Blood-based multiplex kinase activity profiling as a predictive marker for clinical response to checkpoint blockade in advanced NSCLC

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Background

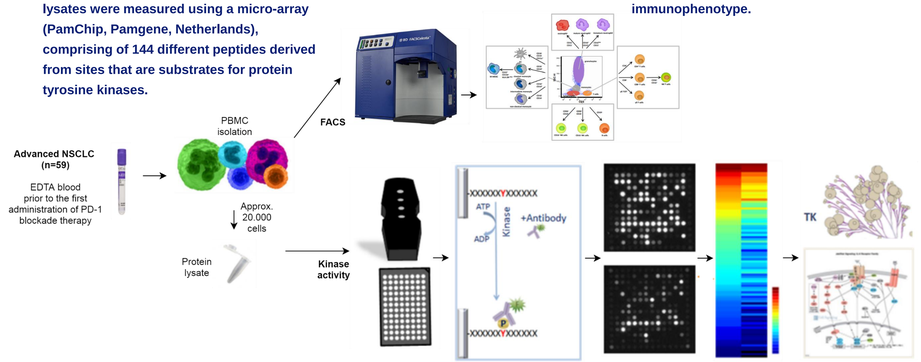
Only a minority of non-small-cell lung cancer (NSCLC) patients benefit from treatment with immune checkpoint inhibitors (ICIs), therefore, there is an urgent need for response prediction. Previously, the potential of using tyrosine kinase activity profiling of baseline peripheral blood mononuclear cells (PBMC) was demonstrated in an analysis of ICI-treated advanced melanoma patients[1].

Objective

To evaluate the predictive/ prognostic value of tyrosine activity profiling of baseline PBMC for early progression after PD-1 blockade in NSCLC patients.

Methods

- **59 ICI naïve advanced NSCLC patients who were treated with anti-PD-1 ICIs were included (MULTOMAB; NTR7015), 3 outlier samples were excluded from the analysis.**
- **PBMC were isolated from blood samples (EDTA) obtained prior to treatment with ICIs.**
- **Kinase phosphorylation signatures of PBMC lysates were measured using a micro-array (PamChip, Pamgene, Netherlands), comprising of 144 different peptides derived from sites that are substrates for protein tyrosine kinases.**
- **Correlation of the profiles with PFS (RECIST v1.1) and OS was analyzed using univariate cox-regression.**
- **Predictive multivariate (GLMnet) analysis was performed by binary survival grouping of patients with a cut-off at 140 days for PFS.**
- **The predictive performance of the models was estimated using cross validation.**
- **Multiplex flow cytometry, enumerating 18 immune cell subsets and assessing expression for 28 T cell markers[2], was performed for a selection of patients to provide further insight in the immunophenotype.**



Results

Baseline characteristics	
Total evaluable, n (%)	56
Age, median (range)	63.1 (35-81)
Gender, n (%)	
Male	36
Female	20
Primary tumor, n (%)	
Adenocarcinoma	37
Squamous cell carcinoma	17
Large cell carcinoma	2
Treatment regimen, n(%)	
Nivolumab	50
Pembrolizumab	6
Prior therapy lines, n (%)	
0	1
1	46
2	7
>2	2
Prior immunotherapy, n (%)	
No	56
Yes	0
LDH, median (range)	269 (133-860)

Table 1: Baseline characteristics of all evaluable patients (n=56). All NSCLC received anti-PD1 monotherapy and were not treated with any prior line of immunotherapy.

- **Evaluation of the predictive value of GLMnet models resulted in estimates for the Correct Classification rate (CCR) of 69% (90% CI 58-80%) and 70% (90% CI 56-82%), for respectively early progression (progression < 140 days after initiation of PD-1 blockade; Figure 2: right) and early death (OS < 1 year; data not shown).**
- **The predicted-high-risk group displayed significant poorer median PFS and OS than the predicted-low-risk group ([56 vs. 246; HR 2.3, 95%CI 1.2-4.7, p=0.02] and [171 vs. 488 days; HR 2.7, 95%CI 1.1-6.6, p=0.03], respectively; Figure 3A and 3B).**

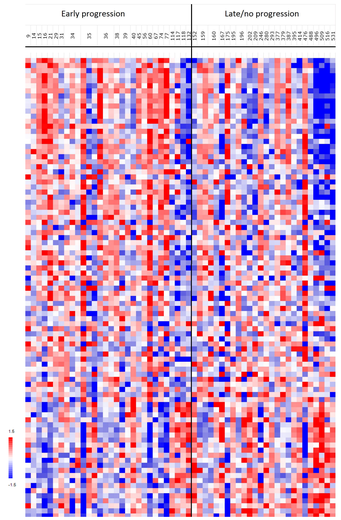


Figure 1 (on the right): Data visualization showing the correlation of the kinase activity profiles with PFS. The columns represent subject, sorted from left to right to increasing PFS. The header indicates early progression (if PD occurred before 140 days after start of treatment) and late/no progression.

- **An upstream kinase interpretation was performed of the phosphorylation signature of patients with early progression compared to patients with late/no progression. The results of this analysis were mapped to a kinase phylogenetic tree (Figure 4), and indicate that T cell function related proteins are differentially activated in these groups.**
- **On a subset of 28 patients in the present cohort, FACS analysis was performed to enumerate 18 immune cell subsets[2] showing that the PBMC composition is heterogeneous between patients (Figure 2: left). Here, no significant differences applicable for response prediction could be determined based on the immunophenotype.**

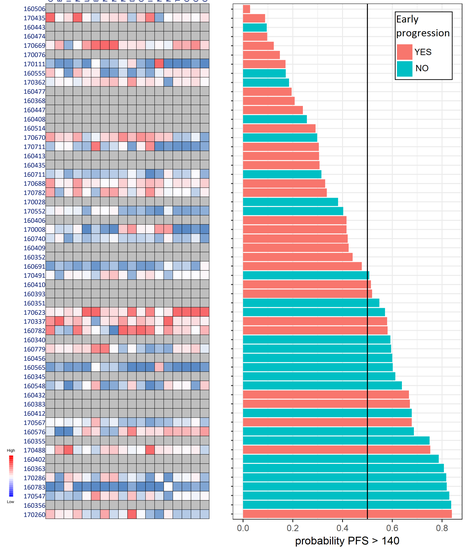


Figure 2: Immune cell enumeration by FACS analysis of a subset (n=28, colored squares) of patients (left) and the predicted probability (based on the kinase phosphorylation profile; n=56) of late/no progression (PFS > 140 days) for each patient - obtained by 25-fold cross validation. The bars in the graph are colored according to the known class of the subjects (see legend).

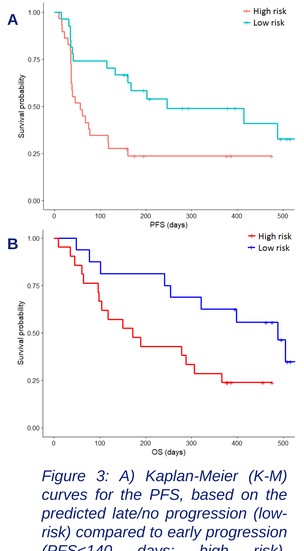


Figure 3: A) Kaplan-Meier (K-M) curves for the PFS, based on the predicted late/no progression (low-risk) compared to early progression (PFS<140 days; high risk). Similarly, B) K-M curves for OS are shown, based on the probability of early death compared to long survival (OS > 1 year).

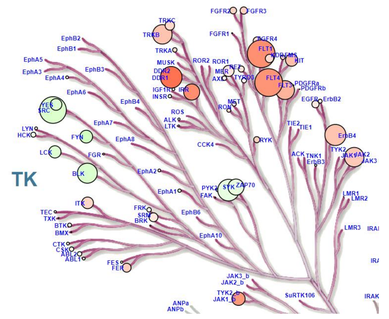


Figure 4: Result of upstream kinase interpretation comparing patients with early progression to patients with late/no progression, mapped to a kinase phylogenetic tree (image reproduced courtesy of Cell Signaling Technologies Inc). The color of the circle represents the effect size with green/red indicating lower/higher activity of the corresponding kinase in the group of patients with late-no progression. The size of the circles represents the significance of the corresponding kinase in the analysis.

Conclusion

Kinase activity profiles of baseline PBMC samples of advanced NSCLC patients predict the likelihood of early progression to PD-1 blockade and may serve as a predictive[3] and/or prognostic biomarker.

References:

1. D.P. Hurkmans, E.M.E. Verdegaal, S.A. Schindler et al., J Clin Oncol 36, 2018 (suppl; abstr 9579)

2. A. Kunert, E. . Basak, D.P. Hurkmans, et al.. J Immunother Cancer. 2019 Jun 8;7(1):149

3. Understanding prognostic versus predictive biomarkers, BEST Resource, FDA-NIH Biomarker Working Group, published 2016, https://www.ncbi.nlm.nih.gov/books/NBK402284/

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