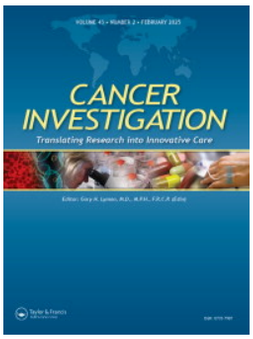

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Clinical Validation of Local Versus Commercial Genomic Testing in Cancer: A Comparison of Tissue and Plasma Concordance

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ABSTRACT

Genomic sequencing of tumours improves patient outcomes through implementation of precision oncology. At present, genomic testing is mainly confined to research settings, with samples sent to biopharmaceutical companies for analysis. The ever-expanding catalogue approved of targeted therapies has created an urgent unmet need for local genomic testing facilities, to enable upscaling of testing. Here, we compare the outcomes of local (IonTorrent™) and commercial (Foundation Medicine) genomic testing collected from 30 cancer patients in from plasma and tissue samples. Overall concordance was high in both tissue (98%) and plasma (94.2%). Variants identified by both platforms had a strong correlation in variant allele frequencies (VAF%): plasma: $r = 0.99$ $p < 0.0001$, tissue: $r = 0.91$ $p < 0.0001$. However, numerous low VAF% variants resulted in low positive percentage agreement (tissue 78.8% plasma 16.1%) and positive predictive values (tissue 56.3% plasma 71.4%). Local sequencing demonstrated higher fidelity in detecting fusions but low fidelity in detecting indels. Overall, this study supports the use of local genomic testing for routine molecular diagnostics but highlights outstanding issues before widespread implementation. Processing of variants detected at low VAF% and the limit of detection of assays needs to be addressed. Construction of gene panels requires careful consideration, including incorporation of markers of genomic instability.

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Genomics, Next-generation sequencing, Liquid biopsy, Cancer, Precision oncology

Introduction

Genomic testing of cancers provides the potential to revolutionise modern cancer management. The NHS Long Term Plan sets out to widen access to genomic testing such that it is routinely available to cancer patients through NHS Genomic Medicine Services (1). The ability to sequence a cancer's genome enables identification of the underlying genetic alterations, including detection of less frequent genetic changes and actionable mutations.

At present, the availability of genomic testing in clinical practice is limited and is mainly confined to research settings. More widespread availability of genomic testing can be predicted to improve patient outcomes through implementation of precision medicine and enhanced prognostication (2), whilst also providing a powerful technology to deepen our understanding of cancer biology. Current challenges include sample preparation, data privacy, sample processing times, rare variant interpretation and data analysis (2–4).

Next-generation sequencing (NGS) technologies read DNA fragments in parallel enabling sequencing of large numbers of nucleotides simultaneously. These high-throughput methods enable large quantities of genetic material to be sequenced quickly and at relatively low per-base costs.

The most widely used NGS technologies used are Illumina® platforms, based on sequencing by synthesis (5,6). The nucleotide sequence is determined through sequential cycles of incorporation

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of fluorescently labelled deoxynucleotide triphosphates (dNTP) into the growing DNA strand, followed by optical analysis and cleavage (6).

Other emerging short-read sequencing technologies have been developed. Ion semiconductor sequencing is based on the detection of a change in pH with release of a hydrogen ion during nucleotide incorporation. Nucleotides (A/C/G/T) are sequentially flooded into microwells on a semiconductor chip, in order to determine the sequence of bases of reads attached to ion sphere beads (5–7). This offers a relatively more affordable method of NGS, with fast run times and automated workflows (7,8).

NGS enables sequencing of large amounts of the genome. In a research setting, whole genome sequencing (WGS) or whole exome sequencing (WES) may be performed by NGS. Wider panels enable the identification of rarer genetic aberrations and the opportunity to advance our knowledge of tumour biology. However, this comes at the cost of greater expense and longer sample turnover time and hence this approach is impractical for mainstream clinical use at present. The majority of genomic testing occurs in the form of targeted panels offering a more practical and affordable approach. Gene capture panels are designed to incorporate genes of clinical or biological significance. The number of genes incorporated can vary widely from tens to hundreds of cancer-related genes (3). Panels may be tumour-specific or tumour-agnostic; tumour-agnostic panels refer to those designed for testing across a range of cancer primaries (9) and facilitate batching of samples to enable bulk testing.

Traditionally, genomic testing has been performed on tissue samples, obtained through diagnostic biopsies or surgery. Liquid biopsy refers to the molecular analysis of a tumour from material derived from the peripheral blood. Circulating tumour DNA (ctDNA) is the most common analyte sequenced (10). ctDNA are cancer-derived fragments of cell-free DNA released into the blood during apoptosis and necrosis. It is cleared by both the liver and kidneys as well as DNase digestion in the blood with a short half-life of up to $2\frac{1}{2}$ h (11,12).

ctDNA has many advantages as a cancer biomarker and offers the opportunity to advance clinical practice. Most notably, the minimally invasive nature of testing is of much greater convenience and would facilitate repeat measurements over time to enabling longitudinal tracking of molecular changes during treatment (2,10). The short half-life of ctDNA means it provides real-time information on current molecular disease status. Moreover, liquid biopsies could theoretically provide a complete picture of the tumour molecular profile, containing genetic variants from emerging clones and distal metastases not represented in a spatially limited tissue biopsy (10,11).

Despite these vantages, a number of limitations remain in ctDNA analysis. ctDNA comprises only a minor proportion of cell-free DNA, thus sensitive methods are required to enable its detection (11). Clonal haematopoiesis of indeterminate potential (CHIP) are non-tumour derived somatic mutations in haemopoietic cell lines, bringing the possibility of false positive results. These somatic variants occur with age, and can be found in up to 10% of people over the age of 70 (13). Moreover, a subset of patients do not have detectable ctDNA ("non-shedders"), even in advanced disease, which may limit widespread application (14).

At present, genomic testing is mainly performed commercially, with samples sent to biopharmaceutical companies for analysis, particularly in the context of clinical trials. The increasing demand for genomic testing in routine clinical care will require upscaling testing. Hence there is an urgent unmet need for validation of sequencing methodology locally in clinical laboratories. Introduction of liquid biopsies into clinical practice could be predicted to increase this demand further.

Validation of genomic sequencing in local clinical laboratories would facilitate up-scaling of genomic testing to meet the ever-increasing demand for genomic testing of tumours from both tissue and plasma samples. This would enable the delivery of personalised medicine through prescription of targeted therapies, set to improve clinical outcomes of cancer patients (2,3). Increased availability would improve patient access for more equitability and widespread use and integration of testing in mainstream clinical practice beyond clinical trials. Local testing would enable more rapid sample processing, enabling clinically meaningful turnover times for timely initiation of therapy. Moreover, local testing

would provide a more affordable alternative to commercial testing, reducing costs to healthcare systems to ensure required testing levels is feasible.

To this end, we conducted a prospective, observational pan-cancer study evaluating upfront genomic testing in cancer patients undergoing curative surgery as part of local validation for in house genomic testing. Tissue and plasma samples were sequenced in-house using ion semiconductor based NGS and compared with commercial testing performed as orthogonal testing for independent validation. Comparison of patient-matched samples was performed in order to cross-validate in-house genomic analysis.

Methods and materials

Study design and participant recruitment

The MARTINI study, is a prospective observational study "A Trial Using Liquid Biopsies in Solid Malignancies" (NCT04853420) supported by the Leicester ECMC. Inclusion criteria were patients over 18 years old diagnosed with solid tumours requiring surgical resection with a high risk of post-surgical relapse. Exclusion criteria were those not fit for surgery, unwilling to donate blood and tissue samples, pregnancy or lactation or involvement in another research study involving an investigational product <3 months prior to recruitment.

Patients were followed up from recruitment to progression, death or censoring at five-years. Follow-up was conducted through electronic patient records, chemotherapy prescription charts and radiology reporting systems. Data collection for trial participants is ongoing and due to be complete in June 2027. The study formed part of a local proof-of-concept trial demonstrating the ability of ctDNA for detection of minimal residual disease and local validation of NGS; sample size calculations analysis were not performed (15).

The study was registered with the NHS Health Research Authority (IRAS 285462) and was approved by the Liverpool Central Research Ethics Committee (REC 20/NW/0467). All patients were receiving treatment at the University Hospitals of Leicester NHS Trust, UK. Patients signed a written consent form for participation in the study (15).

Sample collection and processing

Peripheral blood samples were collected pre-surgery in Cell-Free DNA Collection Tube® (Roche) containing EDTA. Pre-surgery blood samples (~40 mL) were collected at any point up to surgery. All blood samples were collected a minimum of two weeks after any chemotherapy dose. Formalin-Fixed Paraffin-Embedded (FFPE) tissue slides were obtained from surgery; where tissue samples from surgery were insufficient archival tissue from diagnostic biopsies surgery was sent.

Plasma was separated by centrifugation at 2300 g for 10 min at 20 °C at which point red blood cells and buffy coat were obtained. Plasma was then isolated by a further centrifugation at 3200 g for 10 min at 4 °C to remove remaining cell debris. All samples were processed within four hours of collection. Samples were stored at −80 °C until DNA extraction.

DNA extraction

Cell-free total nucleic acid (cfTNA) was extracted from 4 mL of plasma using the MagMAX cell-free Total Nucleic Acid isolation kit (ThermoFisher™ Scientific) as previously described (16). Plasma samples were thawed on the bench and individual aliquots pooled into 15 ml Falcon tubes. The samples were then centrifuged at 1000 g for 5 min at 20 °C to remove any other cell debris. The supernatant was transferred to a new 15 mL Falcon tube and the volume was adjusted to 4 ml with Dulbecco's phosphate-buffered saline. Samples were treated with proteinase K and incubated at 65 °C for 30 min at 1000 rpm, after which they were cooled on ice for 5 min. Digested plasma was then transferred to 96 deep-well plates for extraction on the KingFisher™ Flex Purification system (ThermoFisher™ Scientific).

FFPE tumour tissue sections were marked by a histopathologist to select an area of tumour tissue suitable for analysis. Tissue samples were then extracted using the using the MagMAX FFPE DNA/RNA Ultra extraction kit (ThermoFisher™ Scientific) according to manufacturer's instructions.

Extracted DNA was quantified using the Qubit™ 4 Fluorometer with the Qubit™ 1xdsDNA HS Assay kit (ThermoFisher™ Scientific). Quality of cfDNA

was checked with the High Sensitivity D5000 ScreenTape (tissue), Cell-Free ScreenTape (plasma) or Genomic DNA ScreenTape (buffy coat) on a TapeStation 4200 instrument (Agilent).

Local sequencing: Ion Torrent™

Sequencing by synthesis remains the most widely used methodology adopted in commercial genomic sequencing, including by Foundation Medicine. However, ion semiconductor sequencing offers characteristics that may be advantageous to clinical laboratory settings, including more rapid sample processing with shorter run times and automated workflows requiring minimal technician input (17). This technology has demonstrated favourable analytical performance measures in validation studies (18) and was the assay of choice in large-scale national genomic clinical trials (18,19). Thus, in order to evaluate performance and feasibility locally, we selected to use ion semiconductor based sequencing for the in-house analysis of samples.

Genomic sequencing was performed at the University of Leicester on plasma cfDNA, genomic DNA from the buffy coat and DNA isolated from FFPE tumour tissue sections.

Sequencing was performed using the Ion Torrent™ Genexus™ Integrated Sequencer (ThermoFisher Scientific) in an automated workflow including library preparation, templating, sequencing, alignment and variant calling before being analysed using the ThermoFisher Ion Reporter software.

FFPE samples were analysed by the Oncomine™ Comprehensive Assay v3 (OCA), covering 161 cancer-related genes, detecting hotspot SNV, indels, CNA and gene fusions from extracted DNA/RNA (Supplementary Table 1). Plasma samples were analysed with the Oncomine™ Precision Assay (OPA) (ThermoFisher Scientific), covering 52 cancer-related genes detecting hotspot SNV, indels, CNA and gene fusions (Supplementary Table 2). Tumour-agnostic gene panels were selected, owing to the incorporation of different cancer primaries within the trial (9). This would enable the potential of a pan-cancer approach locally to facilitate bulk sequencing of samples for convenience in delivering local sequencing and evaluate the performance of a narrower panel to reduce costs with increased coverage. Moreover, these assays permitted low sample input, requiring only 10 ng (OPA) (20) or 20 ng (OCA) (21) of extracted DNA (20,21), of relevance when sequencing FFPE and liquid biopsy samples, where DNA contents may be low.

Variant calling was performed on the Genexus™ software according to pre-defined workflows and using GRCh38 as the reference genome. Reported variants met QC thresholds. Variants identified were further evaluated through visual inspection using the Integrative Genomics Viewer (IGV) software (22) for verification and analysis. Variants were classified according to the Human Genome Variation Society (HGVS) guidelines (23).

Foundation medicine commercial genomic testing

Foundation Medicine comprehensive genomic profiling was selected for orthogonal testing as an independent validation assay, as one of the most widely used commercial sequencing platforms and with FDA approval, as well as to enable a direct comparison of sequencing by synthesis to ion-semiconductor sequencing.

FFPE tissue sections and whole blood samples were sent for commercial genomic profiling with FoundationOne® CDx and FoundationOne® Liquid (Roche) respectively. Where tissue from surgery was unavailable or inadequate, archival tissue from biopsies or previous surgery was sent. For plasma testing, 2×8.5 mL of blood was collected in EDTA cell-free DNA collection tubes according to technical instruction and shipped either the day of collection or the following day.

FoundationOne® CDx covered 324 genes (309 with complete exon coverage, 15 with select non-coding coverage) including 36 introns for rearrangements. It also reports tumour mutational burden (TMB), microsatellite instability (MSI) and tumour fraction; loss of heterozygosity (LoH%) is also reported in patients with ovarian cancer (24) (Supplementary Table 3).

FoundationOne® Liquid Commercial detects single nucleotide variants (SNV), insertions and deletions (indels), copy number alterations (CNA) and rearrangements across 324 cancer-related genes with full exon coverage including 15 genes with select non-coding sequencing and

75 genes with enhanced coverage through baited hybrid capture. It also reports microsatellite instability (MSI), blood tumour mutational burden (bTMB) and tumour fraction (24) (Supplementary Table 4).

TMB was defined as the number of somatic variants per million bases sequenced (mt/Mb). Tumour fraction provides an estimate of the percentage of total cell-free DNA (cfDNA) derived from the tumour (ctDNA). Subclonal variants were defined as those detected in <10% of tumour DNA sequenced. The copy number threshold for reporting an amplification was six (except for ERBB2 gene where a threshold of four was used), equivocal CNAs were those just below the threshold providing some but not definitive evidence of copy number alteration (24).

Detectable ctDNA on commercial testing was defined as variants reported on the genomic profiling report.

Concordance testing

Concordance testing was conducted in accordance with the Association for Molecular Pathology and College of American Pathologists Guidelines for Validation of Next-Generation Sequencing Based Oncology Panels (8) from matched samples. Patient-matched tissue and plasma samples were also compared to explore the relative representation of variants in the plasma.

Concordance calculations between assays were performed considering variants present on both gene panels and was defined as either detecting an identical variant or absence of variant detection by the two assays being compared. In order to be considered concordant, the mutation had to be equivalent at a molecular level, and differing molecular alterations in the same gene were considered a discordant result for that gene. Shared variants were considered those reported by both platforms, whereas those reported by only one platform were termed private commercial or private in-house. Commercial sequencing results were considered as the orthogonal testing method. Positive percentage agreement (PPA) and positive predictive value (PPV) were defined according to the guideline above, with commercial sequencing considered as the reference test.

For comparison between sequencing platforms, all genes on the OPA were present on the commercial sequencing panel therefore 52 genes were covered by both plasma assays. 138 genes were covered by both tissue assays. For comparison between tissue and plasma sequencing, 52 genes were covered by both OPA and OCA gene panels and 324 genes were covered by both commercial sequencing panels.

Statistical analysis

The correlation of between variant allele frequency (VAF%) between matched samples was investigated by calculating a Pearson correlation coefficient (r) and corresponding p-value. Statistical analysis was performed on GraphPad Prism version 10.1.2 for Windows, GraphPad Software, San Diego, California USA. p-values <0.05 were considered significant.

Results

Recruitment, sample acquisition and sequencing reports

Thirty patients were recruited, baseline characteristics and cancer information are shown in Table 1. Commercial plasma sequencing was initially unsuccessful in two patients due to inadequate DNA extraction, in one patient a further plasma sample was obtained where reattempted extraction was then successful. Therefore, FoundationOne® Liquid reports were available in 29 patients. Of the patients continued on-trial on the basis of the presence of ctDNA pre-surgery ($n = 23$), 17 patients underwent surgery (Figure 1). FoundationOne® CDx reports were available from 14 patients on-trial. Of these, sequencing was originally unsuccessful in the surgical sample in two patients (necrotic tissue $n = 1$, and insufficient material $n = 1$) and sequencing was performed on tissue from the diagnostic biopsy or previous surgery. Commercial tissue sequencing was unable to be performed in three patients due to insufficient material from surgery ($n = 2$) or technical failure ($n = 1$). In one further patient who was unresectable at surgery, sequencing was also attempted on the diagnostic biopsy, but there was insufficient tumour content. Commercial tissue sequencing was obtained in a further four patients

Table 1. Baseline characteristics.

Patient demographics		
Age at recruitment (years): median (range)		67 (41–77)
Gender (male): *n* (%)		11 (37%)
Ethnicity	White British	26 (86.7%)
	White Other	2 (6.7%)
	Asian	1 (3.3%)
	Mixed other	1 (3.3%)
Cancer primary		
Lower gastrointestinal	Colon	4 (13%)
	Rectal	6 (20%)
Upper gastrointestinal	Gastric	2 (6.7%)
	Oesophageal	3 (10%)
	Pancreatic	1 (3.3%)
Gynaecological	Ovarian	13 (43%)
Head and neck	Larynx	1 (3.3%)
Stage		
II		3 (10%)
III		12 (40%)
IV		15 (50%)
Histology		
Lower gastrointestinal	Adenocarcinoma	10
Upper gastrointestinal	Adenocarcinoma	5
	Squamous cell carcinoma	1
Gynaecological	High-grade serous	11
	Clear cell carcinoma	1
	Carcinosarcoma	1
Head and neck	Squamous cell carcinoma	1

Note: Table to show baseline demographics and cancer information of patients recruited to the MARTINI trial (Minimal Residual Disease: A Trial Using Liquid Biopsies in Solid Malignancies).

who were ctDNA-ve pre-surgery. Therefore overall, 18 FoundationOne® CDx reports were available (ovarian $n = 10$, colorectal $n = 5$, gastric $n = 1$, oseophageal $n = 1$, and larynx $n = 1$).

Ion Torrent™ tissue sequencing was performed on 14 patients (ovarian $n = 6$, colorectal $n = 5$, gastric $n = 1$, oesophageal, $n = 1$, and larynx $n = 1$). In one further patient sequencing was unsuccessful due to technical failures in DNA extraction. Genomic sequencing from the buffy coat layer was performed in these patients, as a control and to check for evidence of germline mutations. Ion Torrent™ sequencing was performed on all plasma samples with insufficient DNA extraction on one pre-surgery plasma sample, therefore Ion Torrent™ plasma sequencing was available in 29 patients.

Foundation medicine commercial sequencing results

Sample turnover time

The median time from the date of order to receiving the genomic sequencing report was 13.5 days (range 9–22) for plasma sequencing and 11 days (range 10–65) for tissue sequencing (Supplementary Figure 1).

FoundationOne® CDx

Variants were reported in all tumours sequenced ($n = 18$) and the number of variants detected in an individual samples ranged between 2 and 24 (median 4). In total 101 variants were reported, (SNV: 42, CNA: 33 indel: 25, structural: 1) (Supplementary Table 5 and Figure 2). VAF% ranged between 0.7% and 91.9%, median 20.4% (Figure 3a).

Tumour mutational burden (TMB) ranged between 0 and 25 (median 2) and could not be determined in two patients (Supplementary Figure 2a). Microsatellite instability (MSI) was detected in one patient with gastric adenocarcinoma; in two patients, MSI could not be determined (ovarian:1 larynx:1), and in all other tissue samples microsatellite status was stable. LoH% in ovarian cancer tumours ranged from 1.8% to 16.2% (mean 9.1%) and was unable to be determined in two patients (Figure 4c). Variants of unknown significance (VUS) were reported in 16 patients (Supplementary Table 11).

FoundationOne® liquid

Twenty-three patients had detectable variants in plasma pre-surgery. The number of variants detected in individual patients varied between 0-10 (median 3.5). In total 76 variants were reported (SNV: 44, indel: 20, CNA: 12) (Figure 2, Supplementary Table 6). VAF% ranged between 0.11% and 71.29%, median 10.9 (Figure 3b).

Blood tumour mutational burden (bTMB) ranged between 0-9 (median 3). (Supplementary Figure 2b) No patients had MSI detected in plasma. Tumour fraction was reported in five patients and ranged between 13% and 61% (mean: 38%) (Supplementary Figure 2c) and could not be determined in the remaining 24 patients. VUS were detected in 28 patients (Supplementary Table 11). Twenty-one variants from 15 patients were reported as being likely related to clonal haematopoiesis (Supplementary Table 12).

Actionable mutations and clinical trials

In FoundationOne® CDx reports, 21 actionable variants were identified across 10 individual patients, identifying EU licenced targeted agents in their tumour ($n = 3$), other tumour types ($n = 2$) or both ($n = 5$). In one patient with

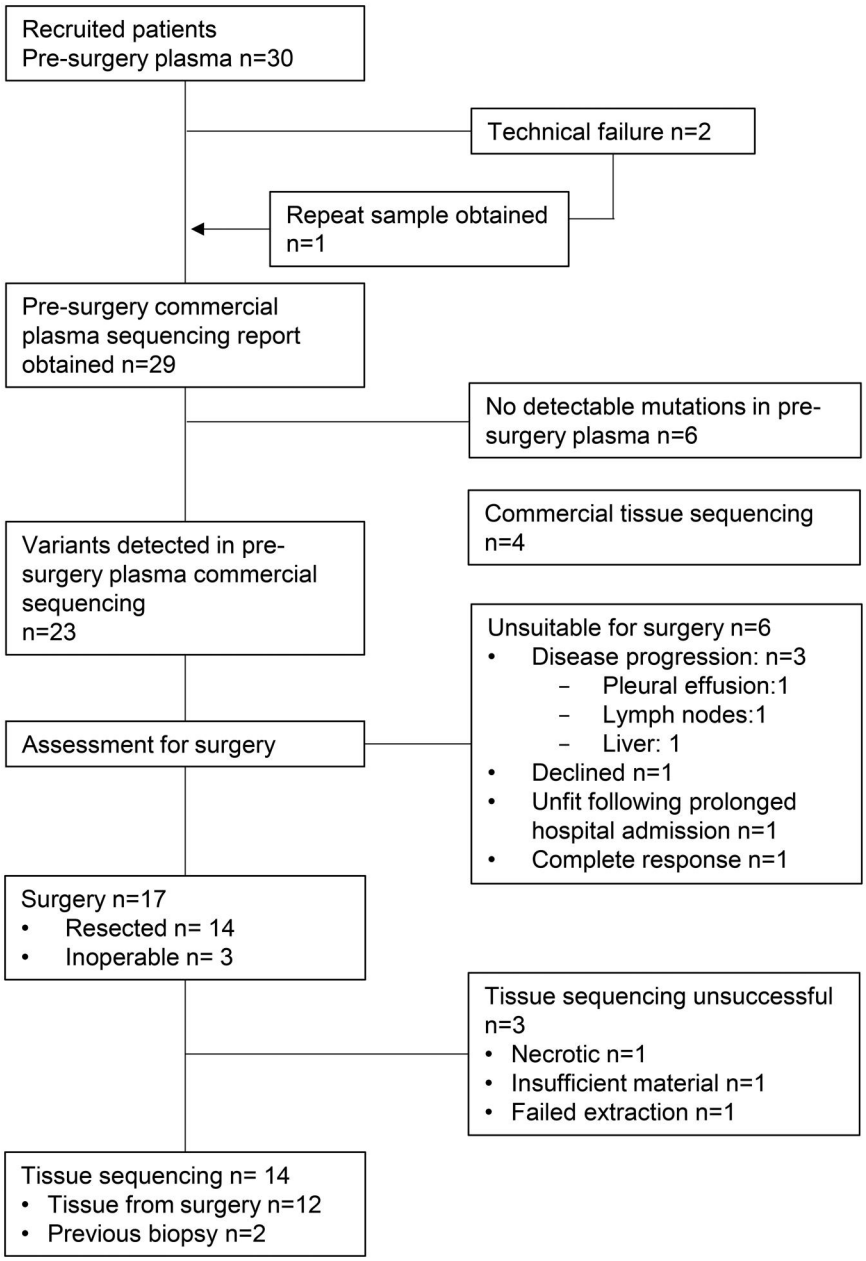

Figure 1. CONSORT diagram. Flow diagram to show patient recruitment and sample collection during the MARTINI trial (Minimal Residual Disease: A Trial Using Liquid Biopsies in Solid Malignancies).

colorectal cancer, a *KRAS* variant was detected, conferring ineligible for *EGFR* inhibitors; in the other four colorectal cancer patients, *NRAS* and *KRAS* wild-type results were reported as disease relevant genes with no reportable alterations. Five patients with ovarian cancer had genomic findings demonstrating homologous recombination deficiency (HRD), conferring eligibility to PARP inhibitors. High TMB and MSI-high were reported in a patient with gastric adenocarcinoma, predicting response to checkpoint inhibition. Other actionable mutations reported were *BRCA2*, *FBXW7*, *PIK3CA*, and *ERBB2* amplification and *MET* amplification (Supplementary Table 10). Seventeen out of the 18 patients (94.4%) where tissue sequencing was performed had mutations detected that would make them eligible for a clinical trial at the time of reporting.

In FoundationOne® Liquid reports, ten actionable mutations were detected in eight individual patients, identifying targeted agents licenced in the EU in their tumour (n = 5) or other tumour types ($n = 3$). Four patients with ovarian cancer had genomic findings demonstrating HRD, conferring

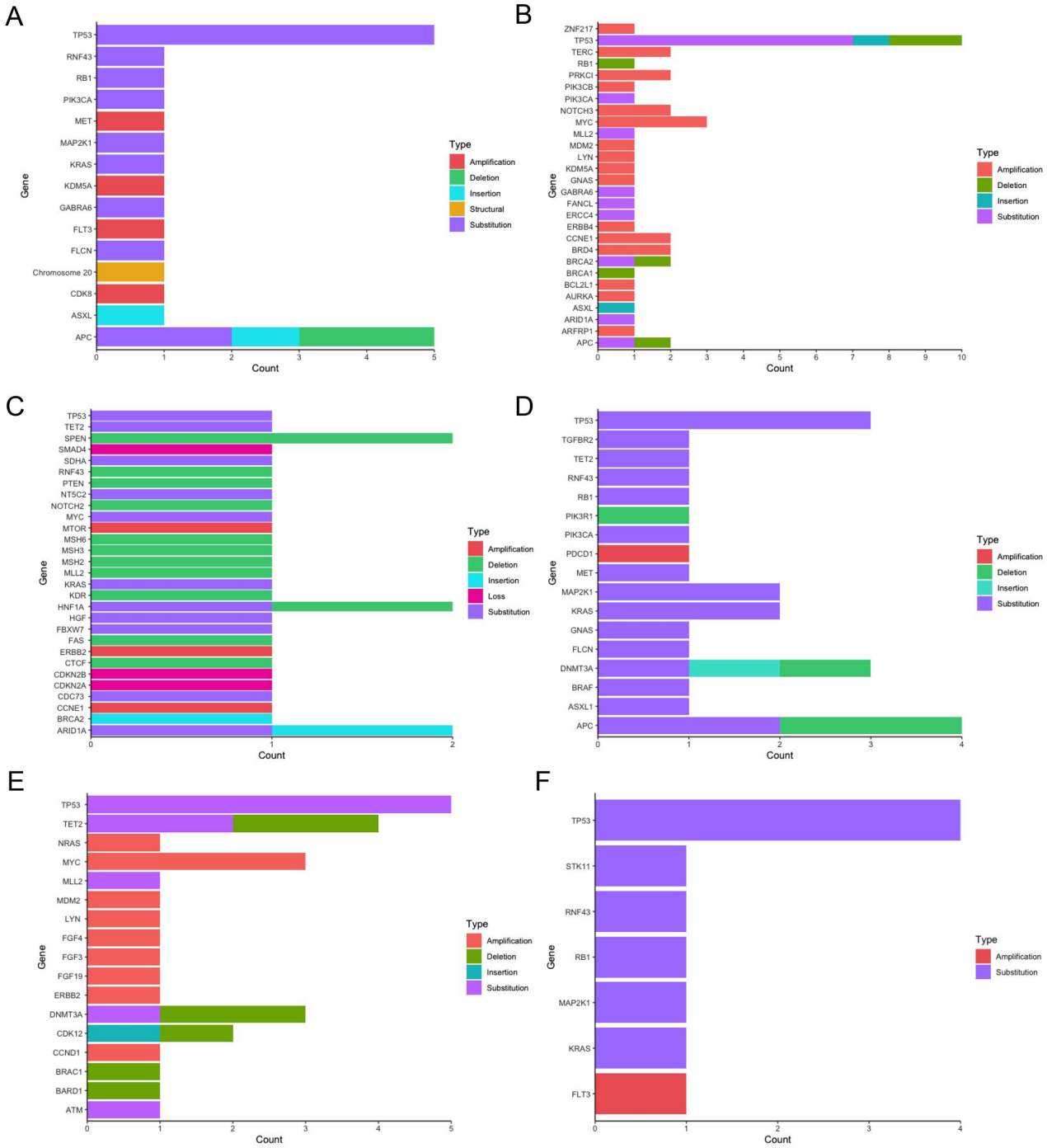

Figure 2. Foundation medicine sequencing. Stacked bar charts showing the variants detected on FoundationOne® CDx (A–C) and FoundationOne® Liquid (D–F) according to variant type from colorectal (A, D), ovarian (B, E) and gastrooesophageal (C, F) primaries. Red – copy number alteration (amplification), pink – copy number alteration (loss), turquoise – insertion, green – deletion, blue – fusion, and yellow – structural.

sensitivity to PARP inhibitors. In addition, in two patients with colorectal cancer, *KRAS* mutations were detected in plasma, which would make them ineligible for *EGFR* inhibitors. Other actionable mutations identified in plasma were *BRAF*, *ERBB2*, *MAP2K1* and *MET* (Supplementary Table 10). Twenty-four variants across 10 patients (10.3%) were identified from plasma sequencing which would make them eligible for a clinical trial at the time of reporting.

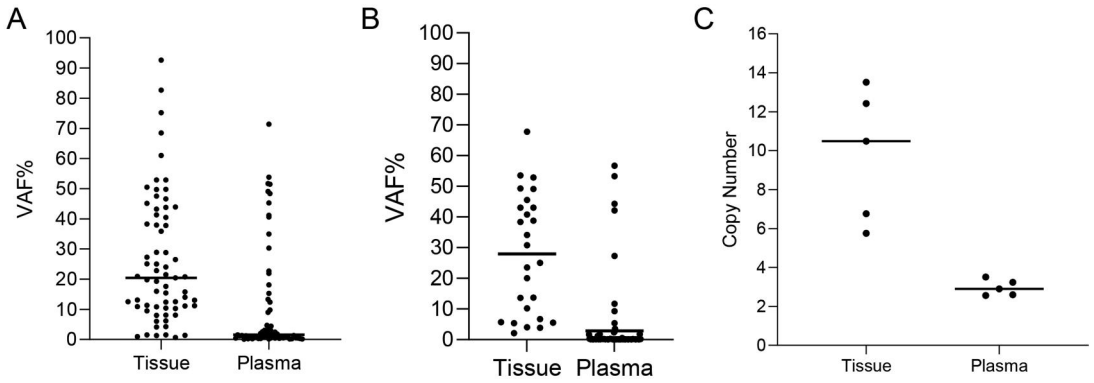

Figure 3. Variant allele frequencies and copy numbers. Scatter plots displaying the distribution of variant allele frequencies (VAF%) of variants detected according to sample type by (A) foundation medicine sequencing, (B) ThermoFisher™ Ion Torrent™ sequencing and (C) copy number of copy number alteration (Ion Torrent™). Line representing median value.

Ion Torrent™ sequencing

Ion Torrent™ sequencing: Tissue

Variants were reported in all tissue samples sequenced ($n = 14$); in total 32 variants were detected: SNV:23 indel:3 fusion:1 CNA:4 (Figure 5). The number of variants detected per sample varied between 1 and 4 (median 2). VAF% ranged between 2.1% and 67.8% (median 27.9%) (Figure 3b and Supplementary Table 7).

Ion Torrent™ sequencing: Plasma

Coverage ranged between 604 and 10,000×, with 96.7% of variants reported at >1000x coverage.

104 variants were detected from 26 patients: SNV:95 fusion:4 CNA:5 (Figure 5). VAF% ranged between 0.1% and 56.7% (median 0.1%). Copy number alterations varied between 2.56 and 3.51 (median 2.9) (Figure 3b,c). The number of variants detected per sample ranged between 0 and 7 (median 3). Only 15.8% (15 out of 95 SNV/indels) of the variants had a VAF > 1% (Supplementary Table 8).

Genomic sequencing of buffy coat

Genomic of the buffy coat of peripheral blood was obtained in 14 patients. Six variants were reported (indel = 6) (Supplementary Table 9). One patient with ovarian high-grade serous carcinoma had a *BRCA1* variant detected in the buffy coat also present in commercial and in-house tissue sequencing and was a known germline *BRCA1* carrier. None of the other variants detected in the buffy coat were reported in either plasma or tissue sequencing.

Comparison of commercial and local sequencing

Tissue comparison

Concordance. The overall concordance for variants from matched tissue samples was 98%. The positive percentage agreement (PPA) was 78.8% and the positive predictive value (PPV) was 56.5%. Across the 10 tissue samples sequenced by both assays, 44 variants reported on commercial sequencing were not detected by Ion Torrent™ sequencing. Of these, 24 were not present on the OCA panel. Therefore, there were 20 private commercial variants from tissue sequencing (SNV:5 indel:10 CNA:5), of which 4 variants were subclonal and two amplifications were equivocal (Table 2). There were seven private in-house variants (SNV:5 indel:1 fusion:1; VAF% range 2.1%–20%) (Table 3).

VAF% correlation. Twenty-six variants were detected on matched tumour samples by both assays (SNV:19 indel:2 amplification:4 fusion:1) (Table 4). Pearson's correlation coefficient of VAF% from sample-matched variants was 0.908 (95% confidence intervals 0.783–0.962, $p < 0.0001$) (Figure 4a).

Actionable variants. Twelve out of 19 (63.2%) of actionable variants reported on FoundationOne® CDx were also detected on Ion Torrent™ tissue sequencing. There was agreement in the *KRAS* status in a patient with colorectal cancer and no *KRAS* or *NRAS* variants were detected in the colorectal cancer patients where commercial sequencing reported wild-type results. Of the

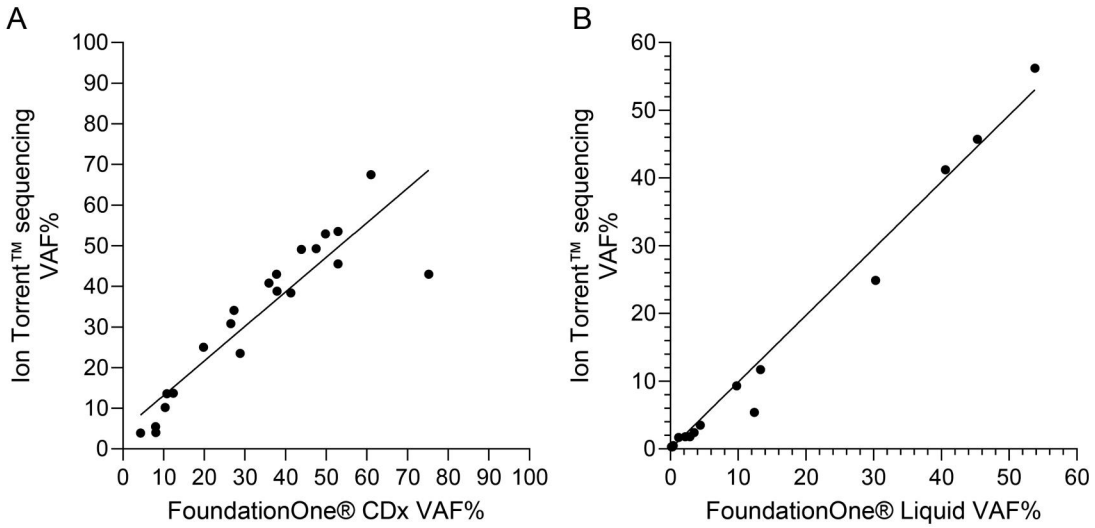

Figure 4. VAF% correlation. Scatter plot to show the correlation of variant allele frequencies (VAF%) of variants detected by both commercial (Foundation Medicine) and local (Ion Torrent™) genomic sequencing from (A) tissue and (B) plasma samples. Line representing the line of best fit. VAF: variant allele frequency.

seven actionable variants not detected, three were either not on the OCA panel or were genomic markers not tested for (*FANCL*, MSI-high, TMB) and the other four represented false negative results *(BRCA2, ERBB2* amplification, *FBXW7*, *MET* amplification).

Fifteeen out of 33 (45.5%) variants identified that would confer eligibility to clinical trial were also detected on Ion Torrent™ sequencing. Of the 18 false negative results, 6 variants were not on the OCA panel (*APC*, *FANCL*, *MTOR* amplification, TMB, MSI, and LoH) and the other 11 representing false negative results (*BRCA2*, *FBXW7*, *KRAS*, *MYC*, *PIK3CA*, *PTEN*, *RNF43*, *TP53*, *ERBB2* amplification, and *MET* amplification).

Plasma comparison

ctDNA detection. Using a definition of ctDNA + ve as any variants detected in pre-surgery plasma sequencing and at any VAF%, 79.3% (23 out of 29) of patients were ctDNA + ve on commercial sequencing and 89.7% (26 out of 29) of patients were ctDNA + ve on Ion Torrent™ plasma sequencing pre-surgery. Of these, 20 were ctDNA + ve on both assays, 2 were ctDNA + ve on commercial sequencing only, 5 were ctDNA + ve on Ion Torrent™ sequencing only and 1 patient was ctDNA-ve on both. ctDNA extraction failed by both testing platforms in one patient (ovarian high-grade serous carcinoma). Concordance for ctDNA classification (+ve/-ve) pre-surgery was 72.4%.

Concordance. The overall concordance for plasma sequencing was 93.9%. The PPA was 16.1% and the PPV was 71.4%.

61 of the variants detected on commercial plasma testing were not detected by Ion Torrent™ sequencing, of which 55 could be explained by not being present on the OPA panel. Therefore, there were six private commercial variants (VAF% range: 0.11%–71.4%) (Table 2).

88 variants were reported on Ion Torrent™ sequencing but not by commercial sequencing (SNV:79 CNA:5 fusion:4) (VAF% range 0.1%–53.3%). Four of these could be explained by not being on the commercial gene panel (*CCDC6* fusion, *ERBB3, VCL* fusion). Therefore, there were 78 private in-house variants, of which only three had a VAF > 1% (*MET* 53.3% gastric cancer, *TP53* 1.2% ovarian cancer, *TP53* 2.6% colorectal cancer) (Table 4).

VAF% correlation. Fifteen mutations were detected in patient-matched plasma samples from both assays (SNV:15) (Table 4). Pearson's correlation coefficient for matched variants was 0.992, 95% confidence intervals 0.976–0.998, $p < 0.0001$ (Figure 4b).

Actionable variants concordance. Of the ten actionable variants reported on commercial plasma

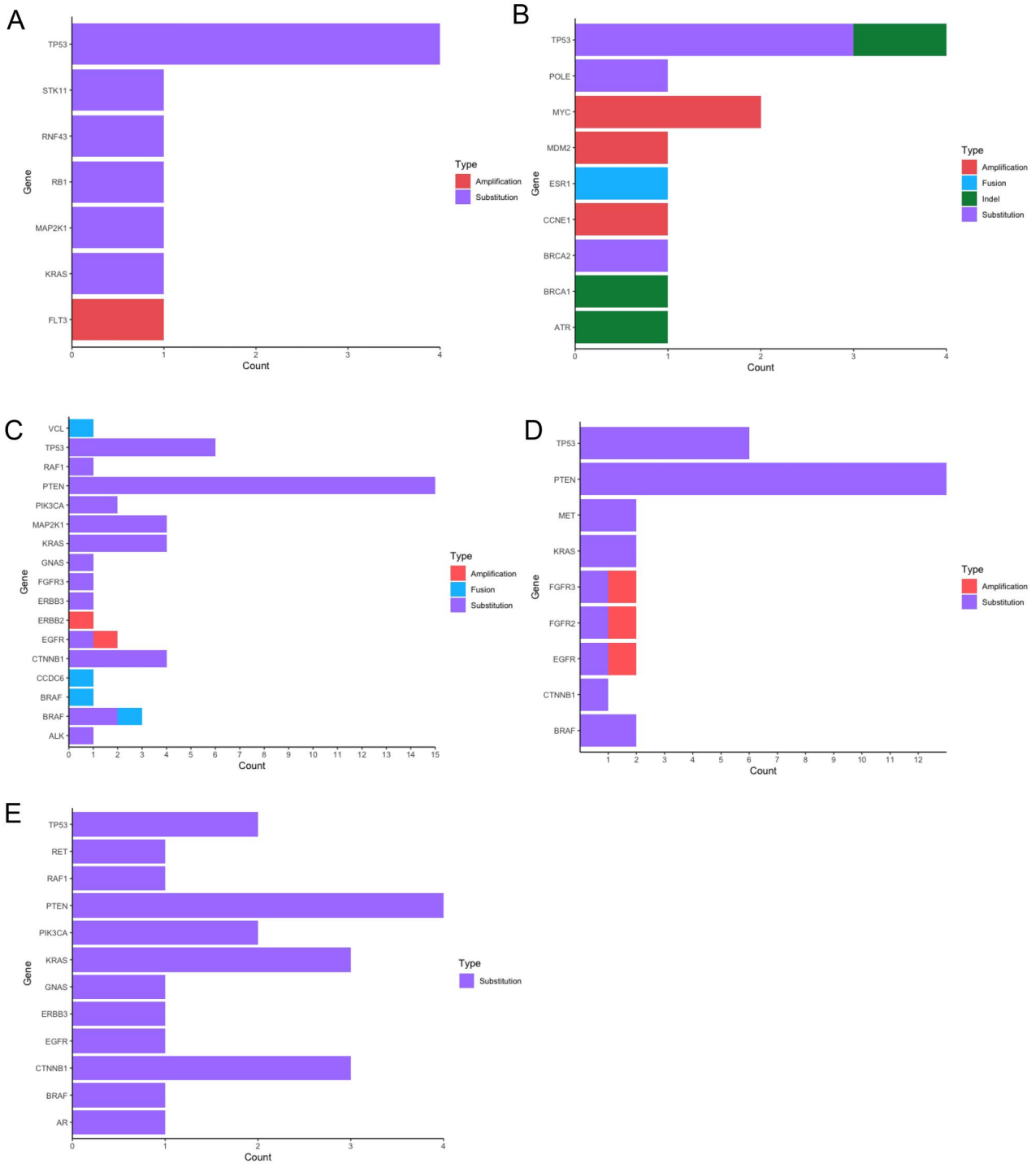

Figure 5. Ion Torrent™ sequencing. Stacked bar charts showing the variants detected on Ion Torrent™ sequencing from tissue (A, B) and plasma (C–E) according to variant type from colorectal (A,C), ovarian (B,D) and gastro-oesophageal (E) primaries. Red – Copy number alteration (amplification), green – indel, and blue – fusion.

sequencing, three (30%) were also detected on Ion Torrent™ sequencing (*BRAF* and *MAP2K1*). Of the seven variants not detected, five could be explained by not being on the OPA panel (*BRCA1*, *ATM*, *CDK12*, and *PALB2*), therefore there were two private commercial actionable variants (*ERBB2* amplification- ovarian high-grade serous carcinoma, *MET*- colorectal adenocarcinoma).

Of the 24 variants detected which confer eligibility to clinical trials, 8 were also detected on Ion Torrent™ sequencing (*BRAF*, *KRAS*, *MAP2K1*, *PIK3CA*, and *TP53*). Of the 16 variants

Table 2. Private commercial variants.

Study ID	Sample type	Gene	Amino acid change	Coding effect	Clonality	VAF%
M01	FFPE-surgery	*PTEN*	N323fs*21	968delA	Subclonal	1.5%
M01	FFPE-surgery	*MYC*	H374R	1121A > G	Subclonal	0.7%
M01	FFPE-surgery	*RNF43*	G659fs*41	1976delG	Clonal	4.1%
M01	FFPE-surgery	*MSH6*	F1088fs*2	3261delC	Clonal	16.%
M01	FFPE-surgery	*NOTCH2*	N1999fs*32	5996delA	Clonal	13.%
M01	FFPE-surgery	*BRCA2*	N863fs*18	2588_2589insA	Clonal	12.5%
M01	FFPE-surgery	*HNF1A*	R271W	872delC	Subclonal	12.5%
M01	FFPE-surgery	*HNF1A*	P291fs*51	811C > T	Clonal	0.93%
M01	FFPE-surgery	*KDR*	P1355del	4063_4065delCCT	Clonal	43.2%
M01	FFPE-surgery	*AID1A*	D1850fs*4	548_5549insG	Clonal	15.4%
M01	FFPE-surgery	*MSH2*	N311fs*5	932_939delACCTTTTT	Clonal	20.9%
M02	FFPE-surgery	*TP53*	C242fs*94	723_750del28	Clonal	28.9%
M03	FFPE-surgery	*FANCL*	NA	Splice site 273 + 2T > G	Clonal	6.2%
M08	FFPE-surgery	*BRD4*	Amplification	Gain	Clonal	Equivocal
M08	FFPE-surgery	*NOTCH3*	Amplification	Gain	Clonal	Detected
M08	Plasma	*TP53*	NA	Splice site 560-1G > A	NA	71.4%
M09	FFPE-diagnostic biopsy	*ERBB2*	Amplification	Gain	Clonal	Detected
M09	FFPE-diagnostic biopsy	*CCNE1*	Amplification	Gain	Clonal	Equivocal
M17	Plasma	*TP53*	E224D	672G > T	NA	1.8%
M18	FFPE-surgery	*CDKN2A/B*	A97V	247C > T	Clonal	8.1%
M19	Plasma	*ERBB2*	Amplification	Gain	NA	0.2%
M20	FFPE-surgery	*PIK3CA*	R88Q	263G > A	Subclonal	1.4%
M26	FFPE-surgery	*MET*	Amplification	Gain	Clonal	Detected
M25	Plasma	*MET*	I1077T	3230T > C	NA	0.11%
M26	Plasma	*TP53*	R282W	844C > T	NA	0.3%
M28	Plasma	*TP53*	H178Q	534C > A	NA	0.3%

Note: Table displaying variants detected on commercial (Foundation Medicine) sequencing not detected on in-house (Ion Torrent™) sequencing.
Abbreviations: FFPE: Formalin-Fixed Paraffin-Embedded; NA: not applicable; VAF: variant allele frequency.

not detected, 13 could be explained by not being present on the OPA panel (*ATM*, *BARD1*, *CCND1*, *CDK12*, *MYC*, *NRAS*, *PALB2*, *PIK3R1*, and *RNF43*) and three represented private commercial actionable variants (*ERBB2* amplification, *MET*, *TP53*).

Comparison of patient-matched tissue and plasma sequencing

Foundation medicine sequencing

Patient-matched tissue and plasma samples from Foundation Medicine sequencing were available in 17 patients. From these, 20 variants were detected in both sample types (SNV: 12 indel: 4 CNA: 4). 73 variants were detected in exclusively in tissue (SNV: 25 indel: 19 CNA: 28 structural: 1) and 15 variants were detected exclusively in plasma (SNV: 10 indels: 4 CNA: 1) (Figure 6). Of the SNV/indels detected exclusively in plasma 10 variants were reported as likely CHIP and 42.9% (6 out of 14) had a VAF < 1% (Supplementary Table 10).

Overall concordance for genes covered by both panels was 99.3%. Overall concordance in MSI was 93.8%, with 15 patients MSI-stable in both tissue and plasma; one patient with gastric adenocarcinoma has MSI-high on tissue sequence despite this not being detected in plasma (Table 5).

There was no correlation between the VAF% of variants detected on both tissue and plasma: $r = 0.1$ 95% CI: 0.19–0.69 $p = 0.22$ (Supplementary Figure 3a).

Ion Torrent™ sequencing

Patient-matched tissue and plasma samples from Ion Torrent™ sequencing were available in 14 patients. 7 variants were detected in both sample types (SNV:7) in *KRAS*, *MAP2K1*, and *TP53*. 40 variants were detected exclusively in plasma (SNV:32 fusion:4 CNA:2) 95% of SNV/indels detected exclusively in plasma were reported with VAF < 1% (Table 5). 25 variants were detected exclusively in tissue (SNV:17 indel:3 CNA:5) (Figure 6), of which 60% (15 out of 25) could be explained by not being present on the OPA panel (*ARID1A*, *ATR*, *BRCA1*, *BRCA2*, *CCNE1*, *FBXW7*, *FLT3* amplification, *MYC* amplification, *MDM2*, *MYD88*, *POLE*, *RB1*, *RNF43*, and *STK11*).

The overall concordance, when considering only genes covered by both panels was 92.2%. There was no correlation between the VAF% from plasma and tissue sequencing ($r = 0.19$ 95% CI-0.89–0.47, $p = 0.33$) (Supplementary Figure 3b).

Table 3. Private in-house variants.

Study ID	Sample type	Gene	Amino acid change	Coding effect	VAF%/ [Copy number]
M01	FFPE-surgery	*CTNNB1*	T41I	122C > T	6.7%
M01	FFPE-surgery	*PTEN*	R335*	1003C > T	5.7%
M01	FFPE-surgery	*PTEN*	R130Q	389G > A	5.5%
M02	Plasma	*MET*	R988C	2962C > T	53.3%
M02	FFPE-surgery	*ATR*	Q2625*	7873_7875delCAGinsTAA	2.1%
M02	FFPE-surgery	*POLE*	Q2217*	6649C > T	20%
M04	Plasma	*EGFR*	G721D	2162G > A	0.1%
M04	Plasma	*KRAS*	A59T	175G > A	0.1%
M04	Plasma	*PTEN*	T131I	392C > T	0.1%
M05	Plasma	*PTEN*	A126V	377C > T	0.1%
M05	Plasma	*TP53*	V197M	589G > A	0.1%
M06	Plasma	*EGFR*	Amplification	Gain	[3.24]
M06	Plasma	*ERBB2*	Amplification	Gain	[2.98]
M06	Plasma	*PTEN*	A126V	377C > T	0.1%
M06	Plasma	*PTEN*	T131I	392C > T	0.1%
M08	Plasma	*FGFR2*	A648T	1942G > A	0.1%
M08	Plasma	*FGFR3*	R399C	1195C > T	0.1%
M08	Plasma	*MET*	R988C	2962C > T	0.1%
M08	Plasma	*PTEN*	A126V	377C > T	0.2%
M08	Plasma	*PTEN*	C136R	406T > C	0.1%
M08	Plasma	*PTEN*	T131I	392C > T	0.1%
M08	Plasma	*FGFR3*	Amplification	Gain	[3.51]
M08	Plasma	*TP53*	NA	Splice site 560-1G > A	71.4%
M09	Plasma	*BRAF*	V600M	1798G > A	0.1%
M09	Plasma	*CTNNB1*	G34E	101G > A	0.1%
M09	Plasma	*CTNNB1*	S33P	97T > C	0.1%
M09	Plasma	*ERBB3*	D297N	889G > A	0.1%
M09	Plasma	*KRAS*	A59T	175G > A	0.1%
M09	Plasma	*RAF1*	S259F	776C > T	0.1%
M10	Plasma	*ERBB3*	V104M	310G > A	0.2%
M10	Plasma	*MAP2K1*	L215P	644T > C	0.1%
M10	Plasma	*PTEN*	A126V	377C > T	0.1%
M11	Plasma	*KRAS*	A59T	175G > A	0.2%
M11	Plasma	*PTEN*	A126V	377C > T	0.1%
M11	Plasma	*PTEN*	R130*	388C > T	0.1%
M12	Plasma	*BRAF*	A598T	175G > A	0.1%
M12	Plasma	*PTEN*	A126V	377C > T	0.1%
M12	Plasma	*PTEN*	R130*	388C > T	0.1%
M13	Plasma	*PTEN*	A126V	377C > T	0.2%
M13	Plasma	*TP53*	R248Q	743G > A	1.2%
M13	FFPE-surgery	*ESR1*	Fusion	Fusion	Detected
M14	Plasma	*CTNNB1*	S33P	97T > C	0.1%
M14	Plasma	*PTEN*	A126V	377C > T	0.2%
M15	Plasma	*EGFR*	Amplification	Gain	[2.6]
M15	Plasma	*FGFR2*	Amplification	Gain	[2.56]
M17	Plasma	*CTNNB1*	G34E	101G > A	0.1%
M17	Plasma	*EGFR*	G721D	2162G > A	0.1%
M17	Plasma	*RET*	R886W	2656C > T	0.1%
M18	Plasma	*CTNNB1*	G34E	101G > A	0.3%
M18	Plasma	*PTEN*	A126V	377C > T	0.2%
M19	Plasma	*TP53*	R175H	524G > A	0.1%
M19	Plasma	*BRAF*	G596D	1787G > A	0.1%
M20	Plasma	*CTNNB1*	G34E	101G > A	0.1%
M20	Plasma	*PTEN*	T131I	392C > T	0.2%
M20	Plasma	*CCDC6*	Fusion	Chr21:chr10	Detected
M21	Plasma	*KRAS*	A59T	175G > A	0.2%
M21	Plasma	*PTEN*	C136Y	407G > A	0.1%
M21	Plasma	*PTEN*	A126V	377C > T	0.1%
M22	Plasma	*PTEN*	A126V	377C > T	0.1%
M22	Plasma	*PTEN*	T131I	392C > T	0.2%
M23	Plasma	*PTEN*	A126V	377C > T	0.2%
M23	Plasma	*PTEN*	T131I	392C > T	0.1%
M23	Plasma	*RAF1*	S259F	776C > T	0.1%
M23	Plasma	*BRAF*	Fusion	Chr7:chr7	Detected
M24	Plasma	*FGFR3*	R248H	743G > A	0.1%
M24	Plasma	*TP53*	G245S	733G > A	0.1%
M25	Plasma	*CTNNB1*	S33P	97T > C	0.1%
M25	Plasma	*PTEN*	C136Y	407G > A	0.1%
M25	Plasma	*PTEN*	T131I	392C > T	0.1%
M25	Plasma	*TP53*	C275F	824G > T	2.6%
M25	Plasma	*VCL*	Fusion	Chr22:chr10	Detected

(*continued*)

Table 3. Continued.

Study ID	Sample type	Gene	Amino acid change	Coding effect	VAF%/ [Copy number]
M26	Plasma	*CTNNB1*	G34E	101G > A	0.2%
M26	Plasma	*PIK3CA*	H1048Y	3142C > T	0.5%
M26	Plasma	*PTEN*	A126V	377C > T	0.2%
M26	Plasma	*PTEN*	C136R	406T > C	0.1%
M26	Plasma	*PTEN*	T131I	392C > T	0.1%
M26	Plasma	*PTEN*	R130*	388C > T	0.2%
M26	Plasma	*BRAF*	Fusion	Chr7:chr7	Detected
M26	FFPE-surgery	*STK11*	Q432*	1294C > T	5.4%
M27	Plasma	*AR*	F877L	2629T > C	0.3%
M27	Plasma	*KRAS*	A59T	175G > A	0.1%
M27	Plasma	*PIK3CA*	E81K	241G > A	0.1%
M27	Plasma	*PIK3CA*	A1046V	3137C > T	0.2%
M27	Plasma	*PTEN*	R173H	518G > A	0.2%
M28	Plasma	*TP53*	R175G	523C > G	0.3%
M28	Plasma	*KRAS*	A59T	175G > A	0.1%
M28	Plasma	*PTEN*	A126V	377C > T	0.2%
M28	Plasma	*PTEN*	C136R	406T > C	0.1%
M28	Plasma	*PTEN*	T131I	392C > T	0.1%
M29	Plasma	*CTNNB1*	S33P	97T > C	0.1%
M29	Plasma	*EGFR*	T790M	2369C > T	0.1%
M29	Plasma	*MAP2K1*	L215P	644T > C	0.1%
M30	Plasma	*ALK*	G1123D	3368G > A	0.1%
M30	Plasma	*BRAF*	D594N	1780G > A	0.1%
M30	Plasma	*KRAS*	A59T	175G > A	0.1%
M30	Plasma	*PTEN*	A126V	377C > T	0.2%

Note: Table displaying variants detected on in-house local (Ion Torrent™) sequencing not commercial (Foundation Medicine) sequencing.
Abbreviations: FFPE: Formalin-Fixed Paraffin-Embedded; NA: not applicable; VAF: variant allele frequency.

Discussion

The development of next-generation sequencing (NGS) over the preceding decades has provided the ability to uncover large amounts of a tumour's genome (4,5). This provides an unparalleled opportunity to deepen our understanding of the genetic mechanisms underlying carcinogenesis. Moreover, genomic testing can improve patient outcomes through the implementation of precision medicine with the potential to revolutionise cancer management (2). The ever-increasing catalogue of approved targeted therapies has created an unmet need for local provision of genomic testing. At present, genomic sequencing is mainly performed commercially. However, this is expensive with long sample turnover times and hence has limited capacity for upscaling. Here, we outline the comparison of results from two different sequencing platforms and to evaluate the utility of upfront genomic testing in cancer patients awaiting surgical resection. We compared reports from patient-matched tissues and plasma samples between in-house sequencing using ion semiconductor-based technology against commercial sequencing with the Foundation Medicine platforms, performed as orthogonal sequencing for independent validation

Overall, we demonstrate good concordance, supporting the use of local genomic sequencing for routine molecular diagnostics within this small single-centre exploratory study. Where matched variants were identified in both assays, there was good quantitative agreement, with a strong correlation between VAF%; this relationship was observed in both tissue and plasma. This effect has previously been observed between PCR and NGS-based techniques, different NGS platforms and varying sequencing depths (25,26) and here we demonstrate that this is also observed between commercial and local genomic sequencing.

The majority of discordances were seen at VAF $< 1\%$, highlighting the need for improved assay performance at the lower limit of detection (LoD). Concordance of plasma sequencing was lower than that of tissue sequencing and the low PPA in plasma sequencing reflects numerous variants reported at low VAF% in local sequencing. These may represent either detection of variants below the LoD of commercial sequencing or sequencing errors in in-house sequencing from mutational biases of the sequencing technology, and demonstrate the requirement for further bioinformatic processing before validation and clinical assimilation.

Table 4. Variants detected by both commercial and in-house sequencing.

Study ID	Cancer primary	Sample type	Gene	Amino acid change	VAF% tissue	VAF% plasma
M01	Gastric adenocarcinoma	FFPE	*ARID1A*	R1722*	12.4%	13.7%
M01	Gastric adenocarcinoma	FFPE	*KRAS*	A146T	10.4%	10.2%
M02	Ovarian high-grade serous carcinoma	FFPE	*BRCA2*	S1855*	35.9%	40.8%
M03	Ovarian high-grade serous carcinoma	FFPE	*BRCA1*	Q1111fs*5	52.9%	53.5%
M03	Ovarian high-grade serous carcinoma	FFPE	*TP53*	N239fs*1	10.8%	13.6%
M05	Ovarian high-grade serous carcinoma	FFPE	*TP53*	R248W	37.8%	43%
M05	Ovarian high-grade serous carcinoma	FFPE	*MYC*	Amplification	NA	NA
M08	Ovarian high-grade serous carcinoma	FFPE	*MDM2*	Amplification	NA	NA
M08	Ovarian high-grade serous carcinoma	FFPE	*MYC*	Amplification	NA	NA
M09	Oesophageal adenocarcinoma	FFPE	*FBXW7*	R505C	52.9%	45.5%
M09	Oesophageal adenocarcinoma	FFPE	*TP53*	P151S	75.2%	43%
M13	Ovarian high-grade serous carcinoma	FFPE	*TP53*	R248Q	49.8%	52.9%
M18	Laryngeal squamous cell carcinoma	FFPE	*CDKN2A*	H83Y	8.1%	4%
M18	Laryngeal squamous cell carcinoma	FFPE	*MYD88*	L260P	8%	5.5%
M18	Laryngeal squamous cell carcinoma	FFPE	*PIK3CA*	E542K	4.3%	3.9%
M20	Colorectal adenocarcinoma	FFPE	*TP53*	G245C	61%	67.5%
M22	Ovarian high-grade serous carcinoma	FFPE	*TP53*	V272G	47.5%	49.3%
M22	Ovarian high-grade serous carcinoma	FFPE	*CCNE1*	Amplification	NA	NA
M23	Colorectal adenocarcinoma	FFPE	*MAP2K1*	K57N	19.8%	25%
M23	Colorectal adenocarcinoma	FFPE	*RNF43*	R40*	43.9%	49.1%
M23	Colorectal adenocarcinoma	FFPE	*TP53*	R248Q	26.5%	30.8%
M26	Colorectal adenocarcinoma	FFPE	*TP53*	R282W	28.8%	23.5%
M29	Colorectal adenocarcinoma	FFPE	*FLT3*	Amplification	–	–
M30	Colorectal adenocarcinoma	FFPE	*KRAS*	K117N	41.3%	38.4%
M30	Colorectal adenocarcinoma	FFPE	*RB1*	E48*	37.9%	38.8%
M30	Colorectal adenocarcinoma	FFPE	*TP53*	P250L	27.3%	34.1%
M05	Ovarian high-grade serous carcinoma	Plasma	*TP53*	R248W	9.8%	9.3%
M06	Colorectal adenocarcinoma	Plasma	*BRAF*	V600E	30.3%	24.9%
M06	Colorectal adenocarcinoma	Plasma	*PIK3CA*	H1047R	45.3%	45.7%
M06	Colorectal adenocarcinoma	Plasma	*TP53*	R273G	53.8%	56.2%
M09	Oesophageal adenocarcinoma	Plasma	*GNAS*	R201S	0.18%	0.3%
M15	Ovarian high-grade serous carcinoma	Plasma	*TP53*	E286K	40.6%	41.2%
M19	Ovarian high-grade serous carcinoma	Plasma	*TP53*	G245S	4.4%	3.5%
M20	Colorectal adenocarcinoma	Plasma	*TP53*	G245C	0.43%	0.5%
M23	Colorectal adenocarcinoma	Plasma	*MAP2K1*	K57N	12.4%	5.4%
M23	Colorectal adenocarcinoma	Plasma	*TP53*	R248Q	13.3%	1.17%
M25	Colorectal adenocarcinoma	Plasma	*KRAS*	G13D	2.9%	1.8%
M25	Colorectal adenocarcinoma	Plasma	*MAP2K1*	K57N	3.5%	2.4%
M28	Oesophageal adenocarcinoma	Plasma	*TP53*	G266R	1.2%	1.7%
M30	Colorectal adenocarcinoma	Plasma	*KRAS*	K117N	0.31%	0.3%
M30	Colorectal adenocarcinoma	Plasma	*GNAS*	R201H	2.2%	1.8%

Note: Table displaying the variants detected by both commercial (FoundationOne® CDx) and in-house (Ion Torrent™) sequencing from patient-matched samples.

Abbreviations: FFPE: formalin fixed paraffin embedded; NA: not applicable; VAF: variant allele frequency.

Discordance in variant detection between methodologies may arise due to biological or analytical factors. Biological factors include heterogeneity, CHIP and germline mutations. In tissue samples, intra-tumour heterogeneity from clonal evolution causes regional differences within a tumour. Tumour cellularity varies across the tumour, affecting variant detection rate between samples (8) and in plasma, variation in tumour shedding cause differences in the proportion of tumour-derived cell-free DNA. Inclusion of matched non-malignant samples can be used to identify germline mutations (2,27), and is not usually offered in commercial sequencing packages. In plasma sequencing this can also be used for identifying CHIP variants (28). In this study we demonstrate the ability of buffy coat sequencing to identify false positive plasma variants of germline origin, however buffy coat sequencing did not identify any variants from CHIP.

Analytical properties of the testing platform that can introduce discrepancies in genomic reporting including library preparation, LoD and bioinformatic pipelines (3,29). Technical errors during sequencing, base-calling errors or PCR amplification errors. Base calling errors may arise due to optical errors or cytosine deamination during sample preparation (27) Lower LoD enables greater sensitivity and detection of emerging clonal variants. This is of relevance in clinical settings where tumour bulk is low, including minimal residual disease and early cancer detection,

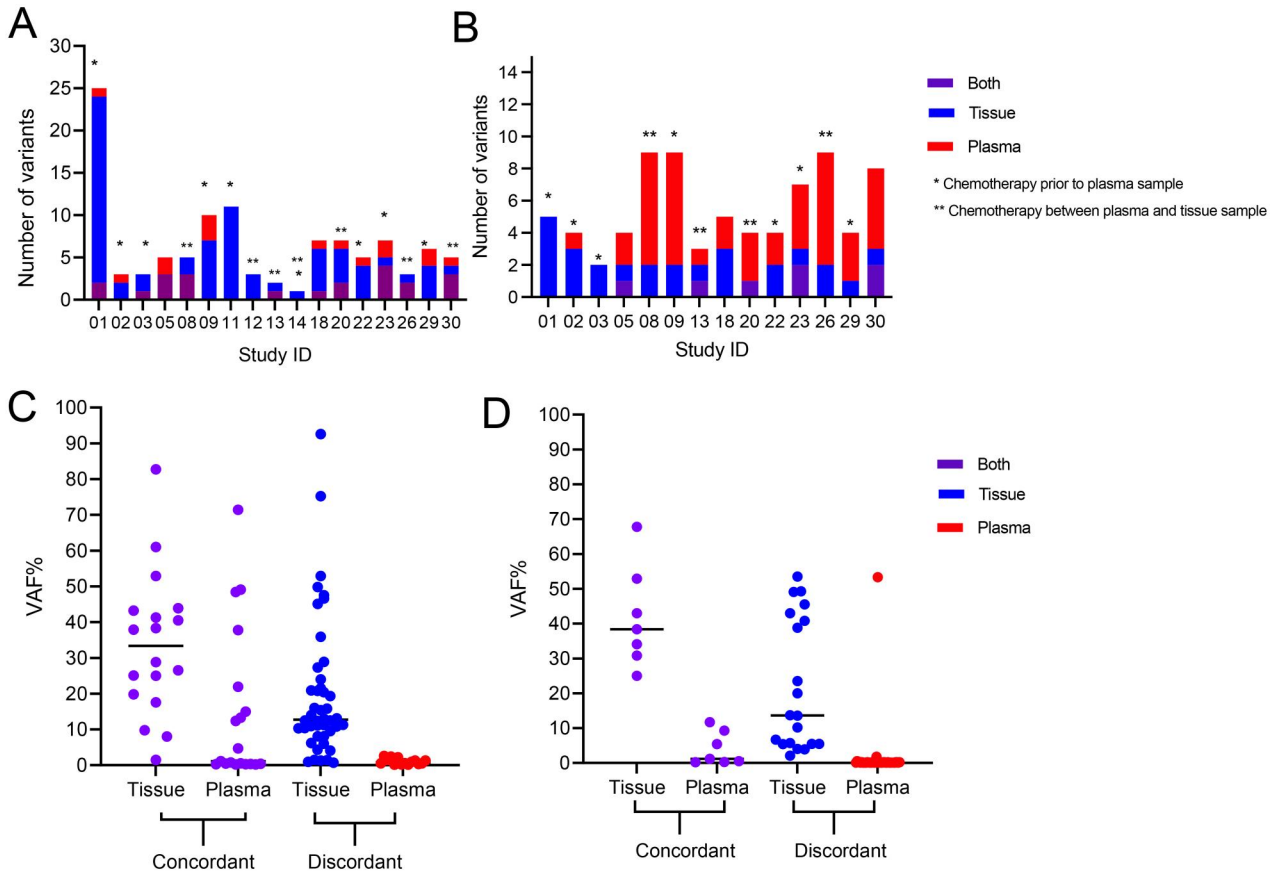

Figure 6. Tissue and plasma sequencing comparison. Stacked bar chart displaying the number of variants detected in tissue (blue), plasma (red) or both (purple) from matched patient samples from (A) Foundation Medicine and (B) Ion Torrent™ sequencing. *chemotherapy prior to plasma sample **chemotherapy between plasma and tissue sample. Scatterplots displaying variant allele frequency (VAF%) according to the sample type variant detected in (red – plasma, blue – tissue, andpurple – both) from patient-matched samples from C) Foundation Medicine and D) Ion Torrent™ sequencing. VAF: variant allele frequency.

where tumour-derived ctDNA will only constitute a small proportion of the total cell-free DNA. However, lower LoD comes at the cost of false positive results, with artefacts arising from the detection of sequencing errors.

Concordance between commercial sequencing platforms has previously been investigated in a number of settings. Comparison of plasma NGS analysis across four independent commercial platforms was evaluated, through analysis of plasma of 24 patients with early-stage cancer. They observed significant discordance particularly at lower VAF% and rarer genetic variants, with mutational biases specific to individual tests (30), and conclude that technical factors are a major contributor to disconcordance (31). Similarly, comparison of two widely used commercial plasma sequencing tests: Guardant 360® (Guardant Health) and Foundation One® Liquid (Roche™) across nine cancer patients reported low concordance rates (22%) when comparing genes covered by both gene panels, with greater discordance at lower VAF%. They also report low agreeance (25%) in reporting suitable therapeutic agents (32), highlighting the need for a focus on determining drug eligibility in the standardisation and validation between commercial testing platforms. However, differences in sample collection times may have contributed to the differences seen.

Comparison between laboratory sequencing assays has also been investigated. Sequencing of 44 tissue samples performed to validate GeneReader (QIAGEN) compared to reference sequencing with Ion Torrent™ (ThermoFisher™) demonstrated 100% concordance for detection of variants and strong correlation between reported VAF% (26).

To our knowledge this is one of the few papers published describing comparison of local sequencing against a commercial platform. The AVENIO

Table 5. Variant detected in patient-matched tissue and plasma.

Patient ID	Cancer primary	Sequencing platform	Gene	Amino acid change	VAF% commercial sequencing	VAF% in-house sequencing
M03	Ovarian high-grade serous	Commercial	*KDR*	P1355del	43.2%	49.1%
M03	Ovarian high-grade serous	Commercial	*BRCA1*	Q1111fs*5	52.9%	48.4%
M03	Ovarian high-grade serous	Commercial	*TP53*	R248W	9.8%	37.8%
M03	Ovarian high-grade serous	Commercial	*TP53*	splice site 560-1G > A	82.7%	71.4%
M03	Ovarian high-grade serous	Commercial	*MLL2*	P3091fs*29	25.1%	0.77%
M23	Ovarian high-grade serous	Commercial	*MYD88*	L265P	8%	1.1%
M23	Ovarian high-grade serous	Commercial	*APC*	E1309fs*4	38.3%	0.37%
M23	Ovarian high-grade serous	Commercial	*TP53*	G245C	61%	0.43%
M23	Ovarian high-grade serous	Commercial	*MAP2K1*	K57N	19.8%	12.4%
M23	Ovarian high-grade serous	Commercial	*RNF43*	R40*	43.9%	21.9%
M23	Ovarian high-grade serous	Commercial	*FLCN*	splice site 1177-1G > T	25%	15%
M23	Ovarian high-grade serous	Commercial	*TP53*	R248Q	26.5%	13.3%
M23	Ovarian high-grade serous	Commercial	*APC*	W421*	17.6%	0.21%
M23	Ovarian high-grade serous	Commercial	*TP53*	R282W	28.8%	0.23%
M30	Ovarian high-grade serous	Commercial	*KRAS*	K117N	41.3%	0.31%
M30	Ovarian high-grade serous	Commercial	*APC*	L1488fs*19	40.5%	0.54%
M30	Ovarian high-grade serous	Commercial	*RB1*	E48*	37.9%	0.29%
M01	Gastric adenocarcinoma	In-house	*KRAS*	P1355del	43%	9.3%
M05	Ovarian high-grade serous	In-house	*TP53*	R248W	52.9%	1.2%
M20	Colorectal adenocarcinoma	In-house	*TP53*	G245C	25%	11.7%
M22	Ovarian high-grade serous	In-house	*TP53*	P177T	67.8%	0.5%
M23	Colorectal adenocarcinoma	In-house	*MAP2K1*	K57N	30.8%	5.4%
M23	Colorectal adenocarcinoma	In-house	*TP53*	R248Q	34.1%	0.2%
M30	Colorectal adenocarcinoma	In-house	*KRAS*	K117N	38.4%	0.3%

Note: Table displaying variants detected in patient-matched tissue and plasma samples from commercial (Foundation Medicine) or in-house (IonTorrent™) sequencing.
Abbreviations: FFPE: formalin fixed paraffin embedded; NA: not applicable; VAF: variant allele frequency.

ctDNA assay (Roche™) was compared against commercial testing in 12 cancer patients. They also report high rates of discordances at lower LoD, demonstrating 100% sensitivity in detecting variants with VAF > 0.5%, and only 50% sensitivity at 0.1% (33).

Study provided a comparison NGS technologies, sequencing by synthesis (Illumina® platforms) and ion semiconductor sequencing. Ion semiconductor sequencing has lower DNA input requirements and shorter run times, favouring its use in clinical laboratories (7,34). However, this method has a higher error rate in homopolymer regions (5,34), which may account for some of the low-level variants seen. Sequencing techniques differ in their ability to detect different variant classes (8). In this study, commercial sequencing displayed a higher fidelity in detecting indels, particularly in plasma, with none of the 20 indels reported across the cohort detected in local sequencing. Gene fusions are technically challenging to detect; of note, no fusions were reported on commercial sequencing from any of the samples, with a total of 5 (plasma-4 tissue-1) fusions detected on in-house sequencing, of which 2 were inter-chromosomal. Inclusion of RNA in extraction in Ion Torrent™ sequencing facilitates more powerful detection of structural rearrangements, as opposed to Foundation Medicine DNA-based assays. Conversely, a chromosome duplication in a patient with colorectal adenocarcinoma was detected by commercial but not local sequencing. Also of note, in CNA analysis, commercial sequencing reported both losses and gains, whereas in local sequencing only copy number gains were reported. Upfront genomic testing has been demonstrated to improve overall survival, through enhanced identification of clinically actionable mutations (35,36). In cancer primaries with multiple targeted therapy options available, the high multiplexing capacity of genomic testing can reduce costs and save laboratory staff time (37). In this study, 76.7% of patients had clinically relevant results (actionable or conferring eligibility for trials) in either plasma or tissue, demonstrating a high degree of potential clinical utility in upfront genomic testing in this setting.

This study also provided the opportunity to investigate the concordance between patient-matched tissue and plasma sequencing. The representation of variants in plasma remains relatively undetermined, yet has significant implications for the clinical applicability of liquid biopsy testing.

Limitations of this analysis were that plasma and tissue samples were obtained at different time points, particularly where patients had received chemotherapy in the interim. Overall concordance between tissue and plasma sequencing was high, however this was mainly due to consensus in reporting the absence of variants. Additional variants were identified exclusively in either tissue or plasma in all patients. Variants not detectable in the plasma may reflect low tumour shedding or VAF% below the LoD of the assay, as ctDNA comprises only a small proportion of total cell-free DNA. This suggests an ancillary role of a liquid biopsy for genetic profiling of cancers, as opposed to being adopted as the exclusive testing method. The majority of variants detected exclusively in plasma were detected at VAF $< 1\%$. These may arise due to regional heterogeneity within a tumour with varying genomic profiles across a tumour as a result of clonal evolution or false positive variants from CHIP.

Gene panel construction remains a key consideration in establishing local genomic testing (3). Tumour-agnostic panels facilitate batching of samples providing scalability and are thus more likely suitable for widespread adoption. WGS/WES are unlikely to be feasible and panel testing prioritises clinically relevant variants offer lower cost testing with increased coverage. Here, we explored the utility of 161 (tissue) or 52 (plasma) tumour-agnostic gene panels for upfront genomic sequencing pre-surgery. Differences in gene panel accounted for 40.9% and 55.9% of initial incongruities between sequencing platforms in plasma and tissue respectively. The narrower gene panels compared to commercial platforms omitted to detect 17 currently therapeutically actionable variants across the cohort, suggesting the need for careful evaluation of genes to be incorporated. Gene panel construction requires continuous re-evaluation as further novel targeted therapies gain approval. Genomic testing can also incorporate genomic characteristics, including tumour TMB, MSI and homologous recombination markers. These markers of genomic instability are increasingly becoming of clinical significance; TMB and MSI predict response to immunotherapy and can be used to determine treatment eligibility (38,39). Evidence of HRD, such as LoH predicts sensitivity to PARP inhibition (40). In this study, these markers of genomic instability were tested for in the commercial but not the in-house laboratory analysis and resulted in identification of potential therapeutic targets in two patients. In addition, variants of unknown significance (VUS) were reported by commercial sequencing and the classification of pathological mutations requires continuous iteration.

Liquid biopsy is a novel and emerging cancer biomarker which holds significant potential for advancing cancer investigation and in a range of different clinical applications (10). In addition to determining treatment eligibility, as outlined above, the presence of ctDNA can have prognostic implications. In particular, post-operatively, the ongoing presence of ctDNA indicates minimal residual disease and is associated with higher rates of disease relapse (10,41). The non-invasive nature of testing also makes it amenable to longitudinal testing by facilitating repeat sample collection, providing utility in treatment monitoring during systemic therapy, enabling early detection of disease progression or drug resistance (10).

Sample turnaround time for commercial testing was in the duration of two weeks from sample collection to obtaining the report. Local testing could potentially process samples within 36 hours and bypasses transport delays. However, this does not incorporate time for data analysis, and the bioinformatic processing that would be delivered at a local level remains largely undetermined (2,42) Following this, there remains the additional challenge of producing meaningful clinical reports interpretable to clinicians, to ensure clinical utility (2,3).

Local provision of NGS in clinical laboratories would bring significant cost-benefits, providing a lower cost alternative to commercial genomic sequencing. This will minimise the financial burden on healthcare systems in delivering precision medicine and enable an expansion in patient testing. Local testing mitigates transport costs and batch testing of samples would also further reduce costs. This would facilitate upscaling of testing, increasing patient access and equity in testing availability between centres (43,44).

Intra-laboratory and inter-run variability may also exist. Automated sequencing workflows will help minimise inter-operator and inter-run variability (18); in this study, local sequencing combined a single automated workflow for library preparation, sequencing and variant calling (17). Variant annotation and quality control should also be objective where feasible (27). Requirements for internal validation and verification processes to assess the performance metrics of the assay will also help mitigate inter-laboratory variation (45) with cross-site assessment of performance measures including reproducibility, sensitivity, specificity and limits of detection (18).

Overall, this study highlights a number of issues that need to be addressed before validation of local NGS testing. Assay performance at the lower LoD remains the most critical challenge to be addressed. Coverage remains fundamental to correct identification of low-level variants (11). Gene panels incorporating only the most clinically relevant genomic regions would maximise efficiency of the sequencing capacity towards accuracy of pertinent regions. This will also minimise bioinformatic processing and data storage requirements and incidental findings (4). Wider exploratory panels are better suited to research settings. Ancillary tests for specific genomic alterations could be available on clinicians' request, particularly those relevant to rare tumour types (44), for example actionable fusions can be more accurately assessed by RNA-based assays (2). However, tumour-agnostic biomarkers that confer eligibility across cancer types including TMB should be included (44).

Given the need to optimise coverage, DNA extraction upstream to sequencing should be rigorous (27), particularly when analysing FFPE samples which yield lower DNA inputs due to degradation and fragmentation during the fixation process (2). A minimum tumour purity threshold could also be applied (18).

Following variant detection, confirmatory testing with PCR-based methods can be applied. Alternatively, read-level molecular barcoding through unique molecular identifiers (UMI) offers a power technology to discriminate sequencing errors and PCR duplicates from true variants (11). This would incur additional costs and technical processing and make the sequencing data more computationally complex to process, so would likely be impracticable to implement as routine initially. We propose that these complementary testing methods could be utilised for variants with VAF $<$ 1%, particularly when arising in difficult-to-sequence regions and where detection would alter clinical management or at the treating clinician's request.

In addition, we propose that matched buffy-coat sequencing should be adopted as standard in local liquid biopsy sequencing for identification of germline and CHIP variants. This will enable identification of positive results as well as having wider clinical implications where testing includes genes with a hereditary risk.

The main limitation of this study was the small sample, with observations based on a small number of events. This was further compounded by heterogeneity arising from inclusion of different cancer types; however this provided generalisability to the findings and further testing in other cancer primaries would further increase the generalisability. Samples were collected as part of a pilot trial with no sample size calculations and larger studies are required to draw more robust conclusions. Technological constraints included multiple comparative factors made been the two methodologies (i.e. local/commercial, gene panels, NGS sequencing technology) and comparison of local sequencing with a more similar NGS platform may help better define the accuracy of this assay. Advantages of the study design were that genomic comparisons were performed on patient-matched samples collected at the time.

Conclusions

Overall, this study supports the use of local genomic testing with ion semiconductor-based technologies for routine molecular diagnostics in this small single-centre study. NGS technologies exhibit differing mutational biases and abilities to detect categories of variants particularly structural alterations, with local sequencing demonstrating higher fidelity of detection of gene fusions but lower fidelity in detecting indels. We highlight some of the challenges that need to be addressed before widespread implementation, including panel

design, requirements for germline testing and consensus on the bioinformatic processing that will be available. Analytic processing of variants detected at low VAF% requires urgent attention and remains the most critical issue. To this end, we recommend clinical gene panels are initially narrow to maximise coverage in pertinent regions, rigorous DNA extraction and future consideration of complementary technologies for distinguishing errors or confirming variants reported. Tumour-agnostic panels are more applicative for batch testing in clinical practice but must incorporate all actionable targets for the individual patient. Gene panel construction should also consider genomic regions of interest (eg. non-coding regions), variant types (ie. SNV, indels, CNA, fusions, structural rearrangements), classification of VUS and incorporation of markers of genomic instability.

Author contributions

Conceptualisation: LH, AT, JS. Funding acquisition: LH, ZS, AT. Data curation: LF, SL, CC, ZS. Project administration: ZS, LF. Abstract writing: LF, LH, AT. Supervision: AT, JS.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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Data availability statement

Data that supports the findings of this study are available in Tables 2–5 and Supplementary Tables 3–12 of this article. Further details are available from the corresponding author lucy.faulkner2@nhs.net upon reasonable request

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