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Comparing comprehensive NGS panel(s) based approach to tissue whole exome sequencing (WES) using matched tissue and circulating tumor DNA from late-stage colorectal cancer patients.

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INTRODUCTION

Developments in next generation sequencing technology, both, in targeted panel approaches and whole exome sequencing (WES) have allowed for the advancement of cancer research[1]. However, comparison of such technologies is lacking and in many cases the concordance between tissue-based sequencing approaches and liquid biopsy-based approaches are not present[2]. The cfDNA shedding rate, concentration, and stability are associated with tumor type, stage, cfDNA degradation and clearance mechanisms[3]. Tumoral heterogeneity together with tissue of origin related genomic alterations adds complexity to genomic profiling[4]. A better understanding of the differences between targeted approaches such as the Illumina TS500 panel and whole exome sequencing both in tissue and liquid biopsy samples is crucial. In this study, we systematically conducted performance evaluation of Illumina TruSight™ Oncology 500 gene (TSO500) NGS assay(s) using matched tissue and cfDNA specimens in comparison to Whole Exome Sequencing (WES).

METHODS

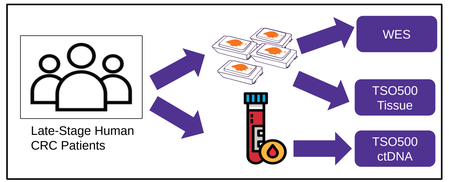

Figure 1: Testing plan schematic. Matched tissue and plasma samples from treatment naive stage III/IV CRC patients were utilized for Illumina TSO500 sequencing and WES. Tissue samples were separately analyzed by both WES and the TSO500 txDNA tissue panel while extracted cfDNA was analyzed by TSO500 cfDNA panel.

Illumina BaseSpace TruSight Oncology 500 platform (*CombinedVariantOutput.tsv*) was applied to select non-synonymous (frameshift, in-frame, nonsense, missense, translation start site, nonstop and splice site) variants in both cfDNA and tissue samples. Genes found to be mutated by tissue WES were filtered for corresponding mutated genes detected by TSO500 analysis. Next, using maftools, we characterize the most frequently mutated genes and the mutations observed for each patient sample.

Table 1: Patient demographics for eight CRC patients. Six of these patients had matched plasma and tissue samples. *Patient 7 only had plasma sample and no tissue sample for TSO500 analysis. **Patient 4 only had tissue sample available. For comparative analysis of tissue and plasma, only the six matched samples were used.

Sample Name	Indication	Stage	Sex	Race	Age
Patient 1	Colon Cancer	Stage IIIB	Female	Caucasian	68
Patient 2	Rectal Cancer	Stage IIIB	Female	Caucasian	78
Patient 3	Colon Cancer	Stage IIIB	Female	Caucasian	61
Patient 4**	Colon Cancer	Stage IIIC	Female	Caucasian	75
Patient 5	Colon Cancer	Stage IV	Female	Caucasian	81
Patient 6	Colon Cancer	Stage IVA	Female	Caucasian	65
Patient 7*	Colon Cancer	Stage IVA	Male	Caucasian	55
Patient 8	Colon Cancer	Stage IVA	Female	Caucasian	58

RESULTS

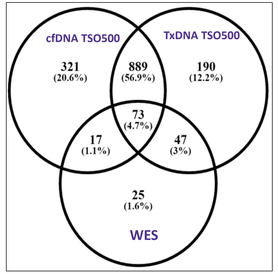

Figure 2: Comparison of the CRC-associated mutations' detection levels across cfDNA TSO500, TxDNA TSO500, and WES platforms. A total of 889 variants (56.9%) were detected in both liquid and tissue biopsy data from cfDNA TSO500 and TxDNA TSO500 analysis, while only 17 variants were common to both liquid cfDNA TSO500 and whole exome sequencing data (WES) and 47 variants were common to both tissue TxDNA TSO500 and WES data. A total of 73 variants were identified to be commonly detected in all 3 datasets.

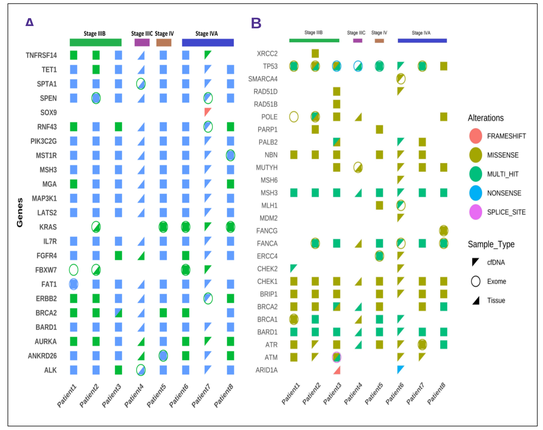

Figure 3: **(A)** Oncoplot displaying the top 20 genes with largest number of variants detected across the three methodologies. This graph depicts mutations in cfDNA with an upper triangle, mutations in tissue TSO500 with a lower triangle and mutation in exome data with a circle. Furthermore, the samples are stratified by tumor stage. **(B)** The same analysis as in (A) was applied to a panel of 35 DNA damage repair (DDR) genes known to have a functional role in colorectal cancer development and progression and previously published[5]. **Note: Patient 7 only had plasma sample and no tissue sample for TSO500 analysis and Patient 4 only had tissue sample available.**

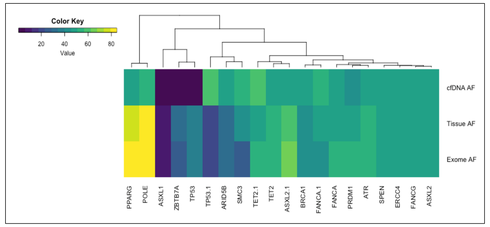

Figure 4. Common mutations in mismatch repair genes detected using cfDNA TSO500, TxDNA TSO500, and WES analysis presented as allele frequency (AF) correlation heatmap across the three platforms. AFs of ZBTB7A, ASXL1, and TP53 genes were lower in case of cfDNA TSO500 approach compared to the tissue-based analysis indicating a potential decrease in shedding of ZBTB7A, ASXL1, and TP53 mutated ctDNA. Note: Color Key bar reflects percent allele frequency.

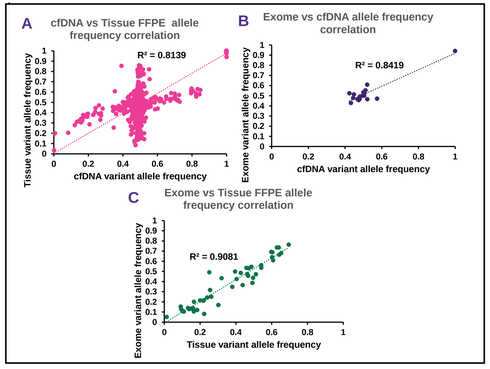

Figure 5: Correlation of allelic frequency of common variants between the three datasets; cfDNA TSO500, Tissue TSO500, and tissue whole exome sequencing. We observed a significant correlation across WES and TSO500-called variants; **A:** TSO500 tissue/TSO500 cfDNA r^2=0.81 (889 variants), **B:** WES/TSO500 cfDNA r^2=0.84 (17 variants), and **C:** WES/Tx TSO500 r^2=0.91 (47 variants). Note: variants at 0.5 (50%) VAF were overrepresented in cfDNA dataset, whereas in the TxDNA TSO500 samples the corresponding variants are distributed across a frequency gradient (5A).

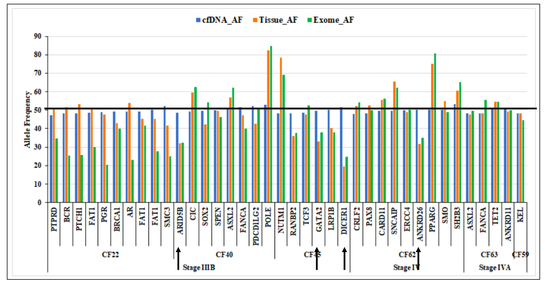

Figure 6. Bar-plot showing allele frequency (AF) of all common gene variants with cfDNA AF >0.47 (47%) and <0.53 (53%) stratified by stage. We previously showed that variants at 0.5 VAF were overrepresented in cfDNA dataset, whereas in the TxDNA TSO500 samples the corresponding variants are distributed across a frequency gradient (See **Figure 5A**). By comparing cfVAF to their frequencies in tissues (TxDNA and WES) and mapping them to specific genes we found that specific DNA variants overrepresented in liquid biopsy compared to tumor tissue correspond to genes DICER1, ARID5B, ANKRD26, and GATA2. These findings suggest that tumors with these mutations might exhibit excessive cfDNA shedding. Alternatively, increased cfVAF of specific mutations might be driven by undetected distal metastatic sites thus suggesting that the tumor may have high possibility for metastatic potential. We also detected DNA variants underrepresented in liquid biopsy compared to their frequencies in tissue. These variants correspond to genes CIC, ASXL2, POLE, NUTM1, SNCAIP, PPARG, and SH2B3. Solid line runs through the 0.5 (50%) VAF cut-off.

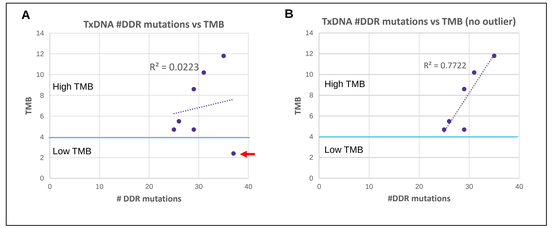

Figure 7. The number of DDR gene mutations and TMB correlation analysis using TxDNA TSO500 data from late-stage CRC samples. Here a larger panel of approximately 200 DDR related genes was used. TMB was calculated using the Illumina TSO500 recommended algorithm. The increase in number of mutations in DDR genes represented on TSO500 panel positively correlates with the increased TMB. TMB is plotted as a continuous gradient and further arbitrarily divided by a tiered TMB cut-off strategy: "Low" (<4) and "High" (>4) TMB (blue line across). Red arrow demarcates an outlier with the "Low" TMB (A). By eliminating a "Low" TMB data point it is possible to achieve a high correlation score between the number of mutations in DDR genes and TMB in "High" TMB CRC samples (B).

CONCLUSIONS

- We demonstrate significant concordance between the TSO500 liquid biopsy and tissue datasets, but lesser concordance between the liquid biopsy and the tissue WES dataset. The evidence we produce supports the potential of leveraging both ctDNA and FFPE based genomic observation to enable precision oncology. A deeper analytical dive into each dataset is on-going.
- Additionally, we leverage our CRC datasets to demonstrate that specific mutated alleles are overrepresented in cfDNA compared to their corresponding mutations in tumor tissue. This observation suggests increased ctDNA shedding by tumors characterized by specific genetic signatures. Alternatively, this observation might imply the existence of undetected distal metastatic sites which contribute specific mutated alleles such as DICER1, ARID5B, GATA2, and ANKRD26 to the cfDNA pool.
- We also observe positive correlation between the number of mutations in DDR genes and TMB. These findings support the notion that increases in TMB can be driven by genomic instability events triggered by the loss of function mutations within the DDR genes.

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DISCLOSURES

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