Poster #222-B

Critical clinical evaluation of plasma to tumor tissue concordance by cancer type using Illumina's cell-free ctTSO500 commercial liquid biopsy assay

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Background

Characterization of circulating tumor DNA (ctDNA) from plasma can provide a secondary source for DNA from solid tumor cancer patients where a biopsy poses a health risk. Plasma-sourced DNA can yield copy number variations, snv/indel, MSI and TMB status comparable to data from tissue-sourced DNA.
Jiang *et al* (2020) exampled a 141-patient lung cancer cohort demonstrating correlations between variants in FFPE and plasma:
Stage I NSCLC patients - 0.12 correlation (N=44)
Stage II NSCLC - 0.58 correlation (N=23)
Stage III - 0.56 correlation (N=18)
Stage IV - 0.74 correlation (N=56)
Guo *et al.* (2018) demonstrated 0.55 correlation between FFPE and plasma DNA for Stage I/II NSCLC patients and 0.80 correlation in Stage IV/metastatic NSCLC patients
Zoughbi *et al.* (2021) showed 77% of alterations (fusions, amplifications, variants) were concordant between FFPE and plasma in patients with a primary non-metastatic tumor across 8 different tumor types while patients with metastases showed a 45% correlation between FFPE and plasma.
Nakamura *et al.* (2022) reported MSI (MicroSatellite Instability) from plasma having a 71.4% positive predictive value from 637 MSI-Low and 15 MSI-High patients.
Willis *et al.*, (2019) reported 71/82 MSI-High Stage III/IV colorectal adenocarcinoma patients as measured by solid tissue NGS matched Guardant's ctDNA assessment.
Lee *et al.* (2022) and Friedlaender *et al.* (2020) showed a pan-cancer TMB positive predictive value between tissue and plasma at ~75%.

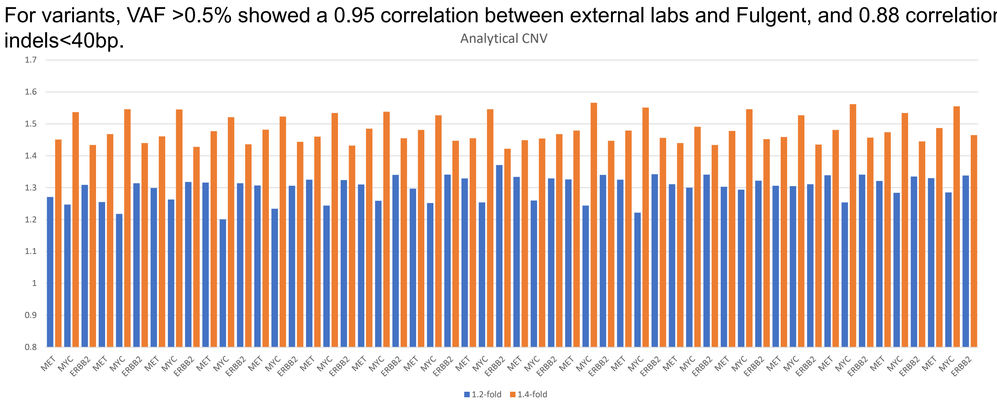
We examined 113 patient samples where a FFPE biopsy was available and plasma was taken within 72 hours of the biopsy, pre-treatment. No other screen was applied. Patients were all Stage III/IV, no mets. We tested Illumina's TSO500 assay for ctDNA to evaluate the concordance across cancer types for snv, indel, copy number, MSI and TMB.

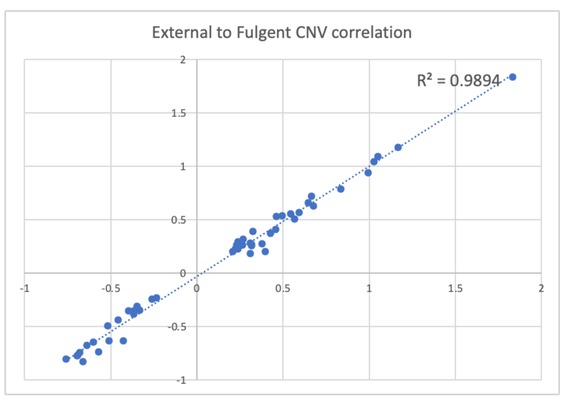
Methods

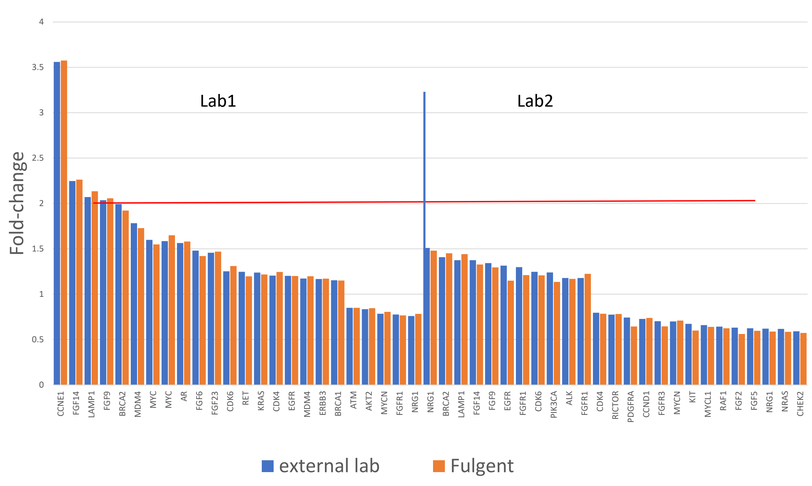
Sample Preparation and Library Generation:
For this comparative analysis: Microdissected samples were selected by receiving pathologist.
DNA Extraction: cf DNA extraction was performed using the Qiagen QIAmp Circulating Nucleic Acid Kit (cat no 55114) according to manufacturer's recommendations. All DNA extractions are logged and measured for quantity.
Library Preparation: TruSight Oncology 500 ctDNA Panel Kit contains the full manufacturer instructions and recommendations. All library construction quantity and quality metrics are logged.
Library Sequencing: NovaSeq 6000 S4 was used for panel library sequencing. All quality metrics are generated by Illumina's software.
Sequence alignment and variant calling was performed using Illumina's DRAGEN TruSight Oncology 500 software version 2.1. Results in this study include small variants (SNVs/INDELs), CNV, MSI, and TMB. All orthogonal testing was done using labs that also followed this recommended preparation and bioinformatic protocol.

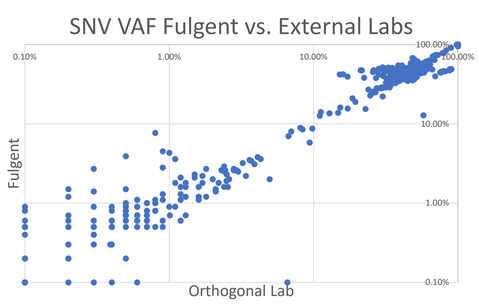
Analytical Results

We wished to establish ctTSO500 consistency regardless of nuisance factors. We examined spike-in samples set to 1.2 and 1.4-fold ratios in MET, MYC, ERBB2 genes, and measured those samples 18 times across days/operators/reagent lots. Reproducibility was 100% for both spike-in levels.
To ensure our lab matches an external lab, we sent 18 cases to lab#1, 23 cases to lab#2, with 1 case being common across both labs to check performance.
CNV's were 100% concordant at ≥2-fold and less.
For variants, VAF >0.5% showed a 0.95 correlation between external labs and Fulgent, and 0.88 correlation for indels<40bp.









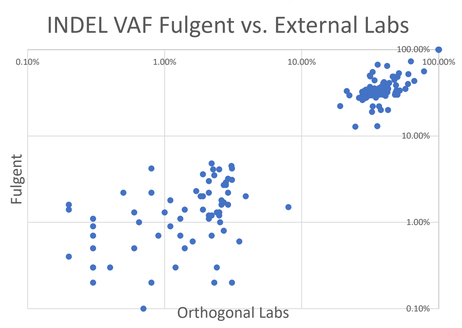


Figure 1: concordance and precision

CNV measurements were measured to ensure sensitivity. Top bar chart shows 100% reproducibility across 18 different runs of the same ERBB2, MET and MYC genes. Middle left scatter plot shows the correlation of CNV's detected in the reportable ctTSO500 genes between Fulgent and the external labs. Middle right bar chart shows the concordance in reported fold-change for the 32 different genes shown from clinical samples.

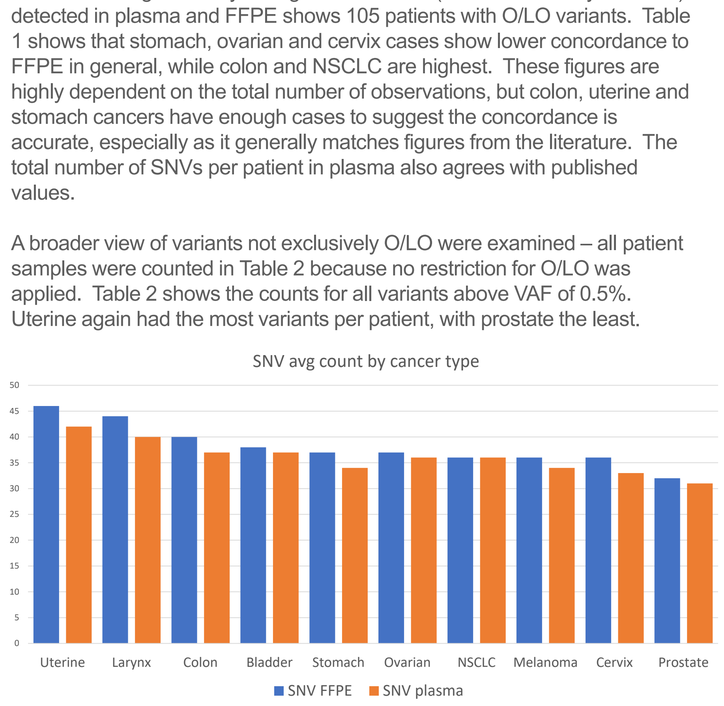
The lower left scatterplot shows the correlation of VAF% for SNVs to the orthogonal lab (R=0.95) and on the right, indels (R=0.88).

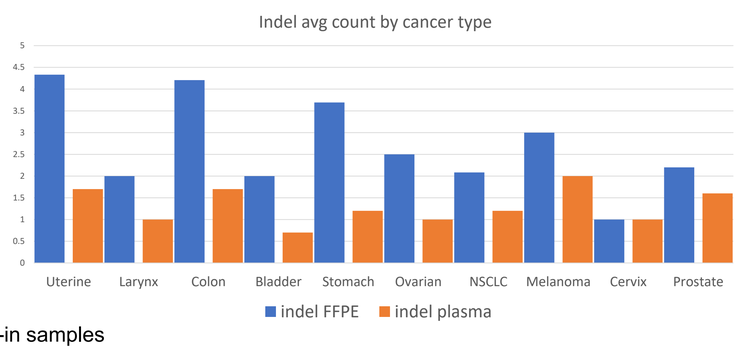
FFPE vs plasma

The sensitivity of ctDNA NGS tests varies based on several factors. We demonstrated the ctTSO500 has high reproducibility and sensitivity down to 0.5% variant allele fraction using analytical samples from SeraCare. We then examined a 113-person cohort of paired FFPE and plasma samples.

A view of oncogenic/likely oncogenic mutations (as determined by OncoKB) detected in plasma and FFPE shows 105 patients with O/LO variants. Table 1 shows that stomach, ovarian and cervix cases show lower concordance to FFPE in general, while colon and NSCLC are highest. These figures are highly dependent on the total number of observations, but colon, uterine and stomach cancers have enough cases to suggest the concordance is accurate, especially as it generally matches figures from the literature. The total number of SNVs per patient in plasma also agrees with published values.

A broader view of variants not exclusively O/LO were examined – all patient samples were counted in Table 2 because no restriction for O/LO was applied. Table 2 shows the counts for all variants above VAF of 0.5%. Uterine again had the most variants per patient, with prostate the least.





Cancer type	# cases	O/LO SNV per patient FFPE	O/LO SNV per patient plasma	Total O/LO INDEL FFPE	Total O/LO INDEL plasma	Accuracy plasma to FFPE
Colon	48	4.8	4.6	4	4	96.90%
Uterine	15	7.5	6.8	5	4	91.10%
Stomach	10	5.4	4.4	1	1	81.50%
NSCLC	6	5.5	5.5	0	0	97%
Bladder	3	4	4	0	0	100%
Prostate	2	5	3.5	0	0	70%
Cervix	1	11	6	0	0	55%
Larynx	1	4	4	0	0	100%
Melanoma	1	6	6	0	0	100%
Ovarian	1	5	4	1	1	80%

Table 1

Oncogenic and Likely Oncogenic genes were counted in FFPE and plasma for SNVs and INDELs. The O/LO variants per patient for FFPE is marginally higher than for plasma, but concordance is generally high.

Cancer type	# cases	Total SNV per patient FFPE	Total SNV per patient plasma	indel FFPE	indel plasma	Accuracy plasma to FFPE
Uterine	18	42	46	4.3	1.7	93%
Larynx	1	40	44	2	1	91%
Colon	48	37	40	4.2	1.7	92%
Bladder	3	37	38	2	0.7	97%
Stomach	13	34	37	3.7	1.2	91%
Ovarian	2	36	37	2.5	1	96%
NSCLC	12	36	36	2.1	1.2	98%
Melanoma	1	34	36	3	2	94%
Cervix	2	33	36	1	1	91%
Prostate	5	31	32	2.2	1.6	96%

Table 2

Rather than restrict to O/LO variants, we counted all reportable variants at VAF>0.5%. The accuracy reflects the overlap of variants found in FFPE that were identified in plasma.

Conclusion

The performance of Illumina's ctTSO500 has been previously published (Verhein *et al.*, 2020; Verma *et al.*, 2020; Pommergaard *et al.*, 2022) for MSI, TMB, SNV and INDELs. Several clinical trials demonstrated a solid link between plasma and tissue variants (Zoughbi *et al.,* 2021; Nakamura *et al.,* 2022; Willis *et al.*, 2019; Lee *et al.,* 2022; Friedlaender *et al.,* 2020). We provide evidence that the analytical sensitivity of the ctTSO500 assay meets or exceeds the performance specifications published by Illumina. We examined a cohort of late-stage patients with plasma taken less than 72 hours from the date of their biopsy, pre-treatment. Observations from a comparison between plasma and tissue include the fact that not all patients had oncogenic or likely-oncogenic variants in their tissue. Tables 1 and 2 have different counts per cancer type because of the few (<5%) patients without actionable mutations. Next, there are two independent measurements that track two different observations. 1) The number of variants per patient in certain cancer types is greater than in other cancer types. 2) The concordance between plasma and tissue differs by cancer type. The first observation is well established from solid tumor studies, where some tumors are driven by copy number changes or fusions, rarely by point mutations. The second observation relates to the capacity of a tumor to shed DNA into the blood stream, and whether or not that patient has multiple tumors each of which are shedding different DNA molecules at different rates. This state will cause a DNA profile from a single biopsy to mismatch DNA from many tumor sites, or from mets distant from the primary tumor.

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