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Comprehensive Evaluation of Illumina's TruSight® Tumor 170 Panel to Estimate Tumor Mutational Burden

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ABSTRACT

Tumor mutational burden (TMB), or the number of mutations within the coding region of a tumor genome, is typically assessed by whole exome sequencing (WES) and has been shown to correlate with efficacy of immunotherapy treatment. Targeted cancer gene panels are broadly used to assess mutational status in cancer related genes but have not historically been used to estimate TMB. Recently, two studies have demonstrated that TMB can be accurately estimated using these cancer gene panels1, 2. Illumina's TruSight® Tumor 170 (TST170, **research use only**; **RUO**) panel is a comprehensive next-generation sequencing (NGS) assay that covers the coding regions of 170 genes associated with solid tumors. TST170 targets DNA and RNA variants from the same FFPE tumor sample in a single sequencing run. Here we evaluate the performance of TMB estimation with TST170.

TST170 is an enrichment-based targeted panel designed to capture mutational changes, including single nucleotide variant, indels, amplifications, splice variants and fusions. The TST170 analysis pipeline is able to call variants with frequencies down to 5%. In the current study, TMB was calculated as the number of reported variants per megabase after germline polymorphism filtering. First, we evaluated the performance of TST170 for TMB estimation using 5336 The Cancer Genomics Atlas (TCGA) samples that had been analyzed by WES. TMB estimated from the TST170 targeted regions showed a high correlation to TMB estimated from WES (R^2=0.91). Next, we evaluated the prognostic value of TMB estimated from TST170 by overlapping the TST170 targeted regions with WES data for 199 subjects treated with CTLA-4 or PD-1 from three clinical studies. Higher TMB estimated from the TST170 targeted regions was observed in subjects who responded to checkpoint inhibitors. Finally, we assessed the correlation of TMB estimation using matched samples profiled with both WES and TST170 and again saw high correlation between the two methods (R^2=0.998). In summary, our analysis indicates that the panel content of TST170 can be used to accurately estimate TMB from tumor samples.

ANALYSIS WORKFLOW

Data from WES studies were subjected to a series of filtering steps based in the targeted regions covered by the TST170 Panel (Figure 1).

Variant calling	Germline variant filtering	TMB calculation
1. TST170 analytical pipeline (2.6% VAF cutoff) 2. Filter out low VAF variants (5% VAF cutoff)	1. Filter with population germline database 2. Add back variants with high COSMIC count	Calculate number of somatic mutations per Mb (targeted region for TruSight Tumor 170 is 0.524 Mb)

Figure 1: TMB Analysis Pipeline with TST170—Variants detected by WES were filtered according to Variant Allele Frequency (VAF) cutoffs and filtered by germline database prior to calculation of TMB as the number of somatic mutations per 0.524 Mb.

LIBRARY PREPARATION AND NGS

For comparing original data from WES and TST170, DNA was isolated and aliquoted from 27 FFPE tumor samples from various tissue types. For each sample, libraries were prepared using the TST170 Library Prep Kit and the TruSeq® Exome Library Prep Kit and sequenced on NextSeq®, HiSeq® 2000 or HiSeq® 2500 Platform.

TCGA Samples

WES data from 5336 TCGA samples were filtered and analyzed *in silico* using TST170. TMB estimated from the TST170 targeted regions showed a high correlation to TMB estimated from WES, with R2 correlation values of 0.91 for total mutations (Figure 2A) and 0.90 for nonsynonymous mutations (Figure 2B).

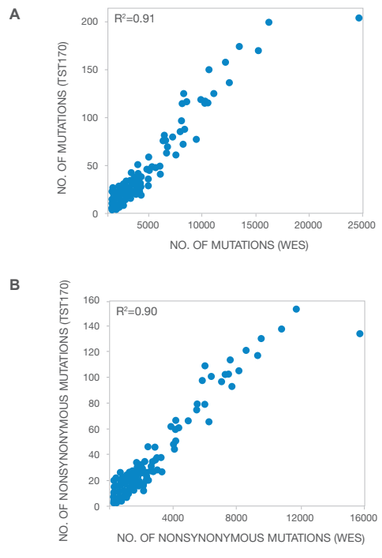

Figure 2: Estimation of TMB with TST170 Analysis of TCGA WES Data — The performance of TST170 was evaluated for TMB estimation using 5336 TCGA samples that had been analyzed by WES. The number of mutations from each method are plotted . A) R2=0.91 for all mutations B) R2=0.90 for same data set filtered for nonsynonymous mutations.

CHECKPOINT INHIBITORS CLINICAL STUDIES

Three previously published studies have demonstrated the prognostic value of differentiating high TMB subjects from low TMB subjects using WES panels. By recreating this study in silico, a high correlation was observed between high TMB subjects and response to checkpoint inhibitors (CTLA-4 or PD-1) using both WES and TST170 (Figure 3).

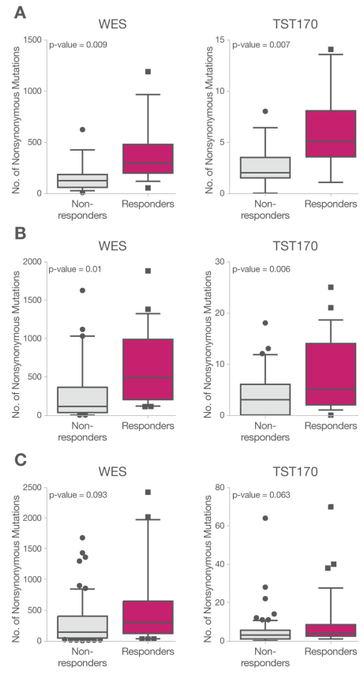

Figure 3: Estimation of TMB with TST170 analysis of Data from Published Studies —*In silico* analysis of coverage by TST170 was applied to WES data from 199 subjects treated with checkpoint inhibitors. Coverage of data from both methods indicated a higher TMB estimation in subjects who responded to checkpoint inhibitors in 3 clinical trials. A) 31 non-small cell lung cancer subjects from study by Rizvi et al.3 B) 58 melanoma subjects from study by Snyder et al.[4] C) 110 melanoma subjects from study by Van Allen et al.[5]

COMPARISON OF WES AND TST170

Twenty-seven FFPE tumor samples were profiled using both WES and TST170 and the correlation of TMB estimation was assessed.

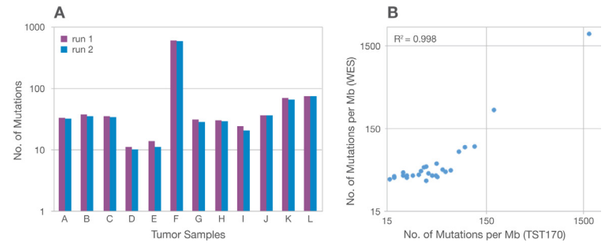

Figure 4: Sequencing and Analysis of 27 Tumor Samples with WES and TST170 — A) A high level of reproducibility of detected mutations was observed on 2 independent runs analyzing 12 FFPE tumor samples using TST170. B) Matched samples profiled using both WES and TST170 showed high correlation following germline and VAF filtering of detected mutations (R2=0.998).

SUMMARY

Results of analysis indicated that TST170, with comprehensive coverage of cancer-related genes, shows high concordance with WES for accurate assessment of TMB. Targeted sequencing panels can provide information at lower cost per sample compared to WGS or WES, which may be a critical consideration in the development of new methods of cancer diagnosis and treatment assessment.

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