27-gene Immuno-Oncology (IO) Score is Associated With Efficacy of Checkpoint Immunotherapy in Advanced NSCLC: A Retrospective BC Cancer Study

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Abstract

Existing biomarkers for ICI treatment in aNSCLC remain suboptimal. DetermaIO is a novel tumor immune microenvironment classifier that has previously been associated with ICI response. DetermaIO was associated with survival in ICI monotherapy treated patients who were PDL1≥50% and among a subgroup of PDL1>50% who were PS 2, suggesting that DetermaIO may have clinical utility over current biomarkers in aNSCLC.

Background: Immune checkpoint inhibitors (ICI) are standard of care in advanced non-small cell lung cancer (NSCLC). However, not all patients benefit, even among PD-L1 tumor proportional score (TPS) ≥50%, indicating an unmet need for additional biomarkers such as those assessing the tumor immune microenvironment (TIME). DetermaIO is a 27-gene assay that classifies TIME and has previously demonstrated association with ICI response. **Methods:** FFPE samples were selected from BC Cancer and West Clinic Cancer Center patients with performance status (PS) ≤2 who received at least 2 cycles of ICI monotherapy in the first (1L) or second line (2L). IO scores were generated and analyzed for association with PFS and OS. **Results:** In the entire cohort (N=147), IO score was significantly associated with OS (HR=0.68, 95%CI 0.47-0.99, $P = .042$) and PFS (HR=0.62, 95%CI 0.43-0.88, $P = .0069$). In 1L treated patients (PD-L1≥50%, N=78), IO score was significantly associated with PFS (HR=0.55, 95%CI 0.32-0.94, $P = .028$). In exploratory analyses, IO score was associated with benefit in 1L PS2 patients for OS (HR = 0.26, 95%CI 0.091-0.74, $P = .012$) and PFS (HR = 0.27, 95%CI 0.098-0.72, $P = .0095$) which was confirmed in PFS subgroup analysis in the independent West Cancer Center study (N=13 HR=0.14, 95%CI 0.027-0.76, $P = .023$). **Conclusion:** These data confirm the association of DetermaIO with ICI clinical benefit in NSCLC, and expand on previous studies by demonstrating that first line treated PD-L1≥50% patients can further be stratified by IO score to identify efficacy. Exploratory analysis suggested that the IO score identifies benefit in patients with poor PS.

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Introduction

Lung cancer remains the leading cause of cancer-related death in both the United States and globally.[1] The majority of incident lung cancers are found in advanced stage, for which the 5-year survival rate historically approaches only 5%, with the majority of patients dying within 1 year of diagnosis.[2] Non-small cell lung cancer (NSCLC), the most common subtype, is responsible for approximately 85% of the total lung cancer burden.[3]

Within the last decade, the cancer-related death rate from lung cancer has decreased in both sexes, due both to screening efforts as well as improvements in targeted therapies and immunother-

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apy.[4] As a result, treatment of advanced stage NSCLC has become increasingly dependent upon predictive biomarkers to guide clinical decisions.[5] Currently, in addition to molecular testing for a growing number of oncogene-drivers at the time of initial diagnosis, PD-L1 testing has become a gold standard for selection of first line Immune checkpoint inhibitors (ICI). However, even among the subgroup with PD-L1 tumor proportional score (TPS) $\geq$ 50%, objective response rates and survival with ICI monotherapy remain suboptimal.[6] These results have led to widespread use of ICI-chemotherapy combinations, even in patients with cancers expressing the highest level of PD-L1 expression. Furthermore, almost all clinical trials conducted to date have excluded patients with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) 2, and those trials conducted in this large subpopulation have shown disappointing results.[7,8] Since greater than 40% of patients in real-world experience have a performance score of 2 score or greater, the resultant unmet need to define those who might benefit from ICI therapy is substantial.[9,10] Nonetheless, due to a better perceived tolerance to ICI treatment compared to cytotoxic chemotherapy, results from landmark clinical trials have simply extrapolated the utility of ICIs to PS2 patients.[11] While additional ICI predictive biomarkers such as tumor mutational burden have been extensively evaluated, none are widely performed in daily clinical practice which emphasizes the limited clinical applicability of current ICI biomarkers.[12-16] Moreover, data demonstrating utility in unbiased cohorts that are most reflective of a real-world clinical setting is lacking.[17]

The 27-gene IO score (DetermaIO) is an RT-qPCR assay that surveys the tumor immune microenvironment (TIME) to generate an IO score which utilizes a previously established threshold for positivity to provide a binary call of IO positive (IO+) or IO negative (IO-).[18 19] The novelty of the 27-gene IO assay and associated algorithm is derived from its ability to holistically assess the TIME through weighted quantification of gene expression of both immune "hot" and immune "cold" genes in a tumor type agnostic setting. As such, this assay has already been shown to be associated with response to ICIs in multiple tumor types of epithelial origin, including NSCLC, without altering the threshold of positivity. In a previous retrospective single institution NSCLC study, we demonstrated that a high IO score was associated with improved progression-free survival from ICI therapy and that this association was independent of both PD-L1 TPS and tumor mutational burden (TMB).[20]

Materials and Methods

Study Cohorts

This retrospective community cohort study was conducted in accordance with the Deceleration of Helsinki. This study was approved by the Research Ethics Board (REB) of University of British Columbia (H20-02635). Individual consent for this retrospective analysis and the use of tumor samples for the IO score assay was required for live patients. Consent was waived by the REB for deceased patients.

BC Cancer Cohort

We obtained archival formalin-fixed paraffin embedded (FFPE) tumor tissue from advanced NSCLC patients encompassing 4 BC Cancer centers. A total of 454 patients were identified as having received ICI therapy initiated between 04/2016 and 08/2020. Patients were then selected for retrospective chart review if they had *EGFR*/*ALK*-negative advanced NSCLC, PD-L1 IHC test result, and had received at least 2 doses of first-line pembrolizumab or second-line ICI monotherapy outside the setting of a clinical trial, had a PS of $\leq$ 2 at the start of immunotherapy and had sufficient pre-treatment biopsy material (FFPE tissue with >20% tumor content) for testing. In total, 147 patients met all inclusion criteria for analysis.

Inclusion of West Cancer Center Cohort for PS2 assessment

The West Cancer Center cohort is a retrospective community cohort study of 67 advanced NSCLC patients treated with 1 of 3 ICIs (pembrolizumab, nivolumab, or atezolizumab) prescribed either as a single agent (57 patients) or in combination with cytotoxic chemotherapeutic drugs pemetrexed and carboplatin (10 patients) as previously described.[20] There were a total of 13 patients who were treated by single-agent ICI therapy with PS2 treated between April, 2015 and February, 2018. The West Cancer Center study was approved by the University of Tennessee Health Science Center Institutional Review Board (18–05806-xp).

Determination of IO score

The 27-gene IO assay (DetermaIO) was used to derive IO scores and produce a binary IO score (IO+ or IO-) based on the pre-defined threshold as previously described.[18,19] Briefly, RNA was extracted using the Qiagen RNeasy FFPE Kit according to the standard protocol. A minimum concentration of 3.57 ng/μL as quantified by Qubit 2.0 fluorometer was required for testing. 50 ng total RNA was used for the cDNA synthesis using the SuperScript VILO cDNA Synthesis Kit (ThermoFisher). cDNA was pre-amplified using the TaqMan PreAmp Master Mix and gene-specific primer pools according to manufacturer's instructions (ThermoFisher). Following pre-amplification, the products were diluted and used as template for the 27-gene IO qPCR assay, which is pre-spotted on a 384-well plate and run with the TaqMan Mulitplex Master Mix in a 10 μL reaction on the QuantStudio 6 (ThermoFisher). Results were exported and processed through the IO algorithm to generate IO scores on the scale of -1 to 1, with the threshold of positivity pre-defined as 0.09.

Data Collection and Clinical Endpoints

Clinical data was extracted retrospectively from chart review. The physician determined response rate (ORR) and progression-free survival (PFS) using the Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1.[21] PFS was defined as the time from the first cycle of ICI to progression or death, and for those without progression, censoring was done at the time of the last disease assessment scan showing no progression. Overall survival (OS) was calculated from the date of the first cycle of ICI to death. PD-L1 TPS was determined using the 22C3 PharmDx assay (Dako, CA, USA). Patients who were still alive at the time of data analysis were censored at the date of last contact.

Table 1 Descriptive Statistics by ICI Line of Therapy for BC Cancer cohort

Characteristic (*P-value*)[b]	first-line (n=78)	second-line+ (n=69)[a]
Age at ICI (*P* = .25)		
Median age	71.5	68
≥ 75	26 (33.3%)	17 (25%)
<75	54 (67.7%)	52 (75%)
Sex (*P* = .55)		
Male	38 (med OS = 14.7 mo)	37 (med OS = 3.3 mo)
Female	40 (med OS = 16.0 mo)	32 (med OS = 5.6 mo)
PS (*P* = .74)		
0	2 (2.6%)	1 (1.4%)
1	50 (64.1%)	48 (69.6%)
2	26 (33.3%)	20 (29.0%)
Histology (*P* = .27)		
Adenocarcinoma	55 (70.5%)	45 (65.2%)
Squamous	19 (24.3%)	23 (33.3%)
NOS	4 (5.1%)	1 (1.5%)
Biopsy site (*P* = .26)		
Primary lung	30 (38.5%)	34 (49.3%)
Lymph node	23 (29.5%)	13 (18.8%)
Other metastasis	25 (32.1%)	22 (31.9%)
ICI drug (*P* < .01)		
Pembrolizumab	78 (100%)	4 (6%)
Nivolumab	0	65 (94%)
PD-L1 IHC TPS (*P* < .01)		
PD-L1 ≥ 50%	78 (100%)	5 (7%)
PD-L1 1-49%	0	15 (22%)
PD-L1 < 1%	0	49 (71%)
IO score (*P* < .01)		
IO+	56	32
IO-	22	37

Abbreviations: IHC TPS = immunohistochemistry tumor proportion score; NOS = not otherwise specified.
Metastatic sites: Brain = 7, bronchial = 22, colon resection = 2, abdomen wall = 1, adrenal = 2, bone = 2, breast = 1, chest wall = 1, liver = 3, skin = 3, trachea = 1, pleural bx = 2.
[a] Three patients were third line of therapy.
[b] Significance assessed by characteristic between line of therapy.

Statistical Analyses

This was a double-blind study, with the sponsor blinded to the clinical outcomes data and the clinical investigators blinded to the IO results prior to the completion of the primary statistical analysis. R (version 4.2.0) and GraphPad Prism V.9.0 were used for data analysis and graphical illustrations. Comparisons between groups were conducted using chi-square. Survival analyses were conducted with Kaplan Meier plots and log-rank test ($P < .05$ threshold for significance). Cox-proportional hazards were used to estimate the association between response to ICI therapy and survival.

Results

Patient Summary

A real-world cohort of 147 advanced stage NSCLC cases treated with monotherapy was assembled from 4 BC Cancer institutions. The overall cohort of ICI monotherapy treated patients stratified into 2 subgroups according to BC Cancer treatment guidelines. Patients who qualified for first line ICI monotherapy all had PD-L1 expression of ≥ 50% whereas patients treated by second-line ICI monotherapy had a variable PD-L1 expression. These subgroups had additional clinical differences and were thus analyzed separately for their relationship to IO score and outcomes (Table 1).

The IO Score is Associated With Response to ICI Monotherapy

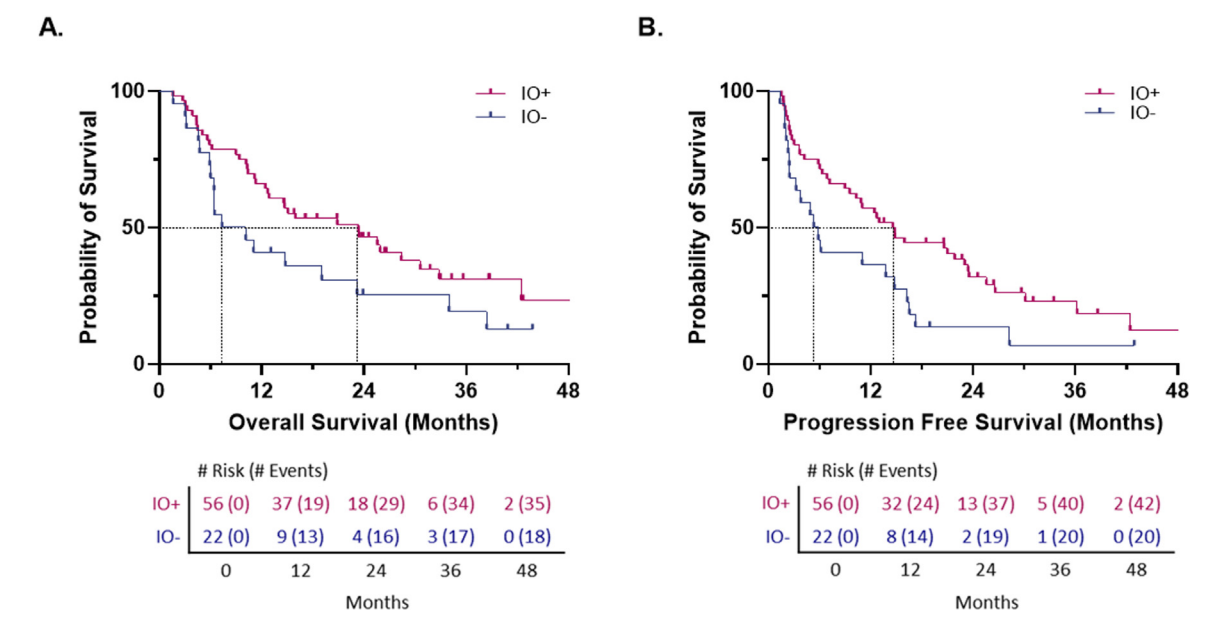
Across the entire BC Cancer cohort (N=147), IO score was associated with OS (HR=0.68, 95%CI 0.47-0.99, $P = .042$) and PFS (HR=0.62, 95%CI 0.43-0.88, $P = .0069$). The objective response rate for first-line patients was 44.9%, whereas it was 17.4% for second-line patients (Table 2). Given the stratification of objective response rate and PD-L1 status by line of therapy, we focused

Table 2 Survival Characteristics

	Whole Cohort N=147	First-line n=78	Second-line +[a] n=69
OS			
Events, n (%)	117 (80%)	53 (67.9%)	64 (92.7%)
Median survival, mo (95% CI)	12.7 (10.2 - 16.6)	15.1 (11.3 - 25.9)	9.7 (6.9 - 14.1)
PFS			
Events	128 (87.1%)	62 (79.5%)	66 (95.7%)
Median survival (mo)	7.0 (5.3 - 10.6)	12.6 (7.2 - 16.6)	4.4 (3.2 - 7.1)
Objective response			
Complete response (CR)	2	2 (2.6%)	0
Partial response (PR)	45	33 (42.3%)	12 (17.4%)
Stable disease (SD)	41	20 (25.6%)	21 (30.4%)
Progressive disease (PD)	59	23 (29.5%)	36 (52.2%)

[a] 3 patients were third line of therapy.

Figure 1 Association of DetermaIO with survival in first-line ICI monotherapy treated patients. Kaplan-Meier plots of A) overall survival and B) progression-free survival among PDL1≥50%, first line ICI monotherapy treated patients



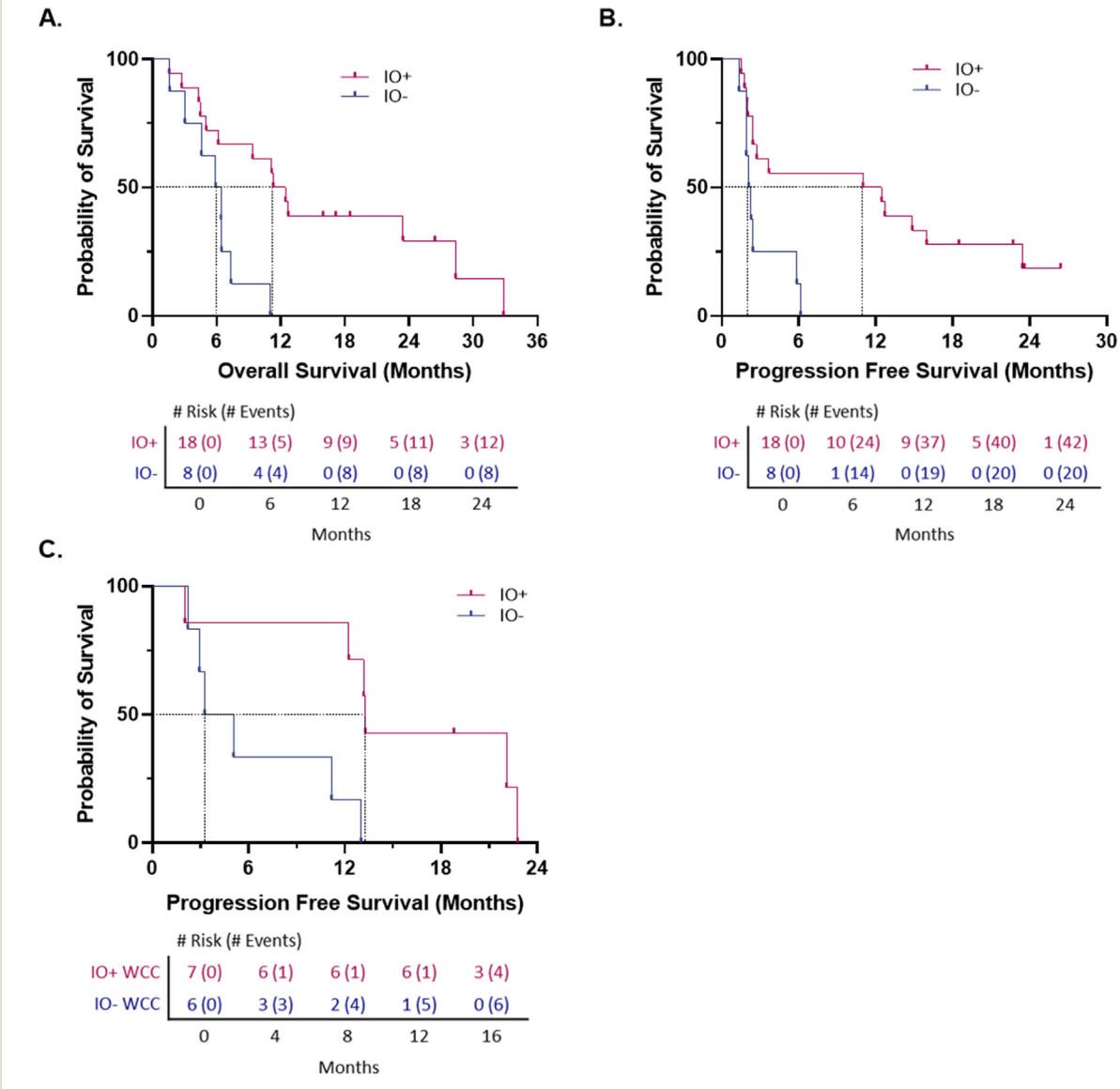
analysis on the first-line therapy patients. These patients (n=78) had a median OS of 15.1 months and a median PFS of 12.6 months.

Within the PD-L1 ≥ 50% first line treated patients, the twenty-two IO score negative (IO-) tumors had a 23% response rate (5 partial responses) whereas the IO score positive (IO+) group had a response rate of 54% (2 complete response and 28 partial responses). By Cox proportional hazard, the IO score was significantly associated with PFS (HR=0.55, 95%CI 0.32-0.94, $P = .028$, Figure 1A), and trended toward significance in OS (HR=0.60, 95%CI 0.34-1.06, $P = .078$, Figure 1B). There was a 14.7-month overall improvement in median OS and 9 month improvement in PFS comparing IO+ to IO- patients (Figure 1A-B). Of the patients who received ICI in second-line or later, 5 had tumors with PD-L1 ≥ 50%, fifteen were PD-L1=1%-49%, and forty-nine were PD-L1< 1% (median OS=9.7 months, PFS=4.4 months). The IO score was not significantly associated with OS or PFS in the second-line and later group.

The IO Score is Associated With Response to ICI Monotherapy Amongst PS 2 Patients

The BC Cancer cohort included twenty-six patients with PS 2 who received first line therapy. In this setting where all patients in

Figure 2 Association of DetermaIO with survival among first line ICI monotherapy treated, PS 2 patients. Kaplan Meier plots are shown for A) overall survival and B) progressionfree survival in patients from the BC Cancer cohort. C) Kaplan Meier plot of progressionfree survival in West Cancer Center cohort



the cohort were PD-L1 ≥ 50% (n=26), the IO score was significantly associated a with both OS (HR = 0.26, 95%CI 0.091-0.74, $P = .012$, Figure 2A) and PFS (HR = 0.27, 95%CI 0.098-0.72, $P = .0095$, Figure 2B).

We explored the association between IO score and response to ICI therapy in an independent cohort of PS 2 patients obtained from the West Cancer Center. The IO score was previously shown to be associated PFS after ICI therapy in this cohort with, however the association in only PS 2 patients had not been examined.[20] Subgroup analysis of the thirteen PS 2 patients confirmed that the IO Score was associated with PFS (HR = 0.14, 95%CI 0.027-0.76, $P = .023$, Figure 2C) in this small but independent cohort (overall survival data was not available).

Discussion

ICI therapy has revolutionized the field of cancer therapy, including for advanced NSCLC. Despite these advances, only a subset of patients benefit and biomarker selection remains suboptimal. In NSCLC, PD-L1 TPS as measured by IHC staining has been adopted as a standard for regulatory and clinical use. However, the overall weakness of the association between PD-L1 expression and efficacy of ICI therapy, as well as differences in staining and

scoring approaches used in clinical trials, have limited the usefulness of PD-L1 as a biomarker. TMB has similarly been explored as a biomarker with promising data in NSCLC but seems to recognize only a fraction of potential responders, has been confounded by inconsistent testing approaches and has had poor adoption as a biomarker to date. Current NSCLC treatment algorithms suggest ICI monotherapy for patients with PD-L1 TPS $\geq$ 50%, and combination therapy for those with PD-L1 TPS $<$ 50%, but in practice, regardless of the first-line therapeutic approach, only a minority of NSCLC patients sustain meaningful and long-lasting efficacy. As newer regimens are developed and physicians confront the challenge of managing patients with poor performance status and or recurrent and resistant disease, the need for better predictive biomarkers has become increasingly apparent.

The 27-gene IO score was developed initially as a classifier of the TIME, distinguishing differentiated gene expression signatures expressed in immunomodulatory, mesenchymal, and cancer-associated fibroblast cell types.[18,22] This intrinsic gene expression pattern has been shown to be conserved in multiple carcinoma tissue types, and to be a binary classifier associated with response to ICI in NSCLC, TNBC, and urothelial carcinomas.[20,23-26] The consistent performance of the assay and uniform threshold in multiple tumor types, suggests that capturing the contrasting physiology from the anti-tumor immune response in the context of the stromal derived immune repressive biology, distinguishes a tumor-type independent phenotype that reflects a biologic state where the addition of checkpoint inhibitor therapy tips the balance in favor of an effective anti-tumor response. The ability of the 27-gene IO score to identify the balance of effector to suppressor components of the TIME may provide an advantage compared to predictive biomarkers that measure only inflammatory components of the TIME and lack the stromal-derived immunosuppressive signal.

Biomarker studies of many "real world" retrospective NSCLC cohorts are difficult to interpret due to the inconsistent use of mono- versus combination ICI-chemotherapy regimens across treatment classes. In contrast, the BC Cancer reimbursement requirement that only patients with PD-L1 TPS $\geq$ 50% are eligible for ICI monotherapy in the first-line setting, allowed investigation of the IO score in a relatively non-biased set of patients who were uniformly high PD-L1. This allowed for evaluation of the IO score amongst first-line treated patients who were uniformly PD-L1 TPS $\geq$ 50%, where we found that the IO score provides incremental association with efficacy that could help inform monotherapy versus combination therapy decisions. As a result, the IO score, either alone or integrated with PD-L1 TPS may be used to maximize the number of patients likely to respond to ICI therapy in routine clinical practice.

Recently, attention has been drawn to PS 2 patients being consistently excluded from phase III clinical trials of ICI therapy in NSCLC, highlighting the lack of data in this patient group.[27] To address this disparity, a recent phase 3 clinical trial randomizing an older patient cohort with PS status 0-1, and a cohort of PS 2 patients regardless of age to ICI combination therapy with nivolumab-ipilimumab versus platinum chemotherapy demonstrated poor results with combination ICI in the PS 2 group but not in the healthy elderly.[8] Conversely, in the IPSOS study, subgroup analysis of PS2 patients who were ineligible for platinum-based chemotherapy demonstrated improved OS on first-line atezolizumab treatment compared to those on single-agent chemotherapy across all PD-L1 sub-groups.[28] These conflicting studies demonstrate why an improved biomarker is necessary to more clearly identify the population who may benefit and implement a regimen containing ICI therapy. In a real-world setting, PS 2 patients may represent approximately 30% to 40% of NSCLC cases.[9,10] In our BC Cancer cohort, we found that the IO score was significantly associated with both PFS and OS and in patients with PS 2 receiving first-line ICI therapy. Similar results were seen in a second, independent, cohort of PS 2 single-agent ICI treated patients at the West Cancer Center (Figure *2*). Together, these data indicate that the IO score might identify the subset of patients who benefit from ICI therapy regardless of performance status, thus addressing an unmet clinical need for actionable ICI biomarkers in poor performance status patients.

Strengths and Limitations

This analysis was a blinded, retrospective study of available FFPE blocks with sufficient tissue from 4 hospitals in the BC Cancer healthcare catchment. There is risk that the selection of blocks with sufficient tissue introduced bias into the cohort and a significant portion of blocks had been consumed beyond the acceptance criteria of the test. The limitation of ICI reimbursement by the BC Cancer agency to only offer ICI therapy to those expressing PD-L1 TPS $\geq$ 50% created an opportunity to remove the confounding variables of PD-L1 status and line of therapy from the study cohort. However, this prohibited the assessment of the clinical utility of the IO score in PD-L1 low patients in the first line setting. Additional limitations to our study include being unable to account for other unmeasured, but potentially relevant, clinicopathological variables and biomarkers (such as TMB) due to the retrospective nature of the study.

Conclusion

This study in advanced NSCLC demonstrates that the IO score (DetermaIO) offers independent and incremental information to PD-L1 IHC for guiding treatment decisions with ICI therapy. DetermaIO is a novel biomarker of the TIME which is associated with ICI efficacy outcomes in this blinded retrospective study, in a similar fashion to promising results in multiple other tumor types. Exploratory analysis also suggests that the IO score may enrich for ICI efficacy amongst PS 2 patients and warrants further study in this population under-represented in clinical studies. Prospective studies of this ICI predictive biomarker are warranted.

Clinical Practice Points

- **What is already known on this topic**

  Immune checkpoint inhibitor (ICI) therapy is the current standard of care for advanced stage NSCLC patients. PD-L1 expression measured by immunohistochemistry (IHC) staining is the current gold standard predictive biomarker for ICI therapy in NSCLC, however many factors beyond PD-L1 expression alone affect the outcome. In view of the multitude of therapeutic choices available for advanced stage NSCLC, there is an unmet clinical need in better identifying those patients likely to have favorable survival outcomes with ICI therapy, and conversely to

select alternative therapeutic regimens in those who are unlikely to benefit.

- **What this study adds**
  Similar to PD-L1 IHC, the 27-gene IO score is associated with both 1 year PFS and OS in ICI treated patients. However, even among the PD-L1 $\geq$ 50% subgroup, the IO score demonstrated that the IO-, first line treated patients are likely to have a poor outcome with single agent immunotherapy. Our results further demonstrate that the IO score is independent of, and incremental to PD-L1 IHC in this clinical setting. Moreover, exploratory analysis suggests that IO score is significantly associated with ICI efficacy in patients with PS2, a subgroup where ICI monotherapy has previously performed poorly.
- **How this study might affect research, practice or policy**
  If additional studies support our findings, the IO score may further inform oncologists in identifying those patients with superior clinical outcomes from single-agent ICI therapy, including those in the PS 2 population. More rigorous selection would thereby help to identify patients who may be spared cytotoxic chemotherapy.
- Caution needs to be exercised in using PPS for patient prognostication, as in some cases the outcome can be variable with the same PPS.

Author Contributions

DLS, RSS, DRH, DTR, GAV, DRG conceived and designed the study. DLS, MGV, HML, ALJ collected the data. DLS, MGV, TJN, RSS, NSC, BLS performed analysis. DLS, MGV, TJN, RSS, DRH, BLS, DTR, DRG, GAV, ALJ wrote and revised the paper.

Ethics Approval

All studies were conducted in accordance with the Deceleration of Helsinki and informed consent was obtained from all living subjects and/or their legal guardians.

The BC Cancer study was approved by the Research Ethics Board (REB) of University of British Columbia (H20-02635). Individual consent for this retrospective analysis and the use of tumor samples for the IO assay was required for live patients. Consent was waived by the REB for deceased patients.

The West Cancer Center study was approved by the University of Tennessee Health Science Center Institutional Review Board (18–05806-xp).

Consent for Publication

All authors consent to the publication of this manuscript.

Data Availability

The data sets generated and analyzed during the current study are not publicly available due to the full clinical files from patients in this manuscript are confidential information from a private clinic but are available from the corresponding author upon reasonable request.

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Disclosure

MGV, TJN, RSS, DRH, BLS, and DTR are employed by Oncocyte Corporation, the commercial entity that markets the 27-gene IO Score as DetermaIO.

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