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Tumor Mutational Burden Estimation and Somatic Mutation Profiling using a Large Next-Generation Sequencing Panel

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INTRODUCTION

Recently, high Tumor Mutational Burden (TMB) was shown associated with significantly longer progression-free survival from immune checkpoint blockade combination therapy in NSCLC[1]. Whilst TMB as computed through whole exome sequencing (WES) is still a gold standard, the high starting material (tumor and germline DNA) requirement and complex bioinformatics refrains exploring this biomarker in individual labs[2,3,4]. Herein, we develop a PCR based targeted panel for computing TMB and detecting important variants from FFPE research samples.

METHODS

We present an integrated solution, utilizing a multiplex PCR-based target enrichment panel with ~1.7 Mb of genomic coverage to estimate TMB and detect variants from FFPE research samples. Oncomine™ Tumor Mutation Load (TML) Assay workflow requires 20 ng of input DNA and can leverage manual or automated library and templating on the Ion Chef. Up to four samples on 540 or six samples on 550 chip can be sequenced to achieve sufficient depth for variant detection and TMB. The analysis pipeline utilizes a custom variant calling and germline variant filtering to accurately quantify somatic mutations in cancer research samples without the need for a matched normal sample.

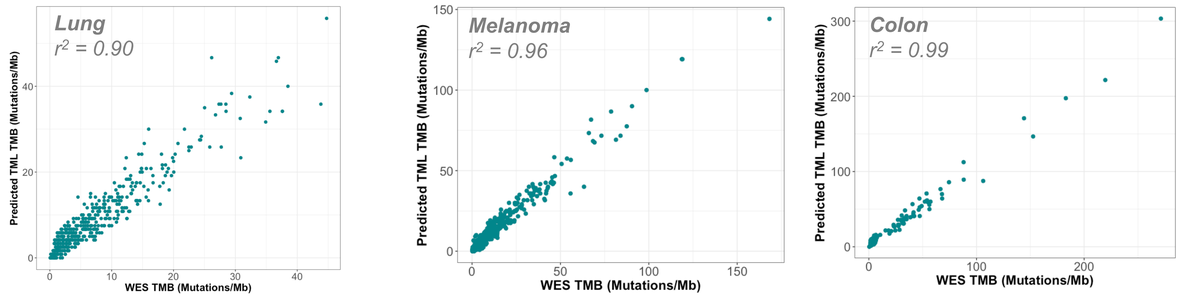
RESULTS

- In-silico analyses using exomes from TCGA MC3 demonstrated Oncomine™ TML panel has adequate size and appropriate targets for TMB estimation.
- Comparison of TML assay TMB with WES (Tumor/Normal analysis) TMB on FFPE samples gave high correlation.
- Analysis on published data demonstrated that the predicted mutation counts associated with the covered regions of the targeted panel could effectively stratify responders and non-responders to immune checkpoint inhibitors.
- TMB estimation in library replicates for a cohort of ten FFPE tumors (Melanoma, CRC, NSCLC) gave high reproducibility.
- The assay was applied to colorectal specimens previously typed for microsatellite instability (MSI) to give high statistical significance ($p = 0.0077$) in separation of MSI and non-MSI groups.
- All six variants were successfully tested by TML assay that were previously detected by orthogonal assays.
- Our pipeline produces a detailed report characterizing mutations consistent with mechanisms such as UV damage and spontaneous deamination of 5-methyl-cytosine, as well as FFPE deamination.

ASSAY OVERVIEW AND PERFORMANCE

- Low input requirement (20 ng DNA)
- Large panel (1.7 Mb total;1.2 Mb Exonic) for quality TMB estimates
- 5% minimum allele frequency for somatic variants detection
- Integrated solution for variant detection and TMB calculation
- Simple workflow with Torrent Suite and Ion Reporter analysis solutions
- 3-day turnaround time (only 60 minutes hands-on time)

Figure 1. In-Silico Comparison of Oncomine™ TML and Whole Exome Sequencing (WES): Whole exomes of lung, melanoma, and colon tumor samples were downloaded from TCGA MC3 dataset. Rate of nonsynonymous somatic mutations was computed for WES TMB. Mutations were limited to TML panel for predicted TML TMB. WES TMB strongly correlated with TML panel TMB on lung adenocarcinoma (left, n=466), skin cutaneous melanoma (middle, n=375), and colon adenocarcinoma (right, n=274) samples.



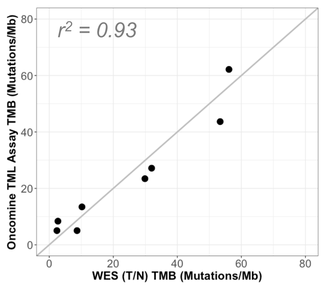
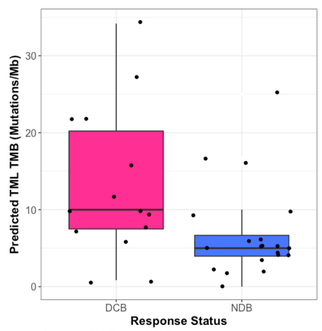


Figure 2. Comparison with WES: WES was performed on eight CRC tumors and their matched normal to compute WES TMB. TML assay was ran on tumor samples only. TMB estimates obtained with the TML workflow had high concordance ($r^2 = 0.925$) with the TMB values obtained from the matched tumor/normal WES analysis.

Figure 3. Estimate on Data from Published Study[3]: Clinical trial, WES data for 31 NSCLC subjects treated with pembrolizumab (anti-PD1) was downloaded with response status[3]. Nonsynonymous somatic mutation were restricted to TML panel targets. Significant difference ($p = 0.0196$) in mutation counts of responders (median 10) and non-responders (median 5) was observed.



*Through Mann–Whitney test

Figure 4. Reproducibility of TMB Estimate on FFPE samples: Assay was ran on two replicates of four CRC (red) and four lung (green), and two melanoma (blue) FFPE samples. High similarity in TMB estimates was observed ($r^2 = 0.99$).

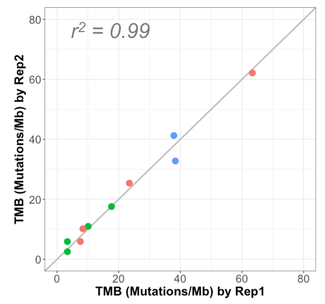


Figure 5. Performance in Separating TMB High (MSI-positive) and TMB Low (MSI-negative) CRC Samples: Assay was ran on nine CRC FFPE samples that were previously typed with MSI. Significant difference ($p = 0.0077$) in means of MSI (mean TMB 37.08) and MSS (mean TMB 6.99) group was observed.

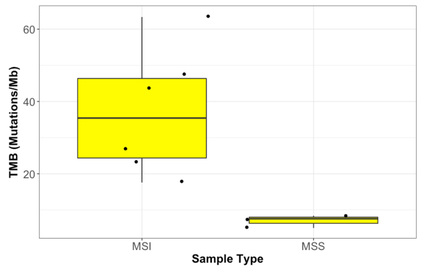


Figure 6. Variant Detection Performance on FFPE Samples: TML assay detected six variants in four FFPE samples. All six variants were separately tested by Sanger Sequencing or dPCR.

FFPE Tumor Sample Type	Gene	Locus	Genotype	Coverage	Allele Frequency	Detected
Uterus	ESR1	chr6:152419923	A/C	693	23.95%	✓
	KRAS	chr12:25398283	ACC/AAC	516	17.25%	✓
	AKT1	chr14:105246551	C/T	1927	40.84%	✓
Lung	EGFR	chr7:55242465	GGAATTAAGAGAAGCAACATC/GACATC	1003	14.76%	✓
Uterus	PIK3CA	chr3:178952085	A/G	1082	18.76%	✓
Lung	NRAS	chr1:115256528	TTG/TCG	1538	14.89%	✓

Figure 7. Analysis Result Report: Two page, PDF result report contains analysis settings, sample information, QC metrics, and analyses results displaying allele ratio distribution, substitution type and context information of somatic mutations. Example report of a lung, FFPE research sample.

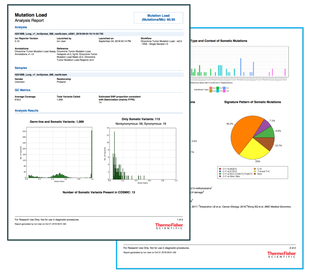
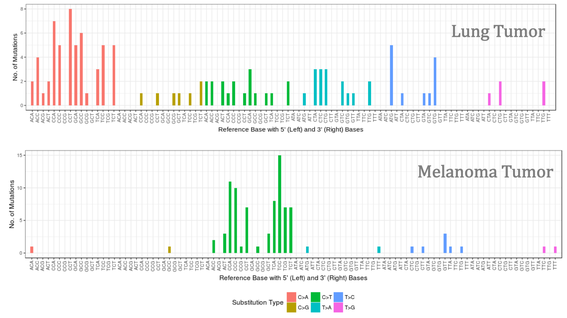


Figure 8. Substitution Type and Context of Somatic Mutations: X-axis represents 96 classes based on 6 substitution types and 16 permutations of bases at 5' and 3' side of altered base. (E.g., CCT and CCG are 2 out of 16 permutations for C>T substitution class.) Y-axis represents the number of somatic mutations of a class type. Lung sample (top) represents tobacco damage as represented by prevalence of C:G>A:T somatic mutations[5]. Melanoma sample (bottom) represents UV damage as represented by prevalence of C:G>T:A somatic mutations at TpC, CpC, and CpC sites, and T:A>C:G mutations[6].



REFERENCES

1.M. D. Hellmann et al. *Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden.* The New England Journal of Medicine, 2018.
2.A. Snyder et al. *Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma.* The New England Journal of Medicine, 2014.
3.N. Rizvi et al. *Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer.* Science, 2015.
4.E. M. Van Allen et al. *Genomic correlates of response to CTLA-4 blockade in metastatic melanoma.* Science, 2015.
5.L. B. Alexandrov et al. Signatures of mutational processes in human cancer. Nature, 2013.
6.N. K. Hayward et al. *Whole-genome landscapes of major melanoma subtypes* . Nature, 2017

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