS-WGS. UF-WGS captured 95% of all clinically actionable variants, and detected 19 additional actionable variants missed by routine GMS-WGS (Fig1). Discrepancies in variant detection were largely attributable to tumour heterogeneity and low variant allele frequency in sequenced tumour regions (Fig 2A, Supp Table 2). The 5% of variants detected only by GMS-WGS would not have altered the diagnosis, prognosis or management of each individual patient, as assessed by three independent clinicians and molecular pathologists. The additional variants detected by UF-WGS altered management for six patients (Fig 2B). For all patients, actionable UF-WGS findings were confirmed by accredited molecular assays prior to any alteration in clinical management, consistent with current regulatory standards for research-only diagnostics.

UF-WGS dramatically reduced the diagnostic timeline from 37 to 3 days (Fig 2C), returning comprehensive clinically interpretable genomic reports in a clinically meaningful time frame. Reduction in TAT was achieved by bypassing labour-intensive steps such as fibroblast culture for germline analysis (required in leukaemia workflows) and by applying crude lysate or extracted DNA directly to the sequencing flowcell surface. The simplified UF-WGS workflow obviated the need for separate DNA extraction or library synthesis, expediting laboratory processing and facilitating decentralised sequencing including end-to-end variant analysis.

Importantly, UF-WGS provided clinically actionable information in both solid and haematological malignancies, leading to a change in management beyond conventional methods. In the prospective cohort, 18/35 children (51%) had improvements in care based on rapid stratification, tailored therapy initiation, and in some cases, avoidance of unnecessary procedures or treatments (Supp Table 3). In the retrospective cohort, three independent clinicians judged that UF-WGS reporting would have altered or improved care pathways in 9/19 (47%) of patients had these results been available at the time of presentation (diagnosis or relapse, Supp Table 4).

Several of our prospective cases illustrate the clinical importance of rapid TAT. Because two children (Patients 22, and 49) presenting with soft tissue lesions were promptly diagnosed with non-malignant conditions, the care team was able to avoid over-medicalisation with surgery, chemotherapy, and associated hospitalisation. Patient 49 was redirected towards rheumatological specialists upon early detection of the *AVCR1* germline variant, pathognomonic of fibrodysplasia ossificans progressiva (FOP), a condition in which even minor trauma (e.g., surgical interventions, biopsy) leads to damaging soft tissue calcification, making prompt diagnosis and avoidance of invasive procedures paramount. Patient 52, with pancreatic acinic cell carcinoma was found to harbor a novel somatic *NKD2::BRAF* gene fusion via UF-WGS, qualifying for precision MEK pathway inhibition. In children with Wilms tumour, UF-WGS rapidly ruled out germline predisposition even in the absence of tumor tissue, enabling confident surgical planning without nephron-sparing strategies reserved for syndromic presentations¹¹. Expedited

molecular diagnosis from pleural fluid obtained from Patient 66 with a life-threatening mediastinal mass (lymphoma) avoided a potentially high-risk biopsy procedure.

UF-WGS also improved molecular diagnosis for leukaemia patients, permitting tumour-only sequencing from BM or PB samples, rather than necessitating time-consuming paired germline analysis. The workflow delivered MRD marker detection, cytogenetic subgrouping (including rearrangement identification), and pharmacogenomic profiling suitable for dose modification within days of presentation. In an infant with Down syndrome and suspected transient abnormal myelopoiesis, UF-WGS confirmed the presence of somatic *GATA1* mutations and germline trisomy 21, guiding monitoring and avoidance of unnecessary AML therapy (Patient 68). A comprehensive molecular diagnosis was obtained from peripheral blood in a subset of leukaemia patients, potentially obviating the need for invasive BM biopsy in selected children (Supp Fig 2).

Technical advantages of UF-WGS include variant phasing and improved detection of variants within repetitive genomic regions. For example, haplotype analysis allowed assignment of *PMS2* variants to separate parental alleles, confirming a diagnosis of constitutional mismatch repair deficiency (CMMRD) and informing eligibility for immune checkpoint inhibition for Patient 69 (Fig 2D). An experimental somatic pipeline that leverages sequencing flowcell proximity enabled sensitive and specific detection of novel gene fusions, exemplified by Patient 46 with an *ATXN1::NUTM2D* fusion brain tumour (Supp Fig 3). These capabilities are particularly pertinent for paediatric oncology where actionable findings often reside in structurally challenging or poorly covered genomic loci.

As shown above, the UF-WGS pipeline was robust across a range of tissue types, sample qualities, and diagnoses, including both archival and freshly collected material. Clinical samples with low tumour cellularity or complex variant compositions were successfully analysed, supporting broad utility in real-world oncology practice. Rapid identification of risk-stratifying variants that alter treatment at the point of diagnosis or relapse, including *BCR::ABL* in leukaemia¹², *MYOD1*¹³, *MYCN* amplification¹⁴, and *BRAF* alterations¹⁵ in solid tumours, are prime examples of the immediate clinical application of UF-WGS as a rapid molecular diagnostic assay that outperforms most standard tests. UF-WGS can lead to safer, timely treatment, avoiding over-medicalisation. Here we demonstrate how delayed WGS can result in avoidable morbidity when treatment decisions must proceed in its absence.

Despite these strengths, the study is limited by its single-centre, non-randomised design. Additional validation across multiple centres will be required to quantify clinical impact, establish cost-effectiveness, and define generalisability for national or international practice.

Implications of UF-WGS extend beyond rapid molecular diagnosis to encompass precision medicine, de-escalation of toxic treatments, and avoidance of over-medicalisation. The workflow supports timely enrolment to clinical trials, genetic counselling, and broader systems-level refinement of diagnostic pathways. UF-WGS has the potential to consolidate multiple single-marker assays into a unified, comprehensive test, streamlining resource allocation and minimising diagnostic delay—a particularly

beneficial aspect in paediatric haematology-oncology, where treatment windows are often narrow and outcomes hinge on timely intervention. Beyond cost savings, this approach of consolidating molecular testing into a single rapid assay is an important consideration in paediatric oncology, where biopsy material is often limited.

In summary, this study presents real-world evidence for the technical feasibility, rapidity, and clinical impact of UF-WGS across a diverse paediatric oncology population. Ours is the first study to deploy this technology in cancer, but similar approaches have been applied in paediatric rare genetic diseases¹⁶. We suggest UF-WGS represents a new standard for time-critical genomics in cancer diagnosis and management, with the potential to reshape care paradigms for children with cancer and related disorders. The workflow can deliver comprehensive, clinically useful molecular information in under 72 hours, facilitating precision management and enabling safer, more effective allocation of therapies. Wider implementation and randomised trials will be essential to validate efficacy, cost, and broader applicability in routine practice.

Online Methods

Methods are available on protocol.io:

<https://www.protocols.io/view/ultrafast-wgs-protocol-j8nlkyn96g5r/v1>

Declarations

Disclosures

DHR, AV, JT, JD, LK, ARM, RG, AS, AG, SW, SL, AM, JA, MG, EB, ED, CB, MJM, JT, CT, CEH, MC, SB, PT - no conflicts of interest.

MM, SC, PG, ZK, JB, TN, IA, IV, MB, LF, JW, MR, DB and SH are or were employees of Illumina at the time of the study, a public company that develops and markets systems for genetic analysis.

Identifying data

This study protocol was approved by the HRA and Health and Care Research Wales (HCRW) as an ethically approved clinical trial (Protocol number PaedWGS22, REC reference: 22/WA/0336).

Registered on ClinicalTrials.gov:

<https://register.clinicaltrials.gov/prs/beta/studies/S000GAM900000019/recordSummary>

Clinical samples and information was collected by AV, JT, LK, ARM, SL, RG, AS, AG, SW, VJ. Sample and data analysis was performed by AV, JT, JD, MM, SC, LK, ARM, SH, PT.

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Figures

Figure 1

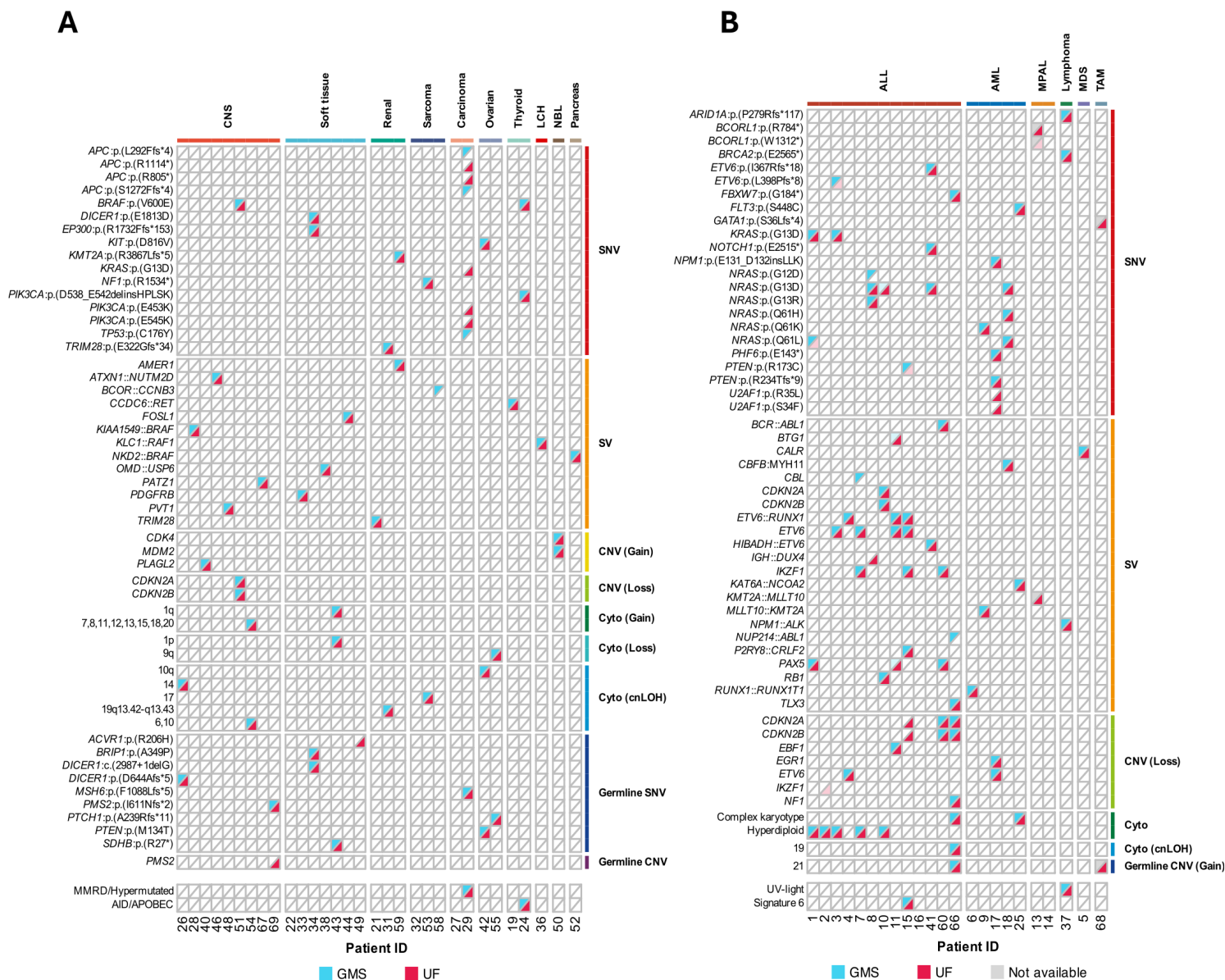


Figure 1: Comparison between GMS-WGS and UF-WGS in detecting clinically actionable variants. Oncoplots listing all somatic and germline variants detected, grouped by variant type. Only clinically actionable variants as per the National Genomics Test Directory are shown. The cohort is divided into patients with (A) solid ($n=31$) or (B) haematological ($n=23$) malignancies. DNA variants detected by GMS-WGS are shown in red and UF-WGS in blue; cases where DNA quantity was not sufficient for GMS-WGS is indicated in grey. Variants detected at low variant allele frequency are shown for GMS (pale blue) and UF (pink).

Figure 1

See image above for figure legend

Figure 2

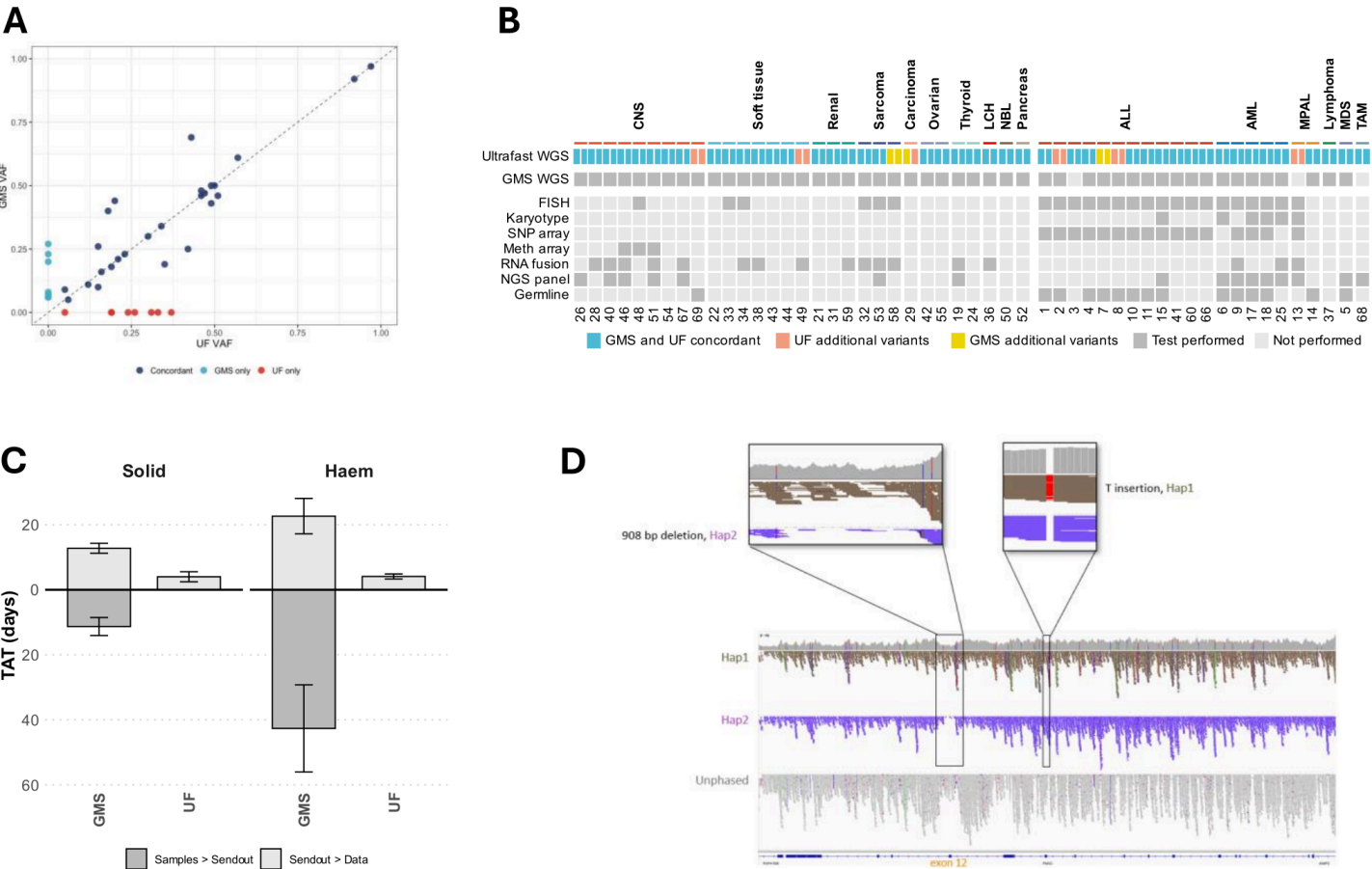


Figure 2: Technical comparison of Ultrafast (UF-WGS) with GMS-WGS and standard-of-care (SOC) assays. (A) Scatter plot representing the variant allele frequency (VAF) for each clinically actionable variant as detected by UF-WGS and GMS-WGS pipelines. Only somatic single nucleotide variants (SNVs) or insertions/deletions (Indels) are shown ($n=41$ variants from 19 patients). (B) Heatmap showing SOC molecular testing for each patient including GMS-WGS. The top line indicates whether UF-WGS was concordant, discordant or added clinically actionable information compared with all SOC testing, and dark grey boxes indicate which SOC test was performed. Germline testing by SOC was performed using NGS panels when indicated. (C) Bar plots representing median time in days from paired tumour and germline samples (or tumour only for haematological malignancies in UF) being received in the genomics laboratory to send out for sequencing and time to return of complete WGS data as a clinically actionable report. (D) Identification and phasing of the germline *PMS2* variant in a patient with CMMRD (Patient 69). Phasing of both haplotypes in a difficult to capture region of the genome by UF-WGS. Constellation flowcell proximity data provided long range mapping for phasing of both haplotypes used in the UF-WGS pipeline.

Figure 2

See image above for figure legend

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [UFWGSVediNatMedSupplementarydata.docx](#)
- [SupplementaryTablesUFWGS.xlsx](#)