Panel-based tumor mutational burden (TMB) analysis of matched tumor and plasma specimens using Illumina's TruSight Oncology 500 next-generation sequencing assay

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Abstract 1346

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Introduction

Checkpoint inhibitor (CPI) therapy demonstrates a remarkable clinical benefit in many cancer types. However, the ability to successfully select patients who will benefit from CPIs is still limited. Tumor mutational burden (TMB), a measure of the number of somatic mutations per coding area of tumor genome, is a putative biomarker of response showing great promise in CPI and immunotherapy combination trials. The ability to measure TMB from tumor biopsies or plasma samples will be important for clinical adoption of this biomarker. Herein we report on initial performance evaluation of Illumina's TruSight™ Oncology 500 gene (TSO500) NGS assay for the analysis of TMB in FFPE tissue and plasma cell-free (cf)DNA specimens.

Illumina's TSO500 assay employs hybrid-capture based approach for target enrichment coupled with unique molecular indices to enable low frequency variant detection of single nucleotide variants and indels. This comprehensive cancer panel interrogates relevant cancer biomarkers in >500 cancer genes (~2 Mb) from as little as 40 ng of FFPE DNA or 30 ng of cfDNA. In addition to variant calls, Illumina's analysis pipeline reports a TMB score and microsatellite instability (MSI) status [1]. Results obtained with the TSO500 TMB assay were compared to our validated whole exome sequencing (WES) TMB assessment for FFPE tissue specimens, with and without matched normal. As previously reported [2], the WES TMB tumor-only pipeline uses somatic variant classifications determined using a random forest model to generate TMB scores from analysis of tumor FFPE specimens.

Methods

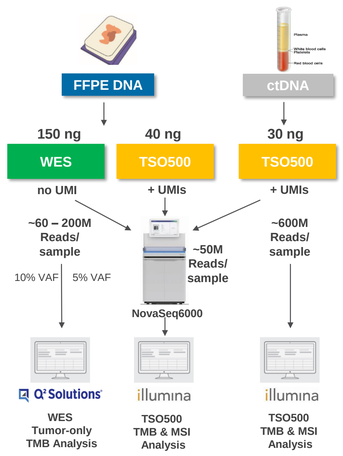

Figure 1. Study design and sample Workflow. DNA from matched FFPE and plasma samples were analyzed for variant detection, TMB and MSI. All FFPE (50) samples were analyzed using TSO500 and a subset (25) were also tested using WES to evaluate biomarker concordance. Plasma samples were analyzed using TSO500 and results were compared to matched FFPE samples on TSO500. Resulting libraries were sequenced on the NovaSeq platform to specified target read depths. Data analysis for TSO500 assays were performed by Illumina and WES by Q² Solutions – EA Genomics.

Samples

Table 1A. Samples used in the study. Fifty (50) matched FFPE & EDTA plasmas from late stage (III+) bladder & CRC patients. Where possible, FFPE samples were enriched by macrodissection to ≥ 50% tumor. Plasma cfDNA inputs ranged from 3 – 30 ng. **1B. Sample QC summary.**

A

	Stage III	Stage IV
Bladder	11	3
Colon	29	7

B

	FFPE PASS	FFPE FAIL
ctDNA PASS	39	8
cfDNA FAIL	3	0

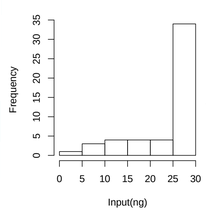

Figure 2. cfDNA Inputs. Over 70% of plasma samples yielded ≥ 30 ng cfDNA for TSO500 analysis. All samples yielded libraries; however, 3 samples failed to meet sample-level sequencing QC metrics.

Results

Accurate & Sensitive Detection of Variants in Reference Standards

Gene	AA	HDx 5% Rep1	HDx %5 Rep2	HDx 1% Rep1	HDx 1% Rep2
EGFR	L858R	4.6	4.7	1.1	0.9
EGFR	delE746-A750	4.3	4.7	0.5	0.7
EGFR	T790M	5.0	4.6	0.7	0.9
KRAS	G12D	6.6	6.5	1.4	1.0
NRAS	Q61K	6.5	7.0	0.9	1.3
NRAS	A59T	4.0	5.1	0.9	1.2
PIK3CA	E545K	6.7	6.0	0.9	1.1

Table 2. Detection of known variants in Horizon Dx reference standards. Observed variant allele frequencies (VAF or AF) shown. Overall, 100% accuracy and sensitivity for detecting 5% and 1% AF variants using the FFPE and plasma workflows, respectively.

Reproducible Detection of Variants at < 1% Allele Frequencies

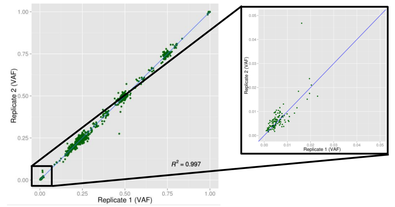

Figure 3. Reproducibility of detecting variants in Horizon Dx reference standards. Unique molecular indices (UMIs) enable low frequency mutation detection in plasma workflow down to ~0.4% allele frequency (R^2 = 0.997).

FFPE Variant and TMB Concordance between WES and TSO500

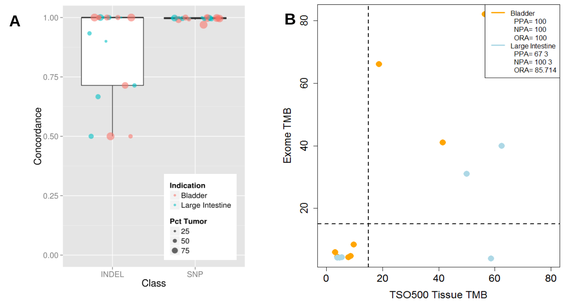

Figure 4A. SNV and Indel concordance. Variant concordance is independent of tumor indication and percent tumor. **4B. TMB concordance**. Strong correlation of TMB status between assays using an example TMB threshold of 15. Large dots represent MSI-high status.

Distribution of Variants and VAFs in FFPE Samples

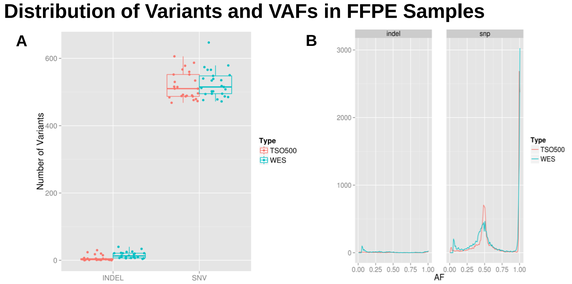

Figure 5A. Number of variants detected in FFPE samples tested on TSO500 and WES. WES analysis was limited to content overlapping TSO500. Each dot represents total number variants per sample. Similar number of SNVs were detected by both assays, whereas as increased number of indels called with WES. **5B. Allele frequency distributions.**

TSO500 Variant Detection in Matched FFPE and ctDNA Samples

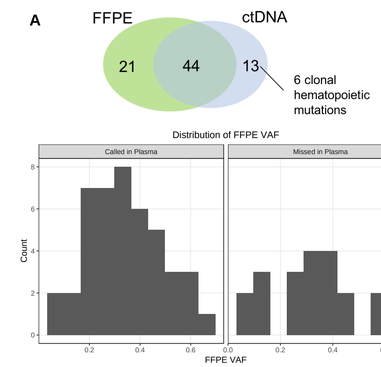

Figure 6A. Example of variant concordance between tissue and plasma samples collected from a bladder cancer patient.. Reporting variants with COSMIC count > 50. **6B. Distribution of FFPE VAF.** Left, concordant variant calls between FFPE and plasma, Right, discordant variants calls between FFPE and plasma.

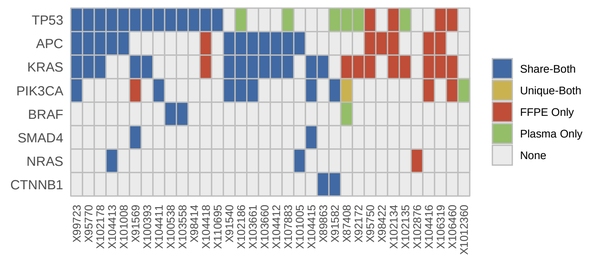

Figure 7. Hotspot mutation concordance between FFPE and Plasma samples. Hotspot genes detected in at least two samples are represented. Plasma only TP53 mutations could be evidence of clonal hematopoiesis.

TSO500 TMB Concordance between FFPE and ctDNA

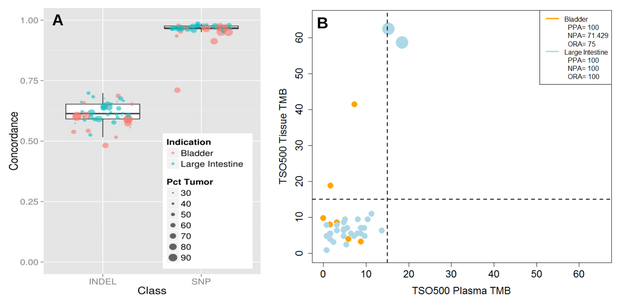

Figure 8A. Variant concordance. Observed high SNV concordance between matched tissue and plasma samples using TSO500 assay. Each dot represents a specific patient sample with size correlated to % tumor content (pathology of FFPE). **8B. TMB concordance.** Using an example TMB threshold of 15, we observed good correlation of TMB statuses in tissue and plasma of patients with CRC (n = 36) and bladder cancer (n = 14).

Correlation between MSI Status and TMB Scores

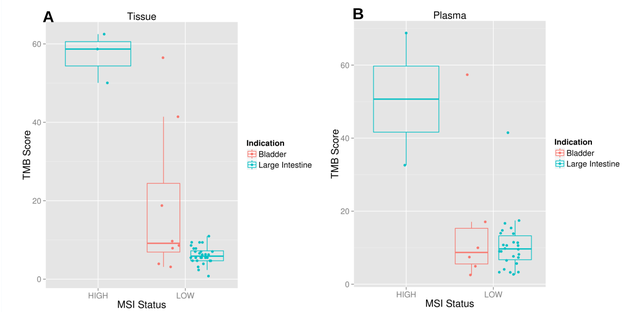

Figure 9. Correlation between MSI status and TMB scores in FFPE (A) and plasma (B). We observed strong correlation between MSI-high and TMB-high status (TMB > 15) in CRC patients. In addition, strong concordance of MSI statuses was observed between matched FFPE and plasma samples from bladder cancer (100%, 8/8) and (96.8%, 30/31) CRC patients.

Distribution of TMB Scores by Assay and Sample Type

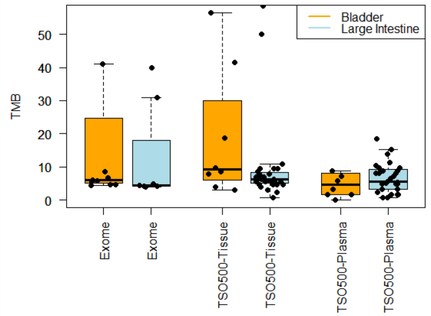

Figure 10. Cross-platform TMB comparison. TMB scores are plotted for each indication and assay. Plasma samples displayed a more narrow dynamic range of TMB scores than matched FFPE tissue.

Conclusions

- TSO500 has a high success rate for sequencing library conversion using 40 ng FFPE DNA or 30 ng EDTA plasma DNA.
- TSO500 FFPE and plasma workflows enable reproducible variant detection down to ~5% and ~0.4% allele frequency, respectively.
- Observed good correlation between TMB scores generated by tumor-only WES and TSO500 methodologies in FFPE specimens.
- Despite inherent biological differences between tissue and plasma, high concordance was observed between TMB statuses in tissue and plasma (ORA = 75% in bladder cancer and 100% in CRC).
- Additionally, strong concordance was observed between MSI statuses in FFPE and plasma patient samples (100% in bladder cancer and 96.8% in CRC).

References

1. Analysis of TMB and MSI Status with TruSight™ Oncology 500. Illumina White Paper, 1170-2018-009-A, 2018. www.illumina.com/tso500.
2. Mola, N and Weigman V. Robust TMB values derived from tumor-only material. SITC 2018. Washington, DC.

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