Different genomic signatures are associated with specific PD-L1/TMB status on lung cancer with potential value for patient screening for immunotherapy.

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P3170

Background

PD-L1 expression and Tumor Mutation Burden (TMB) have independently emerged as prospective biomarkers of response to anti PD1-/PDL1 checkpoint inhibitors. However, TMB has not fully proven its value as a biomarker of immunotherapy responses in lung cancer. Moreover, FDA-approved CDx PD-L1 expression alone is not an optimal biomarker for checkpoint inhibitors, and combined use of MSI, TMB and PD-L1 protein levels has been proposed.
Here we present the correlation between genomic landscape, including TMB and MSI, with PDL1 (22C3) IHC in lung cancer specimens to help identify immunogenomic profiles for stratification of patients for check-point inhibitor therapy evaluation.

Methods

874 FFPE clinical samples across multiple cancer types was characterized in our CLIA/CAP accredited clinical laboratory using a CLIA-validated NGS-based assay that interrogates SNVs, InDels, TMB and Microsatellite Status (27 MS markers) using a 323 gene panel. TMB (mutations/Mb) was categorized as low (≤7), intermediate (7<TMB<15) and high: (≥15). The de-identified aggregated results paired with PD-L1 IHC data (FDA approved test 22C3, Dako) from 424 lung cancer samples were analyzed, and correlations between PD-L1 tumor proportion scores (TPS) and TMB results were made. PD-L1 are reported as follows: TPS <1%: negative, 1-49%: low, ≥50%: high. In-silico analyses were also performed on 5939 lung cancer samples from public databases.

Results

- We found mutational profiles correlating with PD-L1 plus TMB status.
- EGFR and KRAS were found to be more frequently mutated on “PDL1-Neg & TMB Low” NSCLC but are not among the gene signature of PD-L1 Low/TMB High tumors.
- 67% of PD-L1 High-TMB Low tumors presented mutations either on EGFR (12%), KRAS (23.5%) or in genes from known driver TRK/MAPK pathways, whereas only KRAS was part of the frequently mutated gene signature, with 37% samples mutated on PD-L1 High/TMB High samples.
- LRP1B mutations are highest in PDL1 High & TMB-High NSCLC.
- A 5 gene subset from the PD-L1 Neg & TMB High signature (TP53, LRP1B, SPTA1, SMARCA4, GNAS, ALK, FGFR2, SLIT2, ROS1, AMER1, FAT1 and MED12) was associated with reduced overall survival in an additional 5939 lung cancer patients.

Conclusions

- ❖ TMB and PD-L1 results did not correlate in lung cancer specimens, but genomic alteration signatures define subsets of lung cancer tumors with no PD-L1 expression. These signatures might complement TMB and PD-L1 expression as an adjunct to the selection criteria for patients who may benefit from checkpoint inhibitor therapy.
- ❖ Future studies are needed to evaluate how these specific signatures can serve as biomarkers of response in NSCLC patients treated by checkpoint inhibitors. Further analysis to improve the value of PD-L1 low positive or TMB intermediate results is underway.

Data

NeoTYPE Discovery 323 + MSI + TMB Assay Clinical Validation Summary

NeoTYPE Discovery 323 + MSI + TMB	
Panel size	323 genes (all exons)
Genomic size	1 Mb
Chemistry	Amplicon based
Sequencing platform	Novaseq 6000
Microsatellites	27 target regions
Validated samples	FFPE block/slides
Min tumor content	20% (30% MSI)
Recommended DNA input	100 ng
Minimum DNA input	50 ng
Type of test	CLIA validated LDT
Reimbursement Status	CMS covered

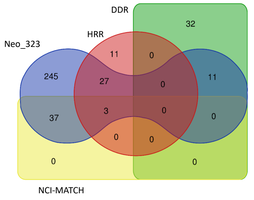
Tumor Mutation Burden		
	TMB scores	TMB category
Mut/Mb	≤7	Low
	>7, <15	Intermediate
	≥15	High

SNV/ InDel Clinical Performance			
variants/ parameter	Sensitivity (5% AF)	Specificity	Reprod.
SNVs	98.60%	98.10%	99.2%
InDel	96.80%	94.30%	

Tumor Mutation Burden Clinical Performance			
metric	Accuracy vs. WES	Reprod. intra assay	Reprod. inter assay
TMB score	91.4% Pearson coeff.	-	-
TMB category	100%	100%	100%

Microsatellite instability Clinical Performance			
Score	Sensitivity	Specificity	Reprod.
MSI (p>0.95)	87.50%	100%	100%

Technical Assay performance	
Average coverage	1908X
Minimum average coverage	≥500X
Limit of Detection SNVs	3% hotspots, 5% all others
Limit of detection InDel	3% hotspots, 5% all others
Analytical Specificity	99.9996%
Analytical Sensitivity (LOD: AF, 95% CI)	2.5% (SNVs), 2.8% (InDel)
reproducibility intra-assay	98.66%
reproducibility inter-assay	99.19%

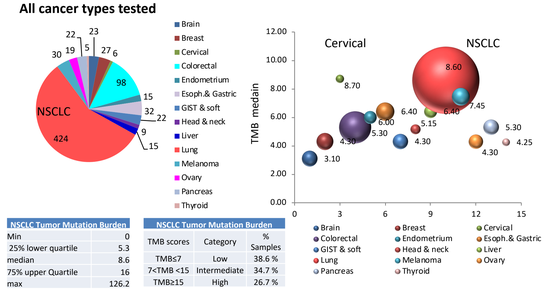


- ❖ 145 solid tumor oncogenes and tumor suppressors
- ❖ 41 Homologous Recombination Repair (HRR) and DNA damage repair genes
- ❖ 40 NCI-MATCH trial arms directly addressed
- ❖ Cancer pathways: EGFR/ RAS/ RAF MAPK/ PI3K/ MTOR, CDK/ Rb/ FGF and other RTK signaling cascades

* Full gene list available upon request

Figure 1. NeoTYPE Discovery 323 +MSI +TMB assay.

Cohort Description And Tumor Mutation Burden Across Cancer Types

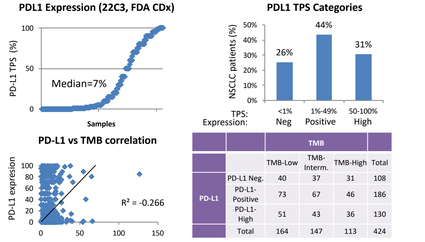


NSCLC Tumor Mutation Burden	
Min	0
25% lower quartile	5.3
median	8.6
75% upper Quartile	16
max	126.2

NSCLC Tumor Mutation Burden		
TMB scores	Category	% Samples
TMB≤7	Low	38.6 %
7<TMB <15	Intermediate	34.7 %
TMB≥15	High	26.7 %

Figure 2. Tumor Mutation Burden (TMB) across cancer types. TMB median for each tumor type from all tumors of known origin was calculated. Microsatellite instability prediction with a p>0.95 was determined in 424 of the 436 lung cancers tested. All lung cancer tumors were Microsatellite Stable (MSS). Cervical and lung cancers presented the highest median values off all tumor sites. Sphere size represents the prevalence of the cancer type.

PD-L1 - TMB Correlation in NSCLC



		TMB		
		TMB-Low	TMB-Interm.	TMB-High	Total
PD-L1	PD-L1 Neg.	40	37	31	108
	PD-L1-Positive	73	67	46	186
	PD-L1-High	51	43	36	130
	Total	164	147	113	424

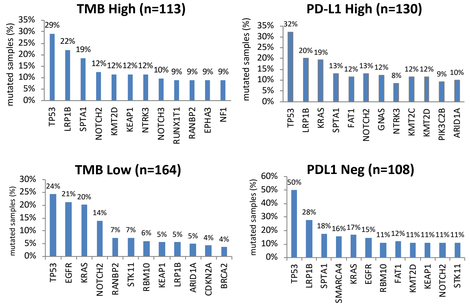


Figure 3. Mutation signatures and correlations with high TMB or PD-L1 expression in a 424 NSCLC patients cohort. TMB and PD-L1 expression lineal regression was performed. Samples were divided according to combined TMB and PD-L1 results. The top 12 most frequently mutated genes in each subcategory were plotted.

EGFR/KRAS Signaling in NSCLC Cohort

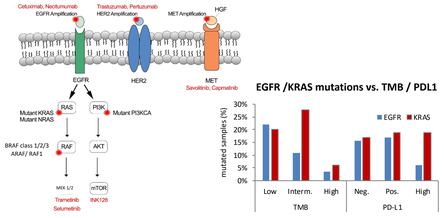
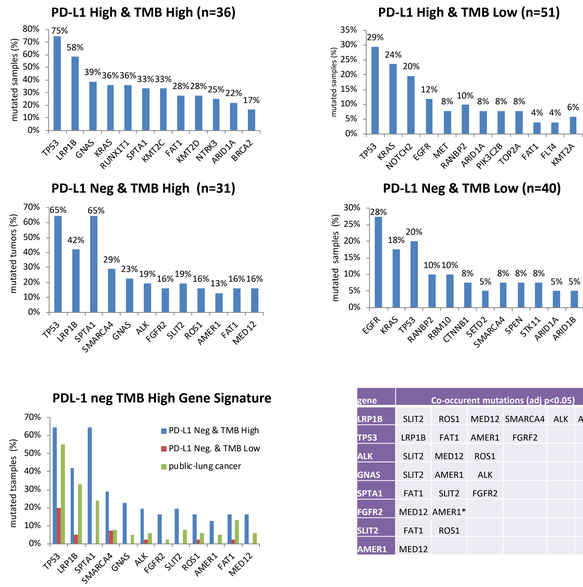


Figure 4. EGFR and KRAS alterations. Mutation frequency of the 2 genes was segregated by TMB or PD-L1 categories.

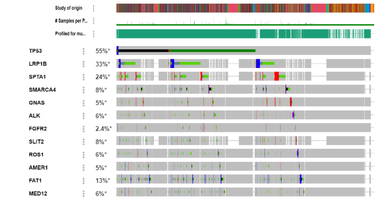
Genomic Signatures Define Combined PD-L1 & TMB Categories

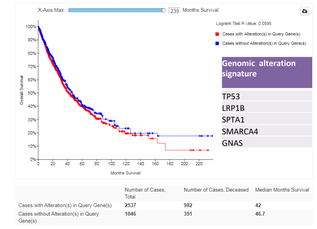


gene	Co-occurent mutations (adj p<0.05)					
LRP1B	SLIT2	ROS1	MED12	SMARCA4	ALK	AMER1
TP53	LRP1B	FAT1	AMER1	FGRF2		
ALK	SLIT2	MED12	ROS1			
GNAS	SLIT2	AMER1	ALK			
SPTA1	FAT1	SLIT2	FGFR2			
FGFR2	MED12	AMER1*				
SLIT2	FAT1	ROS1				
AMER1	MED12					

Figure 5. Genomic signatures on NSCLC samples divided by combined Tumor Mutation Burden (TMB) and PD-L1 expression. The top-12 most frequently mutated genes in each subcategory were included. Mutated samples were counted only once even if more than 1 mutation in the gene was detected. The genes were ordered by the total number of mutations detected. The number of patients on each category is indicated on each plot. The PD-L1 neg &TMB High signature was analyzed in the PDL-1 neg TMB Low subcategory or in a publicly available lung cancer dataset from 5939 lung cancer patients not discriminated by PDL-1 nor TMB values (cbioportal). The co-occurring mutant gene pairs in the public dataset are presented.

The TMB High & PD-L1 Negative Gene Signature Association with Overall Survival





	Number of Cases, Total	Number of Cases, Deceased	Median Months Survival
Cases with Alteration(s) in Query Gene(s)	2537	582	42
Cases without Alteration(s) in Query Gene(s)	1046	391	46.7

Figure 6. Oncoprint of the TMB High PD-L1 low gene signatures. The frequency of mutations on these 12 genes was analyzed in 5939 lung cancer patients (6295 samples) from 22 studies (cbioportal). Neither TMB nor PD-L1 is included on this analysis. Overall survival was estimated with Kaplan-Meyer regression with 1 to 12 genes. The top 5 mutated genes are presented as it was the minimal set showing association with OS.