ASSOCIATION OF MICROSATELLITE INSTABILITY AND TUMOR MUTATION BURDEN

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INTRODUCTION

The targeting of checkpoint inhibitors in colorectal cancer (CRC) has concentrated on the subset of tumors with high Microsatellite Instability (MSI). Moreover, MSI or mismatch repair (MMR) testing is not yet part of the routine clinical workup for all tumors despite potential for successful immunotherapy. The measurement of Tumor Mutation Burden (TMB) by Next-Gen sequencing on the Ion Torrent platform was validated with Whole Exome Sequencing, then compared to MSI results in CRC, endometrial cancer, and non-small cell lung cancer. The objective was to identify the subset of tumors which have high TMB and high MSI, in addition to the subset which have high TMB and are Microsatellite Stable, thus not represented as MSI-High. Herein, we report the results of testing for MSI, TMB and for mutations in Mismatch Repair (MMR) genes and other biomarkers to understand their associations in multiple cancer types.

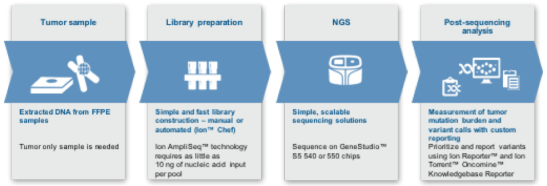
MATERIALS & METHODS

The Oncomine™ Tumor Mutation Load Assay, a research use only, PCR-based target enrichment NGS panel was developed that covers 409 genes over 1.7Mb of genomic DNA with 1.2Mb of exonic coverage. The workflow requires only 20 ng of input tumor DNA and can leverage manual or automated library prep and templating on the Ion Chef and sequencing on the Ion GeneStudio instrument using 540 or 550 chips. No matched normal sample is required. The informatics workflow utilizes a custom variant calling and germline variant filtering algorithm to accurately estimate non-synonymous somatic variants in cancer research samples. A detailed report is provided that includes the normalized mutation load (mutations/Mb), variant profiles, mutation signatures of the somatic variants, and the percentage of mutations consistent with UV damage, tobacco smoke damage, de-amination and base specific substitutions.

MSI/MSS analysis was performed using a multiplex fluorescent PCR fragment analysis assay targeting eight microsatellite loci with an Applied Biosystems® 3500xL Genetic Analyzer and GeneMapper v.5 software. Matched FFPE tumor/normal pairs from CRC and NSCLC were obtained commercially from Biochain, Bioreclamation and Folio Conversant.
TMB results, along with SNV and indel variants in MMR and other genes were reported with Ion Reporter 5.10 software.

Whole Exome Sequencing (WES) was performed to target 50Mb using 100ng of tumor and normal DNA on a HiSeq X instrument.

Figure 1. Oncomine™ Tumor Mutation Load Research Assay sample-to-answer workflow



OVERVIEW

- The Oncomine TML Assay provides accurate estimates of TMB without need for a matched normal sample
- TMB and MSI were highly correlated in CRC for TMB-H / MSI-H and TMB-L / MSS phenotypes.
- However in NSCLC, >95% of tumors are MSS, with low-to-high range of TMB values (Hause et al, 2016)
- TMB expands the value of MSI / MSS testing as a biomarker for PD1/PD-L1 checkpoint inhibition.

RESULTS

Figure 2. GeneMapper electropherogram of sample with microsatellite instability. Example of a sample displaying microsatellite instability at multiple loci (MSI is indicated by red circles). Sample input was 2 ng of FFPE DNA from CRC tumor.

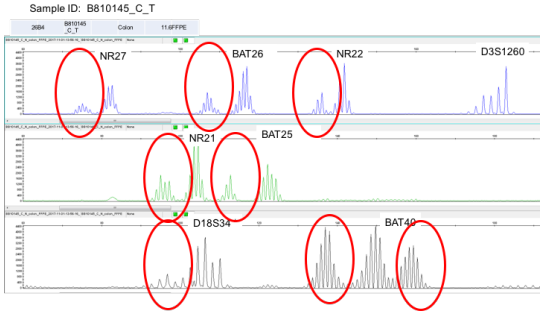


Figure 3A. WES Correlation with TML panel. TMB values from the Oncomine TML panel for 8 tumor DNA samples were compared with WES from tumor + matched normal DNA, showing high concordance (r^2 = 0.925). Whole exome sequencing is in progress for additional samples.

Figure 3B, 3C In Silico Correlation between TML panel and Exome. Somatic variant dataset was derived from COSMICv80 containing exome data derived from colorectal and endometrial cancers. TMB estimates obtained with the Oncomine TML workflow using the targeted panel had high concordance (r^2 = 0.93) with the TMB values obtained from TCGA data by in silico analysis for Colorectal and Endometrial cancers.

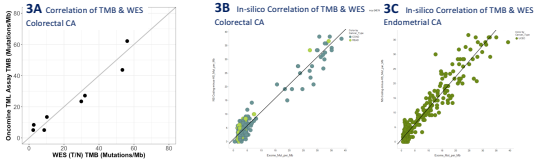
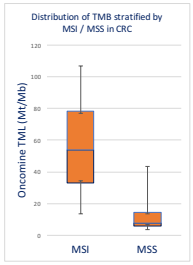


Figure 4. MSI-MSS Stratification with Oncomine TML Assay. DNA from FFPE samples was tested for MSI and for TMB from 14 CRC and 18 NSCLC tumors.



	CRC samples		NSCLC samples	
	TMB-H	TMB-L	TMB-H	TMB-L
MSI-H	7	1	0	0
MSS	1	5	8	18

- TMB was calculated by counting the non-synonymous mutations across 1.2 Mb of exonic region spanning 409 genes, generating the result as mutations/Mb.
- The median TMB was significantly higher in the MSI-H CRC samples compared to the MSS cohort (57.7 vs 14, p-value 0.0094).
- The TMB range for CRC samples was 3.4 – 107 mutations / Mb.
- The TMB range for NSCLC samples was 2.5 – 217 mutations / Mb. All NSCLCs were MSS.
- Almost all MSI-H CRC patients were also TMB-H. However, overlap of TMB-H exists in both MSI-H and MSS samples: 1 MSI-H CRC sample was also TMB-L.
- Likewise, 1 MSS CRC sample was also TMB-H. This type of tumor may be responsive to checkpoint inhibitors.

RESULTS

Variant profiling The minimum allele frequency is specific for different variant types:

- Hotspot SNVs are 3%; De-novo SNVs are 3.5%
- Hotspot indels and De-novo indels are at 10% and 20%, respectively.

Table 1. Variant profiles of MSI and MSS CRC tumors

Sample ID	TMB (Mutations/Mb)		Tumor Type	Mutant Gene	Genotype	amino acid change	Allele ratio
CRC-1	13.44	MSI	Colon	MLH1	C/A	p.Ser193Ter	C=.8796, A=.1204
CRC-2	23.44	MSI	Colon		C/T	p.Arg659Ter	C=0.7805, T=0.2195
CRC-3	47.47	MSI	Colon	BRAF	TTCACTG / TTCTCTG	p.Val600Glu	TTCACTG=0.7821; TTCTCTG=0.2121
CRC-4	104.44	MSI	Colon				TTCACTG=0.8897; TTCTCTG=0.1103
CRC-5	53.11	MSI	Colon				TTCACTG=0.7035, TTCTCTG=0.2965
CRC-6	106.76	MSI	Colon	PMS2	G/A	p.Gln244Ter	G=0.9624, A=0.0376
CRC-7	38.54	MSI	Colon	TSC1	CCTIC	p.Arg901fs	[illegible]
CRC-8	70.6	MSI	Colon	MSH2	C/A	p.Tyr796Ter	C=0.3392, A=0.6608
CRC-9	3.39	MSS	Colon				C=0.4775, A=0.5225
CRC-10	10.06	MSS	Colon	TP53	G/A	p.Arg282Trp	G=0.8032, A=0.1968
CRC-11	8.39	MSS	Colon	KRAS	TT/TC	p.Gln61Arg	TT=0.6321, TC=0.3679
CRC-12	5.03	MSS	Colon	TP53	C/T	p.Trp91Ter	C=0.7059, T=0.2941
CRC-13	50.78	MSS	Colon	None			
CRC-14	43.4	MSS	Colon	EZH2	G/A	p.Arg690Cys	G=0.9524, A=0.0476

Key Variants were identified in 13 of 14 CRC tumors
*4 of 14 CRC tumors have MMR gene variants (i.e., MLH1, MSH2, PMS2)
*1 MSS sample matched to tumor has a germline MMR gene variant
*4 of 14 CRC tumors have driver mutations in BRAF or KRAS.
*1 of 6 MSS samples was TMB-High, providing a biomarker for checkpoint inhibition.

CONCLUSIONS

1. **The Oncomine TML Assay Workflow is quick and easy**
   - 20ng DNA input
   - Simple protocol; can be automated on Ion Chef
   - Correlation to WES shows equivalence of targeted assay performance
2. **TMB and Variant Calling in a single workflow**
   - Large panel size (1.2Mb exonic coverage over 1.7Mb) favorable for Tumor Mutation Burden measurement
   - Variant calling capability shows good sensitivity and specificity.
3. **TMB-H correlates with MSI, defects in mismatch repair genes and/or mutations in driver genes**
   - MSI-High and High TMB correlated in the majority of CRC samples; moreover, samples which are MSS and High TMB are examples where the TMB biomarker provides added value not captured by MSI/MSS testing alone.

REFERENCES
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