Circulating tumour DNA sequencing to determine therapeutic response and identify tumour heterogeneity in patients with paediatric solid tumours

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https://doi.org/10.1016/j.ejca.2021.09.042
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KEYWORDS
Liquid biopsy; Cell-free DNA; Clinical targeted sequencing; Paediatric oncology; Personalised medicine; ctDNA; Cancer heterogeneity

Abstract  Objective: Clinical diagnostic sequencing of circulating tumour DNA (ctDNA) is well advanced for adult patients, but application to paediatric cancer patients lags behind.

Methods: To address this, we have developed a clinically relevant (67 gene) NGS capture panel and accompanying workflow that enables sensitive and reliable detection of low-frequency genetic variants in cell-free DNA (cfDNA) from children with solid tumours. We combined gene panel sequencing with low pass whole-genome sequencing of the same library to inform on genome-wide copy number changes in the blood.

Results: Analytical validity was evaluated using control materials, and the method was found to be highly sensitive (0.96 for SNVs and 0.97 for INDEL), specific (0.82 for SNVs and 0.978 for INDEL), repeatable (>0.93 [95% CI: 0.89–0.95]) and reproducible (>0.87 [95% CI: 0.87–0.95]). Potential for clinical application was demonstrated in 39 childhood cancer patients with a spectrum of solid tumours in which the single nucleotide variants expected from tumour sequencing were detected in cfDNA in 94.4% (17/18) of cases with active extracranial disease. In 13 patients, where serial samples were available, we show a close correlation between events detected in cfDNA and treatment response, demonstrate that cfDNA analysis could be a useful tool to monitor disease progression, and show cfDNA sequencing has the potential to identify targetable variants that were not detected in tumour samples.

Conclusions: This is the first pan-cancer DNA sequencing panel that we know to be optimised for cfDNA in children for blood-based molecular diagnostics in paediatric solid tumours.

1. Introduction

Paediatric cancers are the leading cause of disease-related deaths among children who survive past infancy in the Western world. The degree of genomic complexity is lower in paediatric than adult cancers. Therefore, the impact of individual genomic events on disease development, and the potential for response to targeted therapies, may be greater [1,2]. Tissue profiling studies in childhood malignancies show that a substantial proportion (43%–87%) have potentially actionable alterations [3–8]. Minimally invasive molecular profiling tests could provide a powerful platform to guide clinical decision-making and to deliver precision treatments, particularly at the time of relapse when tumour biopsy may not be possible. Our previous work on tissue profiling [3,9] guided the development of a custom-designed, blood-based 67-gene DNA capture panel focusing on actionable genetic alterations in paediatric solid tumours that can be used to profile cell-free DNA (cfDNA).

cfDNA is fragmented genomic DNA that is present in biological fluids, such as blood or cerebrospinal fluid (CSF). In cancer patients, circulating tumour-derived DNA (ctDNA) is present in blood as a subset of total cfDNA. Molecular profiling of ctDNA has shown great potential in various cancers [10–13]. Emerging studies have applied different methodologies to detect both single nucleotide variants (SNVs) and copy number variants (CNVs) in ctDNA from paediatric cancer patients, including digital droplet PCR (ddPCR) [14–18], whole-exome sequencing (WES) [19,20], low pass whole-genome sequencing (lpWGS) [21–23], cancer type-specific high throughput DNA sequencing panels [24,25] and tissue informed customised DNA sequencing panels [21,26]. However, these methods are limited to the most common paediatric cancer types, and only WES offers an agnostic approach by which SNVs can be detected in ctDNA, albeit with economic considerations limiting sensitivity. We have previously shown a high detection rate of somatic variants in paediatric plasma samples, using a generic adult cancer cfDNA panel [3].
However, due to the differences between the landscape of genomic alterations in adult and childhood cancers, a high number of genomic events enriched in childhood cancers are not present in commercially available adult panels. Therefore, a tailored high sensitivity method for paediatric cancers is needed.

To achieve this, we created a pan-paediatric-cancer solid tumour gene panel using molecular barcoding that can detect low-frequency variants in cfDNA. Our method integrates molecular barcoding and background noise suppression to distinguish true somatic variants from PCR and sequencing errors. This allows variant detection in ctDNA at low allele frequencies, without prior knowledge of the tumour molecular profile, with the potential to enhance sensitivity by including the prior knowledge of tumour variants. Here we describe the development and validation of our 67-gene cfDNA gene capture panel to clinical reporting standards, designed to cover the most clinically relevant genetic alterations in paediatric solid tumours. We also employ lpWGS analysis of the same library to inform genome-wide copy number changes in parallel to variant detection in ctDNA at low allele frequencies, without additional input DNA. We show high concordance between ctDNA and tissue sequencing for SNV and CNV detection in a range of paediatric solid tumours. Finally, we show the potential of liquid biopsy to detect tumour heterogeneity and monitor disease status/progression, outlining potential scenarios where ctDNA findings correlate with clinical parameters, demonstrating the potential utility of this assay in clinical practice.

2. Materials and methods

2.1. Samples and patients

Different sample types with validated variants were used for panel validation:

1. Four cell blends containing 46 cancer-specific SNVs at known variant allelic frequencies (VAF) of 4–30%, 528 background SNVs and 108 background indels (Tru-Q1-4 HorizonDiscovery, Cambridge, UK)
2. Three clinical paediatric fresh frozen paraffin-embedded (FFPE) samples containing 46 known SNVs with 2–87% VAF
3. Two artificial cfDNA controls containing 25 SNVs and 15 indels in a dilution series resulting in 0.125%–5% VAF (SeraSeq Complete and Mutation mix v2, SeraCare, USA)
4. Sixteen clinical cfDNA samples containing 11 SNVs detected by Roche AVENIO cfDNA panel sequencing and 10 SNVs confirmed by ddPCR.

Further validation was performed on paediatric cfDNA samples with matched tissue sequencing (Supplementary Table 2, Supplementary Fig. 4). National Research Ethical approval was obtained for all studies, and participants and/or guardians gave informed consent and age-appropriate assent. Plasma samples were obtained from patients enrolled on the Royal Marsden Hospital (RMH) clinical sequencing pilot study (REC reference 15/LO/077) [3]. A limited number of patients from RMH (patient 1, 2, 6, 14, 19 and 20) were also concurrently enrolled on the Stratified Medicine Paediatric Programme (reference: 246557/264925). All patients had diagnostic tissue sequencing [3]. Additionally, 7 patients were part of the METEOR study (CCLG ref: 2015 BS09). 2–16 ml of blood were collected into Streck Cell-free DNA blood collection tubes, centrifuged twice at 1600 g for 10 min and plasma stored at −80 °C. cfDNA was extracted using QIAamp Circulating Nucleic Acid Kit (Qiagen). DNA was isolated from buffy coats and sequenced to enable the subtraction of germline variants from tumour sequencing data. The patients presented at various stages during the treatment course, including initial diagnosis and relapse.

2.2. NGS library construction and sequencing

Sequencing libraries were generated using cTMT (circulating tumour DNA in Paediatric Cancers) custom design panel (Cell3™Target, Nonacus) kit with Dual Index Unique Molecular Identifier (UMI) adapters (IDT) as per manufacturer protocol (FFPE input of 50–200 ng, cfDNA 5–50 ng). Up to 16 libraries were pooled per capture and sequenced on Illumina MiSeq (v3 150cycle kit), NextSeq (High output 300 cycle kit) and NovaSeq (300 or 200 cycle kits). For lpWGS, pre-capture amplified libraries from the same prep used for the capture were independently pooled and sequenced separately on a different flowcell to a depth of ~1 × (average depth of 1.6 × achieved) using NovaSeq6000 (200 cycle kit).

2.3. Analysis of the quality metrics of the panel

Sequencing reads were aligned to genome build GRCh37, and depth and coverage at each region of interest (ROI) were determined. Sufficient depth was defined as no lower than 2 SD of the mean, with the calculation based on log2 normalised depth to remove effects due to amplification and deletion: Log2(ROI) > mean(log2(ROI))−2 × sd(log2(ROI)).

The repeatability (within-run precision) was determined by comparing four-cell blends’ background variant data in the same run for variant detection and VAF accuracy. An intra-run pairwise correlation was calculated for two runs where the cell blends were prepared by different users and sequenced on different sequencing instruments.

Please cite this article as: Stankunaite R et al., Circulating tumour DNA sequencing to determine therapeutic response and identify tumour heterogeneity in patients with paediatric solid tumours, European Journal of Cancer, https://doi.org/10.1016/j.ejca.2021.09.042
2.4. ctPC pipeline

A custom bioinformatics pipeline was developed, compromising demultiplexing of raw sequencing data, alignment, quality control and variant calling. Demultiplexing was performed using Bcl2fastq2version 2.20.0 (support.illumina.com/downloads/bcl2fastq-conversion-software-v2-20.html" title = 'https://emea.support.illumina.com/downloads/bcl2fastq-conversion-software-v2-20.html'>https://emea.support.illumina.com/downloads/bcl2fastq-conversion-software-v2-20.html). Sequenced reads were aligned to the hg19 genome, and consensus BAM files were generated using the fullcrumgenomics bio (fgbio) suite of tools (http://fulcrumgenomics.github.io/fgbio/), including consensus read generation determined by UMI and clipping of overlapping reads. Variant calling was performed with VarDictJava (https://github.com/AstraZeneca-NGS/VarDictJava), and the output was filtered to remove low quality, synonymous and intronic mutations, as well as any variants below a defined depth or allele frequency (0.1% VAF in UMI consensus reads). High (>90%) on-target rates are chiefly a consequence of the error-correction process.

Variants were then manually curated using IGV software to exclude false positives. Read uniqueness was based on (i) a minimum of 3 UMI reads for tissue informed and a minimum of 5 UMI reads for novel SNV calling, (ii) clearly unique UMIs and unique genomic alignment positions for read pairs, (iii) absence of clear strand bias (iv) absence of mapping to a different genomic location, (v) absence of variant in matched or unmatched deeply sequenced set of buffy coat DNA (DeepSNV version 1.34.1 analysis [27]).

2.5. lpWGS analysis

The base call (bcl) files were demultiplexed using bcl2fastq v2.17.14. Reads were aligned to the human reference genome build GRCh37 (Hg19) using Burrows–Wheeler Aligner (BWA) v0.7.12. The aligned reads were segmented into 500 kb windows and counted using the readCounter function as part of HMMcopy v0.99.0 and the resulting.wig file was used as input for ichorCNA v0.2.0 [28]. IchorCNA utilizes a hidden Markov model (HMM) to predict large-scale copy number alterations and estimate the tumour fraction.

3. Results

3.1. The design of ctPC capture panel

The ctPC panel was designed to enable sensitive and reliable detection of low-frequency variants (<0.3%) in the most clinically relevant genes. The design was based on our clinically accredited 91-gene Paediatric Solid Tumour Panel [3,9], which is currently in routine diagnostic use in the UK. We aimed to maximize diagnostic benefits while maintaining a practical cost for clinical application. To achieve this, we focussed on known hot-spot regions of oncogenes, with full coverage for important tumour suppressors. We included genes with predictive, prognostic and diagnostic SNVs and CNVs in the most commonly reported solid paediatric tumours [1,2], publicly available large-scale childhood cancer genomics data sets (pecan.stjude.cloud/, pedcbio-portal.kidsfirstdrc.org/and cancer.sanger.ac.uk/cosmic) and incorporating consensus opinion from paediatric oncologists. The final panel design covered 67 genes: 33 genes with full coverage of all exons, 25 genes with partial coverage and 9 genes, which are only assessed for copy number. Twenty-two genes with coverage of at least one coding exon had supplementary probes to enable CNV detection (Supplementary Table 1). We adopted a unique dual indexing strategy with UMI’s compatible with a high sensitivity variant detection bioinformatic pipeline.

3.2. Technical validation of the panel

A standardised clinical assay validation framework was followed to determine the panel performance [29], using commercially available reference material. The capture probe performance showed normal coverage distribution for all relevant sample types (Supplementary Fig. 1A–D) with coverage at each region of interest consistent and adequate for the purposes of the test (96.6% of the probes consistently having mean normalised read depth higher than 2 SD of the mean). The mean depth of sequencing for each gene in the panel was consistent between samples, with only one gene, the multicopy HIST2H3C, consistently under-performing (Supplementary Fig. 1E). Sensitivity of SNV detection of 0.999 [95% CI: 94.13%–100%] and specificity of 0.969 [95% CI: 94.03%–100%] was achieved using the standard pipeline (variant allele frequency (VAF) range of 5%–86%). To measure precision, we assessed a set of known mutations supplied by the manufacturer in the control materials (analysis based on 390 SNPs and 96 INDELS per sample). Samples prepared by different users and sequenced on different sequencing runs showed a pairwise correlation of >0.984 [95% CI: 98.0%–98.9%] for SNPs and correlation of >0.896 [95% CI: 86.7%–94.8%] for INDELS (Supplementary Fig. 2). The pairwise correlation of allele frequencies of the variants was consistent among the samples within the same run, with 0.986 [95% CI: 98.3%–99.0%] correlation of SNPs and 0.927 [95% CI: 89.5%–95.9%] correlation of INDELS, indicating a highly repeatable workflow (Supplementary Fig. 3).
3.3. Enhanced limit of detection using UMIs

Since the main application of the panel is to detect tumour derived pathogenic mutations in cfDNA, which potentially could be present at a very low frequency, we enhance the limit of detection by use of molecular barcoding with UMIs and a customised bioinformatics pipeline based on the CAPseq method [30]. This allowed us to achieve a technical sensitivity of 0.965 [95% CI: 90.14%–99.27%] and specificity of 0.815 [95% CI: 68.57%–90.75%] for SNVs and sensitivity of 0.973 [95% CI: 90.70%–99.68%] with the specificity of 0.978 [95% CI: 93.73%–100.00%] for INDELS, at a VAF of 0.125%, based on the requirement of 3 consensus reads (which resulted in no false positives in WT sample) (Fig. 1C and D). Therefore, we established the limit of detection for known variants to be 0.125% with ≥3 mutant reads. De novo variant calling is more challenging due to the background of errors in ultra-deep sequencing. We performed background error suppression [27] trained on buffy coats from paediatric cancer patients and manual variant curation with a threshold of ≥5 variants and >0.3% for novel variant calling.

The performance of deep sequencing with UMIs was further validated using 16 clinical cfDNA samples previously characterised using alternative methods [31]. We detected all of the variants expected in the VAF range 0.18%–47.1% (Fig. 1E and F). In addition, TP53 c.853delG p.Glu287fs mutation was detected by ctPC panel, highlighting the capability of our method to detect frameshift mutations.

3.3.1. cfDNA is detectable in the majority of paediatric solid tumour patients

Our cohort of 39 patients (67 cfDNA samples) represented a range of paediatric solid tumours and showed highly variable yields of cfDNA (5.7–1452 ng/ml) (Fig. 2A). The possibility of genomic DNA contamination was excluded by analysing fragment size. We observed a higher ctDNA fraction in the total cfDNA in patients with active or refractory disease (Fig. 2B).

Sequencing libraries were generated using 5–50 ng of cfDNA and sequenced to a mean depth of 1112 ± 533 unique read families (consolidated based on UMI per target (Table 1)). Unique coverage correlated moderately with cfDNA input (R² = 0.634), but the wide
distribution of coverage with optimal input DNA (50 ng) suggests that DNA quality has an impact (Fig. 2C).

3.3.2. Variant detection in clinical paediatric cfDNA samples compared to matching tumour biopsy

In the 37 patients with tissue available, we found excellent concordance in the variant detection between tumour and cfDNA in patients with active extracranial disease (concordant in 25/29 patients). Consistent with most studies [16,32,33], no oncogenic genomic alterations were detectable in the plasma of patients with brain tumours. Similarly, variants were not identified in cfDNA from patients with minimal or no tumour burden (Supplementary Fig. 5, Supplementary Table 2).

Of 18 patients with active extracranial disease that had SNVs detected in tissue, 94.4% (17) had one or more tissue-confirmed SNV detectable in the matched cfDNA sample (Fig. 3A) with VAF of 0.1%–57% (Supplementary Table 2). The differences between tissue and cfDNA results were most likely related to the large gap between tissue and blood collection that might have led to tumour evolution and/or due to the low purity of some of the cfDNA samples. In four cases, novel SNVs were detected in the cfDNA that were not present in the matched tumour.

3.3.3. Combined SNV, INDEL and copy number detection in ctDNA

We combined SNV and INDEL detection by ctPC panel sequencing with CNV detection by lpWGS from the same library preparation to allow comprehensive molecular profiling without the need for additional DNA. CNVs expected from tissue sequencing were detected in 66.7% (10/15) of patients with active extracranial disease (Fig. 3B). All negative cases had very low ctDNA purity (<10%) except for one (patient 36, Fig. 3B). In this case, there were ~3 years between tumour and plasma sequencing, suggesting that the differences might be due to tumour evolution.

lpWGS also allows estimation of the ctDNA fraction in cfDNA, which can help with clinical interpretation of ctPC results. In our cohort, we detected variants expected from tissue sequencing in all samples with ctDNA purity above 10%, which is consistent with studies in adult cancers where the reported cut-off of ctDNA purity is 5% ctDNA [34–36]. However, it is important to highlight that we would not recommend excluding samples of low purity, as evident by detection of MYCN amplifications in neuroblastoma even in samples with <10% ctDNA purity. We conclude that false negatives are more likely in low purity samples, but detection of variants is still possible.

Table 1

<table>
<thead>
<tr>
<th>Input amount (ng)</th>
<th>Total reads ± SD</th>
<th>Percentage of reads on target ± SD</th>
<th>Mean depth± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD controls (n = 8) 50 ng</td>
<td>5.25 E+06 ± 2.97 E+05</td>
<td>64.2 ± 1.2</td>
<td>756.0 ± 109.9</td>
</tr>
<tr>
<td>FFPE (n = 3) 50–200 ng</td>
<td>6.04 E+06 ± 1.56 E+06</td>
<td>69.1 ± 1.5</td>
<td>1080.3 ± 300.1</td>
</tr>
<tr>
<td>SeraCare controls (n = 11) 50 ng</td>
<td>5.47 E+07 ± 1.42 E+07</td>
<td>93.6 ± 0.45</td>
<td>2333.3 ± 251.3</td>
</tr>
<tr>
<td>cfDNA clinical samples (n = 67) 5–50 ng</td>
<td>5.22 E+07 ± 5.81 E+06</td>
<td>90.2 ± 2.5</td>
<td>1069.1 ± 462.3</td>
</tr>
</tbody>
</table>

Please cite this article as: Stankunaite R et al., Circulating tumour DNA sequencing to determine therapeutic response and identify tumour heterogeneity in patients with paediatric solid tumours, European Journal of Cancer, https://doi.org/10.1016/j.ejca.2021.09.042
3.3.4. Monitoring disease response

Blood-based testing offers the possibility of serial sampling. We identified 15 patients (11 NB, 4 RMS) with ctDNA and/or tissue biopsies taken at more than one time point (1–12 ctDNA samples per patient, Supplementary Table 3). Of these, 3 patients were responding to therapy, and ctDNA findings were consistent with clinical response (Fig. 4). This included patient 17 with relapsed/refractory neuroblastoma with a pathogenic ALK mutation in tissue and cfDNA, which was undetectable after one cycle of lorlatinib therapy, consistent with an objective response on MRI.

lpWGS was also consistent with panel sequencing data with multiple copy number changes detectable in the pre-treatment sample and a silent lpWGS profile in all subsequent serial samples, indicating a very low or non-existent ctDNA fraction.

Patient 11 was a 7-month old girl diagnosed with Stage M, MYCN amplified neuroblastoma and commenced on rapid COJEC induction chemotherapy. CDKN2A deletion and a pathogenic ALK F1174L mutation were also detected in both tumour tissue and ctDNA. The patient had a very good partial response to induction treatment, and coincident with this, the ALK mutation, MYCN amplification and CDKN2A deletion were undetectable after one cycle of lorationib therapy, consistent with an objective response on MRI.

In patient 19 (neuroblastoma), an ATM mutation (7% VAF) was detected in the adrenal primary at diagnosis but not in the post-surgical ctDNA sample. In a subsequent isolated CNS relapse, a SETD2 mutation and the same ATM mutation were detected in tumour tissue (12% and 6% VAF). The SETD2 mutation is not covered by the ctDNA panel, but the ATM mutation was detected at low levels (0.1%) in the time-matched ctDNA sample. Interestingly, additional higher frequency variants were found in ctDNA (TP53, RB1, FGFR3 and ARID1A) that were not detected in the CNS metastasis. The patient subsequently experienced a systemic relapse.

In heterogeneous cancers, more variants can be identified in cfDNA than in tissue biopsy (Fig. 5). More studies are needed to understand the relevance of variants detected at low levels in cfDNA, but wherever found consistent, these might lead to identification of clones resistant to the treatment and have the potential for a systemic relapse.

3.3.5. Monitoring disease progression

Twelve ctDNA samples were analysed from 6 patients who experienced disease relapse following first-line therapy (Fig. 5). Paired diagnostic and relapse ctDNA analyses showed the emergence of novel ctDNA variants in 5/6 patients. Matched tumour samples were available at 12 ctDNA time points, and ctDNA analysis was able to detect all variants detected in tumours where ctDNA purity was >10% (negative cases in Supplementary Fig. 5).

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These case studies, taken together, demonstrate the potential utility of serial ctDNA analyses for response assessment and highlight the importance of future prospective evaluation of ctDNA analysis alongside standard of care investigations.

Fig. 3. Comparison of findings between paediatric cfDNA and tissue samples in extracranial tumours. A. Comparison of SNVs detected in plasma versus tissue sequencing B. Comparison of CNVs detected in plasma versus tissue sequencing. Each column represents a single patient, colour coded for disease type at the top and ctDNA purity and time between tissue and liquid biopsy indicated at the scale on top for each patient. C Venn diagram comparing numbers of alterations detected in tissue versus cfDNA. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
to guide further treatment strategies. The potential of ctDNA to aid clinical decision making is illustrated by patient 20 with neuroblastoma, where a CCND1 amplification was detected in the diagnostic tissue. This was not detected in two relapse tissue samples (the primary tumour and liver metastasis). However, ctDNA from the time of relapse clearly showed the CCND1 amplification, consistent with the molecular findings in the primary tissue, in addition to a novel ARID1A mutation. Both alterations are potentially targetable [37–39].

4. Discussion

Evidence to support the use of liquid biopsies for children with cancer is growing [10–13], but due to differences in the mutational landscape between paediatric and adult malignancies, custom solutions are needed. To
overcome the limitations of adult-focussed panels, we designed a capture panel covering genes most relevant to a wide range of paediatric solid tumours and validated its technical performance using synthetic controls and clinical samples. In line with recent studies [40], the panel showed high technical sensitivity and specificity for SNV detection. In addition, the potential to assess ctDNA fraction and detect large scale copy number changes, as well as confirm focal copy number alterations using lpWGS without any need for additional DNA, was demonstrated in this patient cohort.

Our data supports the clinical implementation of ctDNA sequencing for a variety of paediatric solid cancers. The combined variant discovery and copy number profiling assay can be performed in a routine clinical setting with a turnaround time of under 2 weeks, which is comparable to tissue panel sequencing, and at an affordable cost. ctPC panel sequencing costs approximately 50% more than running a diagnostic 200 gene panel from FFPE, with lpWGS of the already prepared library adding minimally to the cost. However, this is offset by reductions in histopathology costs and potentially offers a faster turnaround time. Where biopsy is difficult or undesirable, plasma-based sequencing is, therefore, a realistic alternative, with the key to achieving cost-effectiveness being to integrate ctDNA sequencing into an existing high throughput workflow to maintain an economy of scale. Practically, even with low volumes of blood, sufficient cfDNA can be obtained for analysis in most cases. For example, in a patient with neuroblastoma, MYCN amplification and an ALK mutation were detected using as little as 5 ng of cfDNA. The clinical sensitivity of the assay is chiefly determined by the ctDNA fraction in cfDNA, which is likely to depend on a number

Fig. 5. Serial liquid biopsy sampling results compared to tissue biopsy at time points where tissue biopsy was available. The top boxes represent tissue sequencing results at a given time point, the bottom ones — ctDNA sequencing. NA — sample was not available. The differences are highlighted in yellow. The samples that had VAF below the limit of detection in tissue sequencing are highlighted in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
of clinical factors, including disease type or disease stage. Further work is needed to clarify the importance of these parameters. Also, patients undergoing treatment or who were in remission showed no detectable ctDNA, except for refractory cases (Supplementary Table 2), where identification may have clinical benefit. Consistent with other studies [16,32,33], we failed to detect the variants in the plasma of patients with brain tumours (Supplementary Fig. 4). For these patients, we are currently collecting CSF samples and hope to evaluate whether it provides a better source of ctDNA.

cfDNA analysis can be performed with or without matched buffy coat analysis. Sequencing matching buffy coats has the benefit of being able to remove variants present in the blood cells and indicate regions of high background noise; however, it adds significant costs to the test. Additionally, we caution against simply filtering out all the variants present in the buffy coat, as highlighted in patient 11. At the first time point, a pathogenic ALK mutation was detected in cfDNA at 47% and at 33% in buffy coats and was, therefore, interpreted as likely germline. In a subsequent blood sample, the same ALK mutation was detected at very low levels in cfDNA but not in the matched buffy coat. Germline testing confirmed that the ALK mutation is of somatic origin. This case highlights the possibility that in patients with a high disease burden, buffy coat contamination with somatic variants at high levels is possible.

We and others have demonstrated the importance of profiling tumours at relapse [3,8,41,42]. Given that mutations detected at relapse may differ from diagnosis, an agnostic profiling method capable of detecting emerging novel variants is needed. There have been several paediatric cfDNA studies to date profiling without prior characterisation of tumour-derived biomarkers, but these were limited to neuroblastoma [26], or in the case of WES, were limited by sensitivity in low purity samples [19,20].

Our method combines the benefits of agnostic profiling with deep sequencing, allowing profiling of cfDNA from patients with different types of solid cancers, as well as detecting low-frequency variants without prior knowledge of tissue variants. Additionally, consistent with previous studies [3,20], we show the potential to detect the emergence of novel variants at relapse using cfDNA. Importantly, some of the variants detected in cfDNA that were missed in matched tumour biopsies are known drivers that may be therapeutically targetable.

5. Conclusions

We demonstrate that our ctDNA panel meets clinical diagnostic standards and shows potential utility as a diagnostic, response assessment and predictive biomarker tool. Prospective validation will facilitate the translation of liquid biopsies into the clinical setting, where it could aid monitoring of treatment response and progression, detection of the emergence of resistance and the biologically informed selection of targeted therapies.

Credit author statement


Funding

This work was supported by the National Institute for Health Research (NIHR) Biomedical Research Centre at The Royal Marsden NHS Foundation Trust and The Institute of Cancer Research, London. Funding was also provided by CRUK, Christopher’s Smile, the Royal Marsden Cancer Charity (RMCC), Royal Marsden CYP Unit and Children’s Unit Fund and research nurse funding by an Experimental Cancer Medicines Centre (ECMC) grant to the Paediatric ECMC network. S. L. G. is funded by CRUK/CRICK Fellowship/Institute of Cancer Research. D. H. is funded by CwCUK/CRUK SMPaeds (17–235/A24566). N. J. is funded by CRUK SMPaeds (A24566). J. A. is funded by Great Ormond Street Hospital Charity Infrastructure award (VSO118) and the Great Ormond Street Hospital NIHR Biomedical Research Centre. A. S. and L. C. are funded by ICR HEFCE. A.S. is also supported by the Wellcome Trust (202778/B/16/Z) and Cancer Research UK (A22909). We acknowledge funding from the National Institute of Health (NCI U54 CA217376) to A.S. This work was also supported a Wellcome Trust award to the Centre for Evolution and Cancer (105104/Z/14/Z). L. M. is funded by Oak Foundation/ RMCC. L. R. P. was funded by Rosetrees project grant. S.A.G. was funded by The Sohn Conference Foundation.
and the Hopkins family. J.C.C. and F.C. are partly funded by the Giant Pledge via the RMCC.

Conflict of interest statement

The authors declare the following financial interests/personal relationships, which may be considered as potential competing interests: Michael Hubank has received consultancy or honoraria or lab support in the last 5 years from the following companies: Amgen, Astex Pharma, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol Myers Squibb, Lilly, Guardant Health, Illumina, Incyte, Merck, Novartis, Qiagen, Roche Diagnostics (each < £2500). Other authors declare no competing interests.

Acknowledgements

The authors thank all participants and their families and the CCLG Tissue Bank for access to samples. We also thank Pfizer for access to lorlatinib on a compassionate named patient basis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2021.09.042.

References


