INTRODUCION

Immune checkpoint inhibitors have improved clinical responses and overall survival for patients with non-small cell lung cancer (NSCLC). However, the response is not equal and known NSCLC biomarkers are not sufficient in predicting therapy outcome. Here, we show results of deep analysis of plasma proteome, using unbiased data independent acquisition mass spectrometry (DIA-MS), from two independent cohorts of late stage NSCLC patients. Both cohorts received anti-PD-1 treatment (Table 1). We have focused our analysis on progression free survival (PFS) as the primary outcome. Overall PFS, for both cohorts is presented in Figure 1. To identify proteomic signatures specific for the PFS we used the approach previously applied by Uhlen et al., 2017 [1] for transcriptomic datasets. In this approach survival data is used to set the most optimal thresholds for candidate biomarkers.

RESULTS

Figure 2: Analysis plan, metrics and methods

All identified proteins provided robust predictive and prognostic signature assessed via hierarchical clustering. Two striking protein clusters (dashed box) were formed across all samples with proteins regulating cell cycle, growth, migration, proliferation (e.g. SAA1, ORM1, C3, C9, F9), acute response (e.g. CCR8, NPEPPS, TMEM198, CENPE, FLT4), and various metabolic processes (e.g. UQCRB, NDUFV3).

Table 1

<table>
<thead>
<tr>
<th>Protein</th>
<th>FoldChange</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRG1</td>
<td>2.0</td>
<td>0.001</td>
<td>High</td>
</tr>
<tr>
<td>MYD88</td>
<td>1.8</td>
<td>0.005</td>
<td>Moderate</td>
</tr>
<tr>
<td>TRAF6</td>
<td>1.5</td>
<td>0.01</td>
<td>Low</td>
</tr>
</tbody>
</table>

Figure 3: Analysis principle and summary of known and potentially new proteomics markers

Table 1 shows all detected proteins signifi- cantly associated with PFS outcome are genomics or proteomic markers in EDRN. 16% of all proteins and 20% known therapy targets like L YPD3.

CONCLUSIONS

• HRM™-MS is providing deep insights into plasma proteome in an unbiased manner.
• The use of clinical information like PFS for high-dimensional proteomic data is possible, and resulted in a unique protein signature which includes known markers like LRG1 or known therapy targets like LYPD3.
• Combining signatures from both timepoints and cohorts resulted in a significant separation of subjects (p < 0.0001).
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Figure 4: Verification of selected proteins

Analysis of LRG1 protein involved in multiple carcinomas [4, 5]. Its intensity was negatively associated with PFS in cohort A after PD-1 treatment (A). It is also reported as a biomarker in EDRN. 16% of all proteins and 30% of proteins associated with PFS outcome are reported as genomic or proteomic markers in Early Detection Research Network - EDRN (B).

Figure 5: Survival analysis of combined signatures

In hierarchical clustering, samples were also clustered column-wise into two clusters. Subjects from these clusters were extracted and survival analysis was performed using the same settings for as individual proteins. The identified proteomic signature was able to separate patients with high and low PFS at p-value = 0.0001, confirming its prognostic and predictive power.

References: