A Blood-Based miRNA Signature Predicts Immunotherapy Response

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ABSTRACT

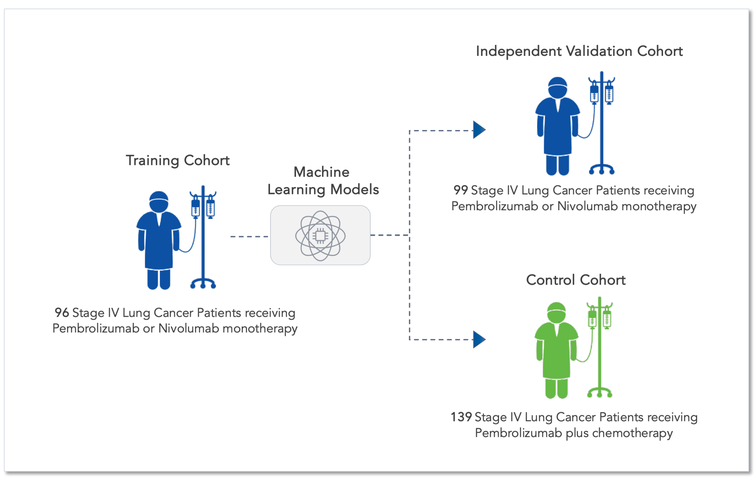
Immunotherapy can be a highly effective therapy in a small subset of patients with late-stage non-small cell lung cancer (NSCLC). Biomarkers are used to help choose which patients may benefit from these drugs however even using the current gold standard (PD-L1 tumor proportion staining (TPS)) only 30-40% of patients will achieve a positive response (Brahmer et al., 2018). **There is pressing need for more accurate biomarkers for immunotherapy response prediction.**

In this study we have sought to identify peripheral blood biomarkers, predictive of response to immunotherapies against lung cancer, based on whole blood microRNA profiling. Using three well characterized cohorts consisting of stage IV NSCLC patients, we have defined a 5 microRNA risk score (miRisk) that is predictive of immunotherapy response in training and independent validation cohorts. We have traced the signature to a myeloid origin and performed miRNA target prediction to make a direct mechanistic link to the PD-L1 signalling pathway and PD-L1 itself. **The miRisk score offers a potential blood-based companion diagnostic for immunotherapy that outperforms tissue-based PD-L1 staining.**

METHODS

Patient Cohorts

We have collected a total of 334 patients across two sites (Heidelberg and Großhansdorf) in three distinct cohorts.



Sample collection and processing

Whole blood was collected from all patients in PAXgene tubes, in order to stabilize whole blood RNA. Total RNA was extracted and captured in small RNA sequencing libraries to generate a small RNA expression profile for each patient.

Survival analysis

A computational pipeline based on that described by Shukla et al., was created to perform initial filtering of features based on Cox univariable association with survival, before a further stepwise multivariable Cox regression was used to create a multiple miRNA signature (Shukla et al., 2016).

The Training Cohort was used to define the miRisk score which is a weighted sum of expression of 5 miRNAs. The miRisk score can stratify patients based on their predicted immunotherapy response using the median risk threshold defined in the Training Cohort.

REFERENCES

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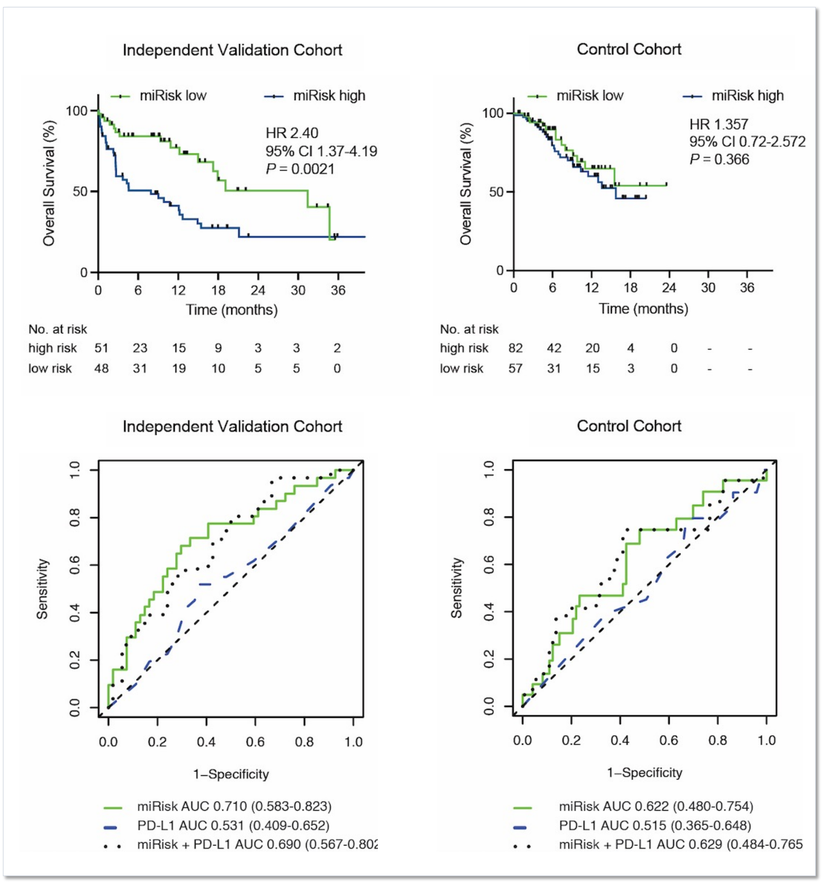
RESULTS

Low miRisk score patients survive longer

99 patients in the Independent Validation Cohort were stratified by miRisk score into low/high risk groups. Patients in the low-risk groups survive for significantly longer.

We have used the clinically relevant timepoint of 6 months to assess the the discriminative ability of immunotherapy biomarkers using time dependent ROC analysis. **We show that the miRisk ROC AUC is significantly higher than that of PD-L1 TPS.**



The miRisk score is specific to immunotherapy response prediction

The miRisk score fails to stratify patients within the Control Cohort, who were treated with a combination of chemotherapy and immunotherapy. This is consistent with other immunotherapy biomarkers that have been shown not to generalize to patients treated with combination therapies (Gandhi et al., 2018). **This is evidence that the miRisk score is not a marker of general prognosis but rather specifically predictive of response to immunotherapy.**

The miRisk score is a significant independent predictor of response

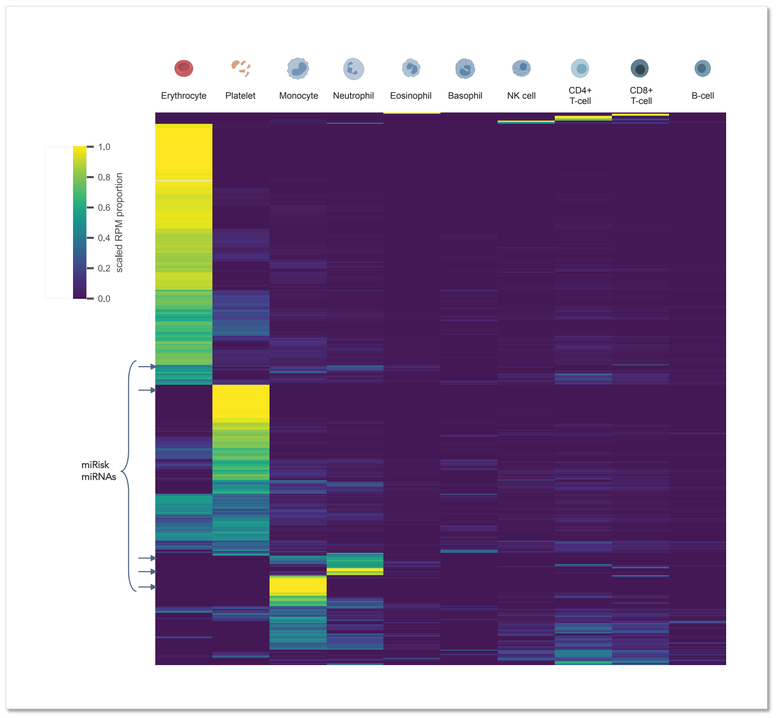
To explore the association between the miRisk score and other clinical covariates with respect to OS, both univariable and multivariable Cox regression were performed within the Validation Cohort. In univariable analysis we observed significant associations between OS and ECOG, absolute neutrophil count (ANC), absolute lymphocyte count (ALC) and the miRisk score. We observed a trend towards increased risk with low PD-L1 TPS in the immunotherapy Validation Cohort; however, this does not reach significance.

In multivariable analysis, the association between blood counts and survival is diminished when controlling for all other covariates. **Both ECOG and the miRisk score remain as the only two significant independent predictors of OS in multivariable Cox regression.**

Overall survival	Univariable Analysis			Multivariable Analysis		
Covariate	HR	95% CI	P-value	HR	95% CI	P-value
PD-L1 (≤ 50%)	1.70	0.97-3.00	0.07	1.85	0.58-5.93	0.30
ECOG (>0)	2.82	1.47-5.41	<0.01	4.61	2.21-9.59	<0.01
Gender (male vs female)	1.07	0.60-1.93	0.82	0.79	0.42-1.49	0.47
Age (>75 years)	1.66	0.85-3.24	0.14	1.84	0.88-3.86	0.11
Therapy line incl. <IV (>2)	1.36	0.73-2.54	0.33	1.31	0.67-2.55	0.43
Substance (Pembro vs Nivo)	0.68	0.39-1.19	0.18	1.36	0.44-4.24	0.59
Histology (non-adeno vs adeno)	1.63	0.93-2.85	0.09	1.65	0.84-3.24	0.15
Smoking status (ever smoker vs other)	1.92	0.46-7.96	0.37	4.62	1.00-21.42	0.05
ANC (>7.5)	1.98	1.08-3.62	0.03	1.07	0.54-2.11	0.86
ALC (>1)	0.57	0.32-0.99	<0.05	0.69	0.39-1.24	0.22
miRisk (high vs low)	2.47	1.36-4.48	<0.01	2.40	1.12-5.14	0.02

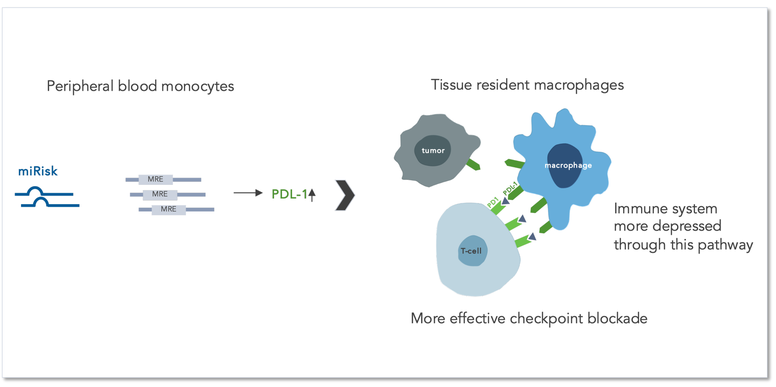
Myeloid enriched miRNAs

Using a blood cell type expression atlas (below), we show that despite the majority of PAXgene detected miRNAs originating from RBCs, **there is a striking cell type specific expression pattern of our 5 miRisk miRNAs in myeloid cells, and platelets.** The miRisk signature, which was defined through a data driven process, has converged upon miRNAs that are specifically expressed in immune cell types that have a plausible relevance to immunotherapy response.



miRNA pathway analysis

Using bioinformatic target prediction and pathway analysis, we have shown an overrepresentation of miRisk miRNA targets in immunotherapy related pathways (PD-L1 and MAPK signalling). Furthermore, the 3' UTR of PD-L1 contains 3 predicted target sites for miRisk miRNAs, representing a direct functional link. Together we propose a mechanism whereby lower expression of the miRisk miRNAs in responder patients corresponds to a derepression of signalling through the PD-1/PD-L1 pathway in peripheral immune cells which is maintained upon their migration into the TME. This establishes a TME in which immunosuppression is mediated through the PD-1/PD-L1 immune checkpoint, and in turn an environment that is particularly susceptible to pharmacological inhibition of this pathway.



CONCLUSIONS

Here we describe the discovery, validation and mechanistic insight into a 5 miRNA risk score (miRisk) that:

- Predicts survival of stage IV NSCLC patients receiving immunotherapy.
- Can be measured in peripheral blood.
- Performs better than the current gold standard, tissue PDL1 assays.

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