Identification of Lung Cancer Mutational Signatures and Tumor Drivers Associated with Specific Bimodal PD-L1/TMB Status

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Background

PD-L1 expression and Tumor Mutation Burden (TMB) have independently emerged as prospective biomarkers of response to anti PD1-/PDL1 checkpoint inhibitors. Combined use of TMB, PD-L1 protein levels has been proposed. However, how the tumor genomic landscape interplays with the tumor microenvironment in defining particular predictive therapy response statuses is not clear. Moreover there is still little real world data available regarding the combined prevalence of these biomarkers in specific genomic landscapes.

Methods

424 FFPE clinical samples from lung cancer patients were analyzed using a CLIA-validated NGS-based assay that interrogates SNVs, indels using a 323 gene panel and by IHC for PD-L1 using the FDA approved PharmDx assay. TMB (mutations/Mb) is categorized as low (≤7), intermediate (7<TMB≤15) and high: (TMB> 15). NGS results were paired with PD-L1 status which was defined by tumor proportion scores (TPS) as: negative (TPS<1%), Low expressing (≥1-49%) and High (≥50%). *In silico* analyses were also performed on 5939 lung cancer samples from public databases. The study was approved by Neogenomics Institution's Ethics Board and external IRB, approval number 420160280.

Results

- We found poor correlation between PD-L1 expression and TMB in NSCLC (r2=0.266) and identified gene mutation signatures specific to groups defined by PD-L1 expression combined with TMB scores.
- KRAS mutations are constant across PDL1 TPS and less frequent on TMB High tumors, while EGFR mutation frequency negatively correlates to TMB and less to PD-L1 TPS..
- In PD-L1 Low/TMB High tumors (n=46) EGFR and KRAS were found mutated on 15% and 9% of samples, respectively (not part of the top 12 genes), while NTRK and NOTCH signaling are altered on 37% and 52%, respectively (65% combined).
- In tumors with an intermediate TMB (7<TMB≤15), we find that KRAS mutations are more frequent (43%) in PDL-1 High expressing tumors..
- EGFR alterations have highest frequency on TMB intermediate/ PD-L1 Low tumors.
- KEAP1 mutations, which have been recently proposed as poor prognosis biomarkers upon immunotherapy and other therapies in NSCLC, are found on up to 22% of lung tumors with TMB intermediate, including on 17% of tumors with TMB-intermediate and high PD-L1 expression.
- STK11 mutations on TMB intermediate tumors are present on 16% of PDL1 negative tumors but are rare (2%) on High PDL-1 expressing tumors.
- KEAP1 mutations predict better PFS than EGFR and STK11, but EGFR mutations predict longer OS than KEAP1 or STK11 according to publically available data
- Mutations on chromatin remodeling /DNA repair SMARCA4 and ARID1A/B genes are frequently found on the TMB intermediate group.
- We also identified a 5 genes set specifically mutated on PDL1-Neg/TMB High tumors with potential prognostic/predictive value.

Conclusions

- Genomic alteration signatures might define subsets of lung cancer tumors with no PD-L1 expression to complement TMB and PD-L1 on the selection criteria for patients whom may benefit from checkpoint inhibitors.
- Further studies are needed to evaluate specific signatures or single genes as biomarkers of immunotherapy response in NSCLC patients as well as to identify the molecular mechanisms enabling cancer cells therapy resistance and disease progression.

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

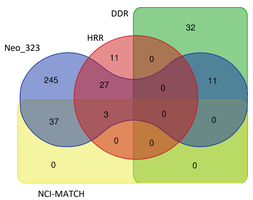


Figure 1. NeoTYPE Discovery 323 +MSI +TMB assay.

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

Tumor Mutation Burden in the Lung Cancer Cohort

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 %

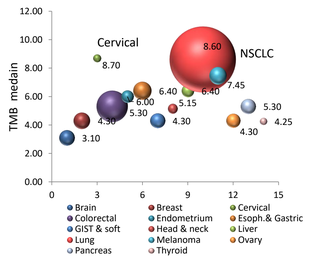
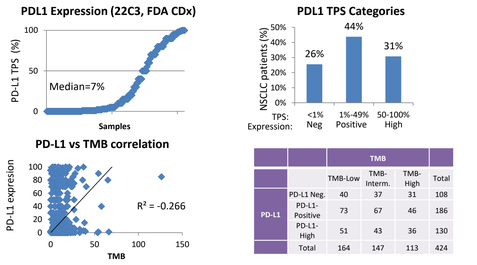


Figure 2. Tumor Mutation Burden (TMB) across cancer types. TMB median of serially tested NSCLC tumors without any preselection criteria and compared with other tumor types processed in the same manner in the same period. In addition, all lung cancer tumors were Microsatellite Stable (MSS) as determined according to the assay specifications. Cervical and lung cancers presented the highest median values off all tumor sites. Sphere size represents the sample size for each cancer type (n=784)

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

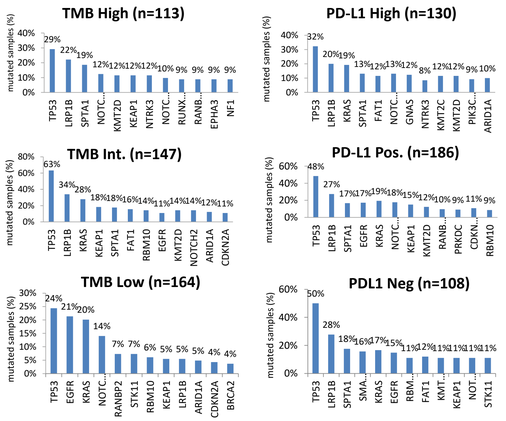


Figure 3. Mutation signatures and correlations between TMB and PD-L1 expression in a cohort of 424 NSCLC patients. TMB and PD-L1 expression lineal regression was performed. Samples were divided according to combined TMB and PD-L1 results. The top 12 most frequently mutated genes in each subcategory were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

EGFR/KRAS Signaling in NSCLC Cohort

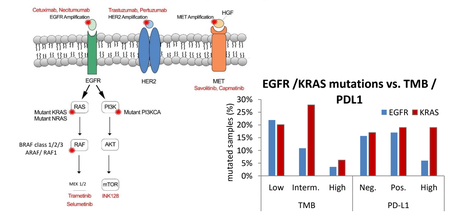


Figure 4. EGFR and KRAS alterations. Mutation frequency of the 2 genes was segregated by TMB or PD-L1 categories.

Genomic Signatures Define Combined PD-L1 & TMB neg/Low vs High Categories

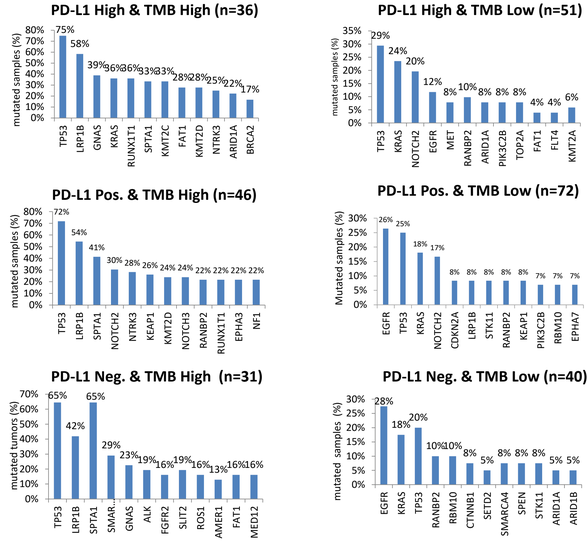
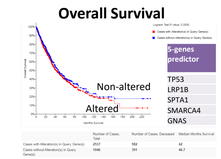
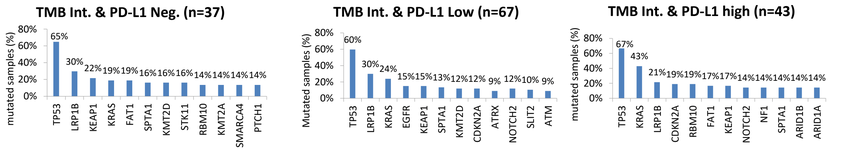


Figure 5. Genomic signatures on NSCLC samples divided by combined Tumor Mutation Burden (TMB) and PD-L1 expression.
The top-12 most frequently mutated genes in each subcategory were included. Mutated samples were counted only once even if more than 1 mutation in the gene was detected. The genes were ordered by the total number of mutations detected. Overall survival from 5939 NSCLC patients with mutations vs wt status on the top 5 mutated genes on PDL1 Neg &TMB High, was estimated using Kaplan-Meyer regressions using cbioportal.



The Genomic Signatures on Different TMB Intermediate NSCLC Scenarios



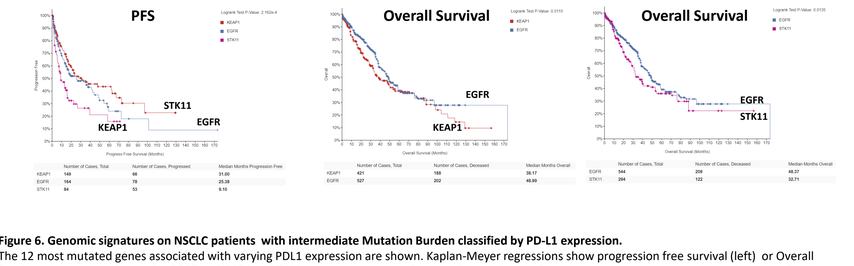


Figure 6. Genomic signatures on NSCLC patients with intermediate Mutation Burden classified by PD-L1 expression.
The 12 most mutated genes associated with varying PDL1 expression are shown. Kaplan-Meyer regressions show progression free survival (left) or Overall Survival (right) on patients with EGFR vs. KEAP1 or STK11 mutant tumors publically available with or without alterations on these genes (cbioportal). Samples with overlapping mutation on these genes are excluded.