Brief Report: A Blood-Based MicroRNA Complementary Diagnostic Predicts Immunotherapy Efficacy in Advanced-Stage NSCLC With High Programmed Death-Ligand 1 Expression

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ABSTRACT

Introduction: Patients with advanced, non-oncogene-driven NSCLC with high programmed death-ligand 1 (PD-L1) expression are eligible for treatment with immunotherapy. There is, however, an urgent medical need for biomarkers identifying cases that require additional combination with chemotherapy. We previously uncovered a myeloid-based 5-microRNA (5-miRNA) signature that identified responders to immunotherapy in PD-L1 unstratified patients; however, its potential utility in treatment guidance for patients with PD-L1 high tumors remained unclear.

Methods: We trained (n = 68) and validated (n = 56) a 5-miRNA multivariable Cox proportional hazards model predictive of overall survival on small RNA sequencing data.

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Steinkraus are employees of Hummingbird Diagnostics and hold company stock options. Dr. Rajakumar, Dr. Horos, Dr. Sikosek, and Dr. Steinkraus are inventors of patent applications related to response prediction for immunotherapy submitted by Hummingbird Diagnostics. Prof. Christopoulos and Prof. Reck serve on the clinical advisory board of Hummingbird Diagnostics. Dr. Mercaldo reports receiving consulting fees from Hummingbird Diagnostics.

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of whole blood samples prospectively collected before the commencement of immunotherapy for stage IV NSCLC with PD-L1 tumor proportion score greater than or equal to 50%, treated with PD-1 inhibitor monotherapy (immunotherapy alone [IO]). Specificity was demonstrated in a control cohort treated with immunotherapy alone (ICT) (n = 31).

**Results:** The revised 5-miRNA risk score (miRisk) stratified IO-treated patients and identified a high-risk group with significantly shorter overall survival (hazard ratio = 5.24, 95% confidence interval: 2.17–12.66, \( p < 0.001 \)). There was a significant interaction between the miRisk score and type of treatment (IO or ICT, \( p = 0.036 \)), indicating that the miRisk score may serve as a predictive biomarker for immunotherapy response. Furthermore, the miRisk score could identify a group of high-risk patients who may benefit from treatment with ICT as opposed to IO (hazard ratio = 0.35, 95% confidence interval: 0.15–0.82, \( p = 0.018 \)).

**Conclusions:** The miRisk score can distinguish a group of patients with PD-L1 high, stage IV NSCLC likely to benefit from adding chemotherapy to immunotherapy and may support treatment decisions as a blood-based complementary diagnostic.

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**Keywords:** NSCLC; Immunotherapy; PD-L1; miRNAs; Biomarker

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**Introduction**

Immunotherapy is revolutionizing the standard of care for numerous cancers; however, an important limitation is the lack of reliable efficacy biomarkers. In advanced-stage NSCLC expressing high levels of programmed death-ligand 1 (PD-L1) (PD-L1 tumor proportion score [TPS] \( \geq 50\% \)), both immunotherapy alone (IO) or in combination with chemotherapy (ICT) are recommended as treatment options in major international guidelines.\(^1\),\(^2\) Nevertheless, in clinical reality, these two options may not be equal, and there are patients unresponsive to IO monotherapy, who need additional chemotherapy. So far, no results from prospective trials can support decision-making on this clinically important issue and there is an unmet need for therapy selection biomarkers.

A positive response to immunotherapy is dependent both on local interactions between cancer and immune cells in the tumor microenvironment\(^3\) and on systemic immune processes that can be assessed in the periphery.\(^4\) Defining the latter has the potential to provide noninvasive therapy guidance, but no blood-based biomarkers have yet entered routine clinical use.

We previously reported the development of a circulating myeloid cell-derived 5 microRNA (miRNA) signature—5-miRNA risk score (miRisk)—to predict overall survival (OS) of patients with advanced NSCLC treated with IO in PD-L1 TPS unstratified patients.\(^5\) Here, we systematically evaluated the ability of a revised miRisk score to identify PD-L1 high patients who are likely to benefit from chemotherapy in addition to immunotherapy.

**Materials and Methods**

This study included a total of 155 prospectively recruited patients with stage IV NSCLC and PD-L1 TPS greater than or equal to 50% whose blood samples were collected before immunotherapy treatment (2017–2020). Patients with actionable mutations in EGFR, ALK, and ROS1 were identified by combined DNA and RNA next-generation sequencing\(^6\) and excluded from this study. All patients provided written informed consent. Samples were obtained from the Lungenbiobank Heidelberg and Biobank Nord within the German Center for Lung Research (DZL) according to the pertinent regulations after approval of the ethics committees at Heidelberg University (S-296/2016, S-089/2019) and LungenClinic Grosshansdorf (AZ 12-238, AZ 19-286). Sample processing and generation of small RNA expression profiles are described in detail in our previous work.\(^5\) All anonymized small RNA sequencing data have been deposited at the European Nucleotide Archive under accession number PRJEB50502. Survival analyses were performed in Python (3.8.8), using the packages scikit-survival (version 0.15.1)\(^7\) and Lifelines (version 0.26.0).\(^8\) miRisk low and high groups were defined based on the median risk score within the training cohort (low risk \( \leq -0.0725 \) < high risk). Relevant clinical confounders to include in multivariable models were selected in consultation with a panel of thoracic oncologists (P.C., M.T., M.R.). Visualization was performed in GraphPad Prism (version 9.3.1, GraphPad Software).

**Results**

This study included 155 patients with stage IV NSCLC, divided into training (\( n = 68 \)) and validation (\( n = 56 \)) cohorts treated with IO, and split according to the time-point of sample collection, and a control cohort treated with ICT (\( n = 31 \)) (Table 1). These three cohorts had similar clinicopathologic characteristics. Fitting a multivariable Cox proportional hazards model to the
training cohort using the five previously identified miRNAs as input features led to a revised linear predictor risk score: miRisk = \(\ln(\text{miR-2115-3p RPM} + 1)\) \(\times 2.190467\) + \(\ln(\text{miR-218-5p RPM} + 1)\) \(\times 0.303095\) + \(\ln(\text{miR-224-5p RPM} + 1)\) \(\times 0.598415\) + \(\ln(\text{miR-4676-3p RPM} + 1)\) \(\times 1.101122\) + \(\ln(\text{miR-6503-5p RPM} + 1)\) \(\times 0.958823\).

We observed significantly longer OS in the miRisk-low patients in the training cohort (hazard ratio [HR] = 3.65, 95% confidence interval [CI]: 1.84–7.24, \(p < 0.001\)) (Supplementary Fig. 1). This finding was confirmed in the validation cohort (HR = 5.24, 95% CI: 2.17–12.66, \(p < 0.001\)) (Fig. 1A). In contrast, the miRisk score was not associated with OS in patients in the ICT control cohort (HR = 1.40, 95% CI: 0.21–9.28, \(p = 0.753\)) (Fig. 1B), consistent with previous reports that IO-specific biomarkers do not predict response to ICT.\(^9\),\(^10\)

To explore the utility of the miRisk score as a complementary diagnostic for IO versus ICT treatment decisions, we merged the validation and control cohorts to explore the interaction between the biomarker and type of treatment in terms of relationship with OS. In the miRisk-low patients, there was no difference in OS between those treated with IO or ICT (HR = 1.22, 95% CI: 0.12–12.25, \(p = 0.849\)) (Fig. 1C). In contrast, in miRisk-high patients, we observed significantly improved OS in patients receiving ICT compared with IO monotherapy (HR = 0.35, 95% CI: 0.15–0.82, \(p = 0.018\)) (Fig. 1D). The interaction between treatment and miRisk score in a multivariable Cox proportional hazards model was significant (HR = 1.62, 95% CI: 1.03–2.55, \(p = 0.036\),
suggesting the miRisk score is both prognostic and predictive for the efficacy of IO (Supplementary Table 1).

Finally, we used multivariable Cox proportional hazards models to investigate the performance of the miRisk score when controlling for other relevant clinicopathological covariates. This revealed that the miRisk score had a stronger association with OS in IO-treated patients (HR = 3.82, 95% CI: 1.29–11.30, p = 0.015) than PD-L1 TPS, histologic subtype, Eastern Cooperative Oncology Group performance status, and therapy line (Table 2).

**Discussion**

Currently, both IO and ICT are recommended as treatment options for patients with stage IV NSCLC and PD-L1 TPS greater than or equal to 50%. Nevertheless, the response rate to IO monotherapy is only 40%, and many apparent nonresponders may benefit from ICT, as the chemotherapy component can sensitize the tumor to concurrent immunotherapy. This additional therapeutic burden comes at the cost of increased frequency and severity of toxicity, with grade 3 to 4 adverse events noted in approximately 70% of patients. Because there is currently no reliable biomarker to predict response to therapy, the decision to treat with IO versus ICT is largely based on clinical judgment, considering factors such as general health status, number of metastatic sites, and disease aggressiveness. Still, the optimal therapy for a given patient often remains unclear.

There is great interest in the discovery of blood-based biomarkers that are predictive of immunotherapy response due to their ease of noninvasive collection and their potential to capture signal both from the peripheral immune system and material shed.
from the tumor itself.16 Efforts to measure soluble PD-L1 status17 and plasma tumor mutational burden18 were found to be promising; however, these are yet to be approved for clinical use. Immune cell RNA profiling and next-generation sequencing-based methods could enable improved prediction with additional integration of signal from multiple sources, such as peripheral effector cell counts.3,19 Encouraging results have been reported from the measurement of circulating miRNAs, known to be master regulators of gene expression, and implicated in multiple processes in immune regulation and cancer.20

The miRisk score exploits these opportunities as a multivariable model that measures the expression of predominantly myeloid-derived miRNAs with predicted interactions with the PD-(L)1 signaling pathway.5 This blood-based biomarker is found to have robust generalizable performance in a validation cohort for survival prediction after IO treatment (HR = 5.24, 95% CI: 2.17–12.66, p < 0.001) and utility as a complementary diagnostic for the decision to treat miRisk-high patients with ICT (HR = 0.35, 95% CI: 0.15–0.82, p = 0.018).

There are limitations to the current study. There was no randomization of treatment between the IO- and ICT-treated cohorts. Therefore, despite controlling for known confounders with multivariable analyses, it is impossible to rule out the influence of potential hidden confounders. We further acknowledge a lack of ethnic diversity in study participants. We aim to address these issues in an upcoming prospective clinical trial.

In summary, the therapeutic landscape in advanced NSCLC is rapidly developing, in large part due to the successes of immunotherapies. The only currently used biomarker, PD-L1 TPS, has several limitations, including poor predictive performance, the necessity for an invasive tissue biopsy, and the subsequent exposure to sampling bias because of tumor heterogeneity and different assay platforms.21 As a result, there is an unmet need for more accurate and noninvasive diagnostics to guide treatment decisions. The miRisk score represents an immune-focused biomarker that is specifically predictive of response to immunotherapy and could serve as the foundation for a complementary diagnostic to guide therapeutic decisions and thereby allow physicians to more accurately choose treating patients between with IO alone versus with ICT.

### CRediT Authorship Contribution Statement

**Timothy Rajakumar:** Conceptualization, Investigation, Formal analysis, Writing—original draft, Writing—review and editing.

**Rastislav Horos, Paul Kittner, Mustafa Kahraman, Tobias Sikosek:** Investigation, Formal analysis, Writing—review and editing.

**Franziska Hinkfoth, Kaja Tikk:** Data curation, Writing—review and editing.

**Nathaniel D. Mercaldo:** Formal analysis, Writing—review and editing.

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**Table 2. Univariable and Multivariable Cox Regression Analysis of miRisk and Clinical Covariates**

| Overall Survival | Univariable Analysis | Multivariable Analysis |
|------------------|----------------------|------------------------|
| Covariate        | HR   | 95% CI | p Value | HR   | 95% CI | p Value |
| IO training cohort |      |        |         |      |        |         |
| ECOG performance status | 1.39 | 0.76-2.52 | 0.281 | 0.87 | 0.46-0.51 | 0.682 |
| Histology (nonadeno vs. adeno) | 1.09 | 0.55-2.17 | 0.806 | 1.40 | 0.69-1.04 | 0.354 |
| Therapy line | 0.75 | 0.38-1.49 | 0.409 | 0.79 | 0.38-0.49 | 0.532 |
| PD-L1 TPS | 0.99 | 0.97-1.01 | 0.433 | 0.96 | 0.93-0.01 | 0.006 |
| miRisk (high vs. low) | 3.84 | 1.86-7.95 | <0.001 | 7.41 | 2.95-2.93 | <0.001 |
| IO validation cohort |      |        |         |      |        |         |
| ECOG performance status | 3.53 | 1.55-8.05 | 0.003 | 3.32 | 1.24-2.18 | 0.017 |
| Histologic subtype (nonadeno vs. adeno) | 1.89 | 0.79-4.50 | 0.151 | 1.79 | 0.69-1.54 | 0.235 |
| Therapy line | 1.57 | 1.05-2.34 | 0.029 | 1.23 | 0.81-0.63 | 0.335 |
| PD-L1 TPS | 1.00 | 0.97-1.03 | 0.753 | 1.00 | 0.97-0.04 | 0.848 |
| miRisk (high vs. low) | 5.37 | 1.96-14.74 | 0.001 | 3.82 | 1.29-2.42 | 0.015 |
| ICT control cohort |      |        |         |      |        |         |
| ECOG performance status | 3.70 | 0.85-16.06 | 0.081 | 6.15 | 1.00-37.99 | 0.050 |
| Histology (nonadeno vs. adeno) | 1.10 | 0.12-9.88 | 0.935 | 1.68 | 0.16-17.36 | 0.665 |
| Therapy line | 0.80 | 0.09-6.75 | 0.838 | 4.00 | 0.18-90.51 | 0.384 |
| PD-L1 TPS | 1.00 | 0.94-1.05 | 0.906 | 1.00 | 0.94-1.07 | 0.925 |
| miRisk (high vs. low) | 1.41 | 0.17-11.91 | 0.754 | 1.17 | 0.13-10.82 | 0.889 |

Note: ECOG performance status, therapy line, and PD-L1 TPS were modeled as continuous variables. Histologic subtype and miRisk were modeled as categorical variables.

Adeno, adenocarcinoma; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; ICT, immunochemotherapy; IO, immunotherapy alone; miRisk, 5-microRNA risk score; PD-L1, programmed death-ligand 1; TPS, tumor proportion score.
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Supplementary Data
Note: To access the supplementary material accompanying this article, visit the online version of the JTO Clinical and Research Reports at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2022.100369.

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