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Comprehensive real-world-data based molecular profiling and mapping of non-squamous NSCLC patients to immune-checkpoint-inhibitor biomarkers

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PURPOSE

Non-squamous non-small cell lung cancer (nsqNSCLC) patients without detectable actionable alterations in *EGFR*, *ALK*, or *ROS1*, have a diverse genetic background. These patients are eligible to and usually receive untargeted, first line immune checkpoint inhibitors (ICI) and/or chemotherapy with heterogeneous outcomes. It is thus of crucial importance to further characterize this sub-population of nsqNSCLC by genetic alterations and other biomarker features to aid treatment decisions. Furthermore, the cross-association of genetic with epigenetic biomarkers, such as PD-L1 protein expression, can be utilized to streamline the molecular profiling and mapping of genetic traits of non-targetable nsqNSCLC.

To identify potential predictive biomarkers for the stratification of nsqNSCLC patients without actionable genetic alterations, a comprehensive molecular profiling of this subpopulation was performed using the FoundationInsights™ web platform. This included the identification of the genetic alteration landscape, downstream pathway analysis for an aggregated interpretation as well as the integration of genetic and epigenetic features.

METHODS

Molecular profiles from 67,001 nsqNSCLC patients were analyzed using the FoundationInsights™ web platform using the March 2022 release.

- A total of 54,205 patients were further analyzed after excluding patients with known and likely functional short variants for *EGFR* and fusions for *ALK* and *ROS1*
- The alterations analyzed included short variants (single nucleotide variants / SNVs, insertions and deletions / indels), copy number variants and gene rearrangements. The prevalence of genes with multiple types of alterations was summarized as 'multiple alterations' in the summary.

Pathway analysis was conducted with Metascape (metascape.org):

- We used all genes as input which displayed at least one variant with an allele-frequency > 1%, which resulted in a total of 85 genes.
- Gene enrichment was conducted in pathways from the GeneOntology database with the Metascape enrichment web platform.
- Pathways with an enrichment p-value < 0.05 were considered significant, and the top 10 most significant pathways are shown.

We obtained from the FoundationInsights™ web platform for each of the top 10 altered genes identified:

- Tumor mutation burden (TMB) = mutations detected per megabase. TMB low = TMB < 10; TMB high = TMB > 10.
- PD-L1 protein expression = scoring with 22C3 Dako PharmDx kit tumor staining. PD-L1 negative (0-1%) and PD-L1 low (1-49%) were combined and called PD-L1 low, and compared with PD-L1 high status group (50-100%).

Statistical significance and enrichment in TMB or PD-L1 biomarker overlap with the respective top 10 altered genes was calculated using a fisher exact test against an alteration negative cohort.

RESULTS

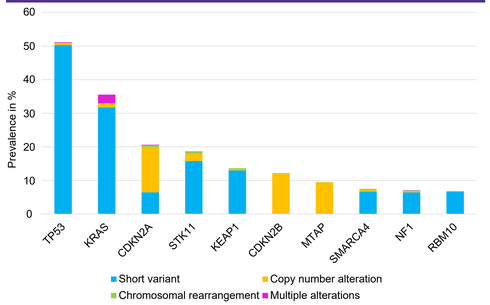

Figure 1. Frequently altered genes in nsqNSCLC patients without targetable genetic alterations. A total of 85 genes with a minimum allele-frequency > 1% were found. Here, top 10 altered genes in nsqNSCLC after excluding patients with alterations in *EGFR*, *ALK* and *ROS1* are shown. Alteration types included in the analysis are short variants (SNV and Indel), copy-number-alteration (CNV) and gene rearrangements.

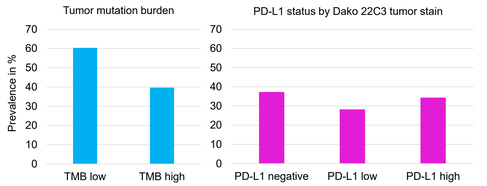

Figure 3: TMB and PD-L1 protein expression do not associate with nsqNSCLC patients lacking targetable alterations. Analysis of tumor mutation burden (TMB), measured as mutations per megabase, and PD-L1 expression status, measured by Dako 22C3 tumor staining. In all nsqNSCLC, there is no clear trend for either TMB high (10+ mut/Mb) status or PD-L1 high (50-100%) status.

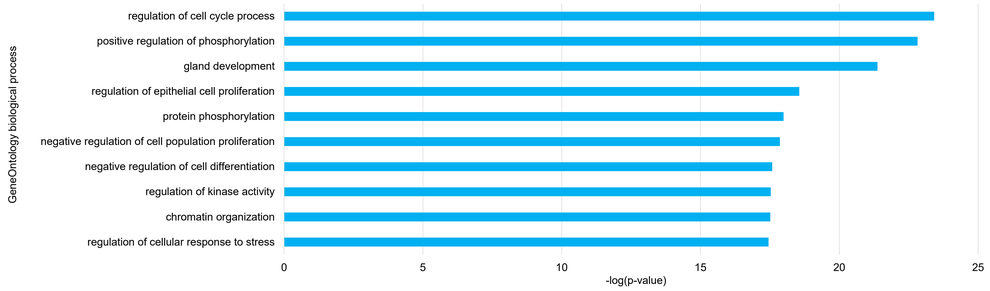

Figure 2: Commonly altered genes in non-targetable nsqNSCLC are associated with cell cycle, growth and proliferation. Pathway analysis conducted by Metascape. To this end, all genes with known or likely oncogenic alterations with allele-frequency >1% were selected for downstream analysis, which was a total of 85 genes. Metascape calculated enrichment and significance with the GeneOntology database and only pathways with p<0.05 were reported. Here, the top 10 most significantly associated pathways are shown.

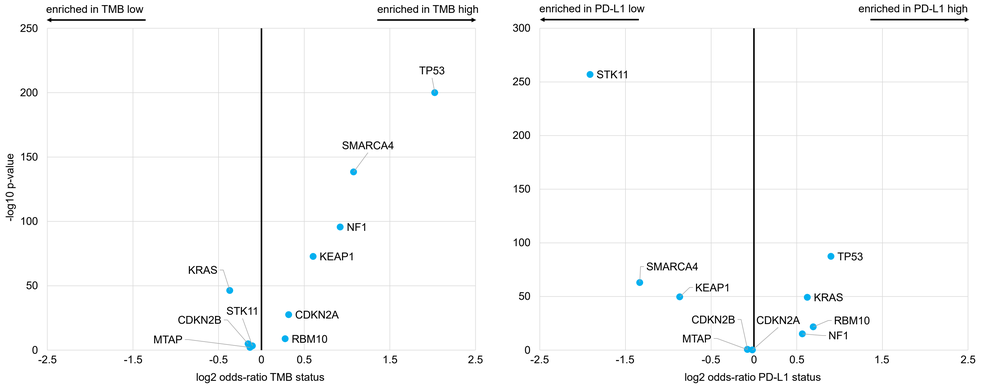

Figure 4: Few commonly altered genes in non-targetable nsqNSCLC are enriched in low or high TMB or PD-L1. Enrichment analysis of TMB (left) or PD-L1 (right) for the top 10 altered genes in non-targetable nsqNSCLC versus an alteration negative cohort. For simplicity, PD-L1 negative (0-1%) and PD-L1 low (1-49%) were grouped into PD-L1 low, and compared with patients displaying high PD-L1 expression (50-100%).

CONCLUSIONS

- Lack of a general association of nsqNSCLC without actionable alterations with either high PD-L1 and high TMB
- We here reported a subpopulation of nsqNSCLC patients described by certain genetic alteration
- Some genetic alterations were associated with ICI-related biomarkers for which patients might benefit from a future precision oncology approach utilizing combined targeted and ICI treatment
- Non-targetable alterations in nsqNSCLC are significantly associated with cellular functions included in cell cycle, proliferation or chromatin organization
- We found *TP53*, *SMARCA4*, *NF1* and *KEAP1* associated with either high TMB or high PD-L1, and *STK11*, *SMARCA4*, *KEAP1* and *KRAS* associated with either low TMB or low PD-L1
- Future research needs to set out to validate a causal relationship between our findings and a potential benefit for the identified patient subpopulation receiving immune-checkpoint-inhibitors.

REFERENCES

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DISCLOSURES

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