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Tumor mutational burden (TMB) ring study: Comparison of multiple targeted next-generation sequencing (NGS) platforms

The right drug or right trial...
For Every Patient

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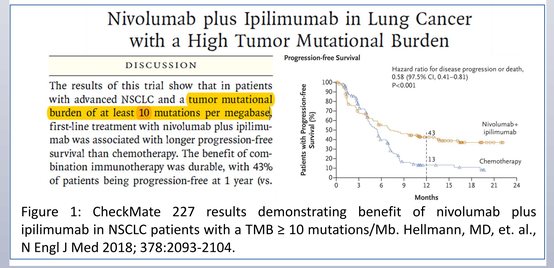
Introduction

Tumor mutational burden (TMB), a measurement of the frequency of mutations in tumor cells, is currently being evaluated as a biomarker to predict response to immune checkpoint inhibitors (Figure 1). Whole exome sequencing is considered the gold standard assay, but is inefficient and too costly to run routinely. Consequently, several targeted NGS assays have been designed to measure TMB. In this study, we compared TMB measurements from four targeted NGS assays using a common source of specimens. Concordance and accuracy of TMB values, cutoffs, and clinical interpretations were assessed.


Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden

DISCUSSION

The results of this trial show that in patients with advanced NSCLC and a tumor mutational burden of at least 10 mutations per megabase, first-line treatment with nivolumab plus ipilimumab was associated with longer progression-free survival than chemotherapy. The benefit of combination immunotherapy was durable, with 43% of patients being progression-free at 1 year (vs.

Progression-free Survival

Hazard ratio for disease progression or death, 0.58 (97.5% CI, 0.41–0.81) P<0.001

Patients with Progression-free Survival [%]

Nivolumab+ ipilimumab

Chemotherapy

Months

Figure 1: CheckMate 227 results demonstrating benefit of nivolumab plus ipilimumab in NSCLC patients with a TMB ≥ 10 mutations/Mb. Hellmann, MD, et. al., N Engl J Med 2018; 378:2093-2104.

Methods

TMB testing was completed or first attempted by Foundation Medicine (FoundationOne®), followed by on-site analysis by OmniSeq (Immune Report Card®), Illumina (TruSight Oncology 500™), and ThermoFisher (Oncomine™ Tumor Mutation Load) from a subsequent central DNA isolation. Genomic DNA from 161 FFPE specimens representing 24 tumor types was extracted following anatomical pathologist review (Figure 2). Each laboratory followed its own protocol for reporting TMB values (Figure 3). Pairwise Pearson product-moment correlations (R) were performed to estimate concordance of TMB values between platforms (Figure 4). 150 gold standards were established (7 TMB-high, 143 TMB-low) for which at least three of four platforms were concordant when using a TMB-high cutoff of ≥ 10. Each platform was assessed for TMB interpretation accuracy at this threshold (Figure 5).

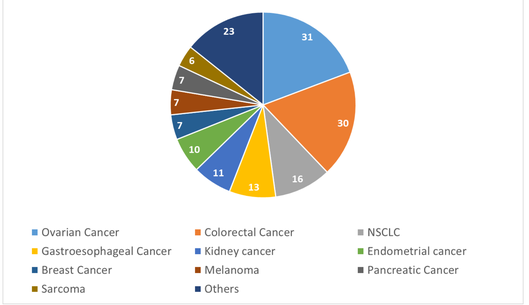


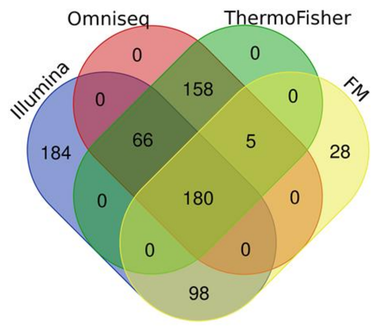
Figure 2: Tumor types evaluated for TMB using several targeted NGS panels

Comparator Platform Study

A

Approach	OmniSeq	ThermoFisher	Illumina	FoundationOne
DNA input (ng)	30	20	40	≥ 50
Fragmentation method	none	none	Sonication	Sonication
Targeted panel size (Mbp)	1.7	1.7	1.9	1.2
Genes/exons	409/6602	409/6602	523/7567	315/4557
Probe length (bp)	50-187	50-187	80	120
Probe design	amplicon	amplicon	hyb-capture	hyb-capture
Probe number	15,992	15,500	39,759	23,685
library chemistry	AmpliSeq	AmpliSeq	TruSight Oncology 500	Custom
Molecular index	No	No	Yes	No
samples per run	16	4	8	32
NGS instrument	Ion torrent S5XL	Ion torrent S5XL	NextSeq DX	Illumina
Coverage	> 120x	> 500x	> 100 MTC	> 250x
TMB value (mut/Mbp)	Non-synonymous	Non-synonymous	Non-synonymous	Synonymous & Non-synonymous

B



C

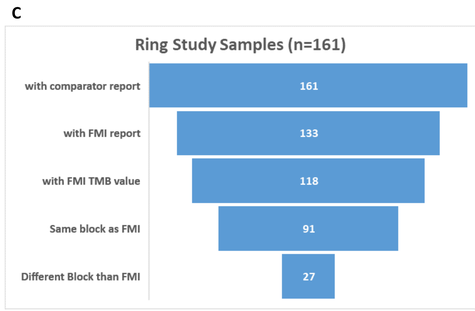


Figure 3: A) TMB platform features, B) overlap of gene content, and C) cases included in ring study.

Assay Robustness

Platform	Samples Attempted	TMB Resulted	Sample Fail	% Resulted
Illumina (TruSight Oncology 500™)	161	153	8	95%
OmniSeq (Immune Report Card®)	213	202	11	95%
ThermoFisher (Oncomine™ Tumor Mutation Load)	161	154	7	96%
Foundation Medicine (FMI)	NA	177	18	NA

Table 1: Performance of TMB platforms across a common set of cases.

TMB Range	OmniSeq (n=213)		Illumina (n=161)		ThermoFisher (n=161)		FMI (n=177)	
	PASS	FAIL	PASS	FAIL	PASS	FAIL	PASS	FAIL
0 - 5	103	11	75	8	109	7	98	18
>5-10	45		53		36		36	
>10-20	11		19		8		17	
>20	2		6		2		8	

Table 2: Assignment and performance of TMB platforms across various TMB ranges

TMB Results

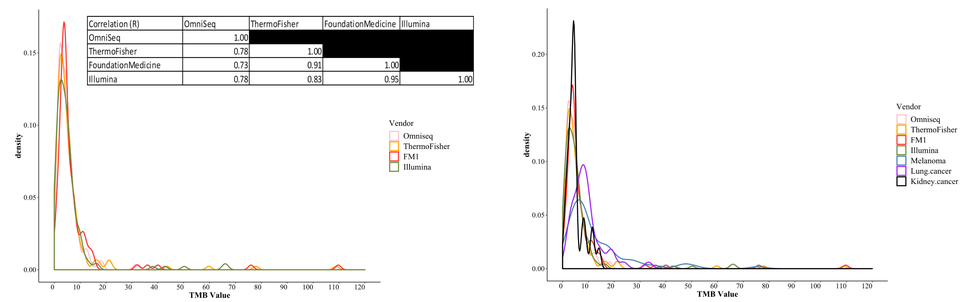
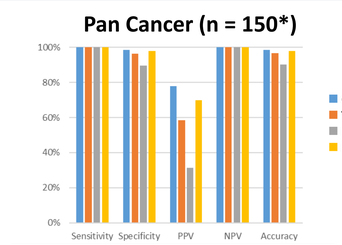


Figure 4: TMB distribution and correlation (R) across platforms (left) and majority tumor types (right).

Analytical Performance

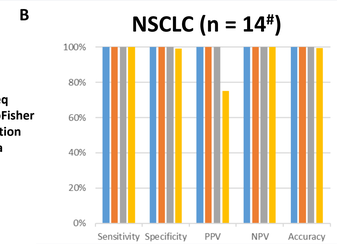
A



Platform	TP	TN	FP	FN
Omniseq	7	141	2	0
ThermoFisher	7	138	5	0
FoundationOne*	5	95	11	0
Illumina	7	140	3	0

*FMI = 111 pan-cancer samples.

B



Platform	TP	TN	FP	FN
Omniseq	3	11	0	0
ThermoFisher	3	11	0	0
FoundationOne#	1	6	0	0
Illumina	3	10	1	0

#FM1 = 7 NSCLC samples.

C

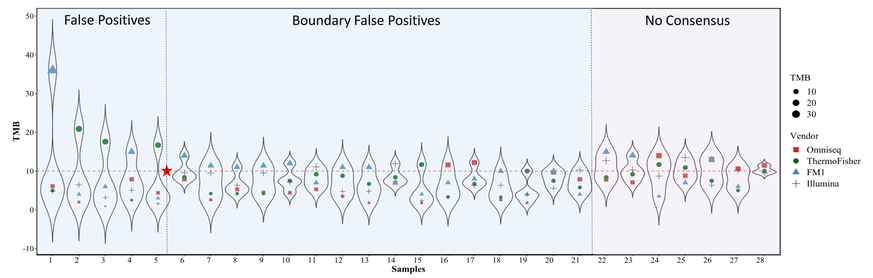


Figure 5: Analytical performance of 150 pan cancer samples (A) and 14 NSCLC samples (B) using consensus >10 as a gold standard true positives (TP) for TMB-high. C) Plot of false positive (FP), boundary FPs and non-consensus calls at the 10 cutoff. ★= FP boundary

Conclusions

- TMB performance is robust across platforms using a wide range of solid tumor specimens.
- There is general concordance between the platforms, but low number of TMB high samples limit statistical analysis.
- Pair-wise linear regression model fits did not significantly improve concordance between platforms (p>0.05)
- Each platform is highly accurate when using a TMB-high cutoff of ≥10, which improves when restricted to NSCLC.
- Majority of FP calls are boundary related to the TMB-high cutoff of ≥10.
- Further studies utilizing additional NGS platforms and gold standard samples are required.