Evaluation of tumor microenvironment and biomarkers of immune checkpoint inhibitor response in metastatic renal cell carcinoma

Landon C Brown [ID],[1,2] Jason Zhu,[1,2] Kunal Desai,[3] Emily Kinsey,[2] Chester Kao,[2] Yong Hee Lee,[4] Sarabjot Pabla [ID],[4] Matthew K Labriola [ID],[2] Jennifer Tran,[5] Konstantin H Dragnev,[6] Laura J Tafe,[6] Farshid Dayyani,[7] Rajan T Gupta,[2] Shannon McCall,[2] Daniel J George [ID],[2] Sean T Glenn,[8,9] Mary K Nesline,[9] Saby George,[10] Matthew Zibelman,[11] Carl Morrison,[4] Moshe C Ornstein [ID],[5] Tian Zhang[2,12]

For numbered affiliations see end of article.

Correspondence to
Dr Tian Zhang;
tian.zhang@utsouthwestern.edu

ABSTRACT

Background Immunotherapy combinations including ipilimumab and nivolumab are now the standard of care for untreated metastatic renal cell carcinoma (mRCC). Biomarkers of response are lacking to predict patients who will have a favorable or unfavorable response to immunotherapy. This study aimed to use the OmniSeq transcriptome-based platform to develop biomarkers of response to immunotherapy.

Methods Two cohorts of patients were retrospectively collected. These included an investigational cohort of patients with mRCC treated with immune checkpoint inhibitor therapy from five institutions, and a subsequent validation cohort of patients with mRCC treated with combination ipilimumab and nivolumab from two institutions (Duke Cancer Institute and Cleveland Clinic Taussig Cancer Center). Tissue-based RNA sequencing was performed using the OmniSeq Immune Report Card on banked specimens to identify gene signatures and immune checkpoints associated with differential clinical outcomes. A 5-gene expression panel was developed based on the investigational cohort and was subsequently evaluated in the validation cohort. Clinical outcomes including progression-free survival (PFS) and overall survival (OS) were extracted by retrospective chart review. Objective response rate (ORR) was assessed by Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1.

Results The initial investigation cohort identified 86 patients with mRCC who received nivolumab (80%, 69/86), ipilimumab/nivolumab (14%, 12/86), or pembrolizumab (6%, 5/86). A gene expression score was created using the top five genes found in responders versus non-responders (FOXP3, CCR4, KLRK1, ITK, TIGIT). The ORR in patients with high gene expression (GE$_{high}$) on the 5-gene panel was 29% (14/48), compared with low gene expression (GE$_{low}$) 3% (1/38, χ^2 p=0.001). The validation cohort was comprised of 62 patients who received ipilimumab/nivolumab. There was no difference between GE$_{high}$ and GE$_{low}$ in terms of ORR (44% vs 38.5%), PFS (HR 1.5, 95% CI 0.58 to 3.89), or OS (HR 0.96, 95% CI 0.51 to 1.83). Similarly, no differences in ORR, PFS or OS were observed

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ There are no validated genomic biomarkers used in clinical practice that can predict response or resistance to immune checkpoint inhibitors (ICIs) among patients with metastatic renal cell carcinoma (mRCC). RNA expression signatures of tumors and the tumor microenvironment could provide prognostic or predictive information in this population undergoing ICI-based therapy.

WHAT THIS STUDY ADDS

⇒ This study sought to develop a gene expression signature associated with ICI response that was detected in a multi-institution cohort of patients primarily treated with single-agent anti-programmed cell death protein-1 checkpoint inhibitor with mRCC. This gene expression signature was not validated in a distinct multi-institution cohort of patients with mRCC treated with the combination of ipilimumab and nivolumab.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ RNA sequencing based predictive gene expression signatures may differ for individual treatment regimens among patients with mRCC and further research is indicated.

when patients were stratified by tumor mutational burden (high=top 20%), PD-L1 (programmed death-ligand 1) expression by immunohistochemistry or RNA expression, or CTLA-4 (cytotoxic T-lymphocytes-associated protein 4) RNA expression. The International Metastatic RCC Database Consortium (IMDC) risk score was prognostic for OS but not PFS.

Conclusion A 5-gene panel that was associated with improved ORR in a predominantly nivolumab monotherapy population of patients with mRCC was not predictive for radiographic response, PFS, or OS among patients with mRCC treated with ipilimumab and nivolumab.

BACKGROUND

Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of metastatic renal cell carcinoma (mRCC). In the front-line setting, five immunotherapy-containing combinations are Food and Drug Administration (FDA) approved for mRCC: ipilimumab/nivolumab,[1] pembrolizumab/axitinib,[2] avelumab/axitinib,[3] nivolumab/cabozantinib,[4] and pembrolizumab/lenvatinib.[5] While these therapies have improved overall survival for many patients with mRCC, biomarkers of treatment response or resistance are urgently needed. Unlike lung cancer where programmed death-ligand 1 (PD-L1) is an FDA-approved biomarker of response to ICI, there are no predictive immunotherapy biomarkers that have been shown to be clinically useful in RCC. While several biomarkers have been explored in mRCC, including tumor mutational burden (TMB), microsatellite instability status, intrinsic molecular subtypes, and composite immune biomarkers, none have been shown to strongly predict for ICI response or resistance.[6]

Angiogenesis, T-effector/interferon-γ response, and myeloid inflammatory gene expression signatures have been retrospectively described based on data from IMmotion150 and validated from tumor samples in IMmotion151.[1 2] IMmotion150 and IMmotion151 examined the efficacy of atezolizumab alone or in combination with bevacizumab, compared with sunitinib in patients with treatment naïve mRCC. In the exploratory biomarker analysis of IMmotion150, patients were categorized into T-effector gene signature high and low subgroups, as defined by the gene expression of CD8A, EOMES, PRF1, IFNG, and CD274. In patients receiving combination atezolizumab plus bevacizumab, those with T-effector high (T_{eff}^{High}) gene signature had improved progression-free survival (PFS) compared with T-effector low (T_{eff}^{Low}) signature (HR 0.50, 95% CI 0.30 to 0.86, p=0.011).[1] This benefit was redemonstrated in the subsequent analysis of IMmotion151 (HR 0.76, 95% CI 0.59 to 0.99, p<0.05).[3] While this gene signature was helpful for patients treated with combination therapy, PFS and the objective response rates were not significantly different for patients treated with single agent atezolizumab in IMmotion150. Separately, our group has shown that the combination of low cell proliferation as measured by RNA sequencing (RNA-seq) expression of 10 proliferation-related genes and PD-L1 immunohistochemistry (IHC) negativity is associated with lower response rates (6.5%) compared with moderately proliferative, PD-L1 IHC negative tumors (30%).[4]

In this study, we aimed to define a new potential tumor microenvironment gene signature which could help identify patients with mRCC who have a higher likelihood of response to single-agent programmed cell death protein-1 (PD-1)/PD-L1 inhibition or combination PD-1/cytotoxic T-lymphocytes-associated protein 4 (CTLA-4) inhibition. In addition, we evaluated established gene signatures and immune checkpoints on the OmniSeq platform within a cohort of patients with mRCC treated with ICIs.

METHODS

Patients and clinical data

Two cohorts of patients were collected and analyzed for this study: (1) initial investigational cohort and (2) subsequent validation cohort. For the initial investigational cohort, patients from five institutions were included based on the following criteria: (1) history of advanced/metastatic RCC, (2) pre-ICI treatment archival formalin-fixed paraffin-embedded (FFPE) tissue for transcriptomic (RNA) sequencing, and; (3) availability of demographic, diagnosis, ICI treatment (line of therapy, single agent or combination), and follow-up data. All patients were evaluated for best response based on the Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1 criteria and were designated as having complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). The objective response rate (ORR) was defined as the rate of CR or PR.

For the separate, subsequent validation cohort, only patients with mRCC treated with combination ipilimumab/nivolumab were included, given evolution in practice patterns away from single-agent PD-1 inhibition to combination therapy over time since the initial investigational cohort analysis. Patients from two institutions (Duke Cancer Institute and Cleveland Clinic Taussig Cancer Center) that met the following criteria were included: (1) history of advanced/metastatic RCC, (2) availability of adequate archival pre-ICI treatment FFPE tissue, (3) treated with combination ipilimumab and nivolumab, and (4) availability of demographic, diagnosis, and follow-up data. All patients were evaluated for best response based on RECIST V.1.1 criteria by each site and were designated as CR, PR, SD, or PD.

Clinical outcomes

Time to event clinical outcomes were extracted by retrospective chart review by the clinical authors for the validation cohort. Overall survival (OS) was defined as the time from initiation of ipilimumab/nivolumab until death. PFS was defined as the time from initiation of ipilimumab/nivolumab until the earliest occurrence of death, radiographic progression, or unequivocal clinical progression as indicated in the chart by the treating physician.

Immunohistochemical studies

PD-L1 expression was assessed in all cases using the Dako 22C3 pharmDx antibody (Agilent, Santa Clara, California, USA). PD-L1 levels were scored by a board-certified anatomic pathologist following FDA guidelines, with a tumor proportion score in neoplastic cells with a value of≥1% considered positive.[5]

Transcriptomic analysis

RNA was extracted from each sample and processed for targeted RNA-seq, as previously described.[6] Gene expression was evaluated by amplicon sequencing of 394 immune transcripts on samples that met validated quality control thresholds. For the investigational cohort,

the average gene expression of every immune transcript was compared between patients who were classified as responders (PR and CR) and non-responders (SD and PD) and the five genes which had the greatest differential in gene expression were used to generate a gene signature. The tumor sample was defined as gene expression high (GE_{high}) if the average of gene expression in the tumor was higher than the corresponding score for all cases in the cohort (value of 46, the average gene expression of all five genes in the cohort) and otherwise, they were defined as gene expression low (GE_{low}).

For the validation cohort, RNA-seq was performed along with PD-L1 IHC. The five-gene panel defined by the investigational cohort was applied to the validation cohort. Additionally, previously described gene expression panels tumor immunogenic signature (TIGS)[7] and cell proliferation (CP)[8] were evaluated in this cohort. Lastly, PD-L1 by RNA expression (high defined as ≥75% rank), CTLA-4 by RNA expression (high defined as ≥75% rank) and TMB (high defined as >10 mut/Mb or >20% rank) were also evaluated.

Data analysis

In the investigational cohort, statistical testing of differences in ORR was performed using χ^2 test without Yates' continuity correction for individual variables. For the validation cohort, ORR was assessed by the proportion test to evaluate differences in percentages and logistic regression to evaluate OR. For survival outcomes (OS and PFS) the Kaplan-Meier method was used to calculate median survival and Cox regression were used to calculate HRs. Cox regression models were controlled using the covariates sex, stage at diagnosis, ECOG (Eastern Cooperative Oncology Group) performance status at ipilimumab/nivolumab treatment, and time from diagnosis to ipilimumab/nivolumab of greater or less than 1 year. Analysis was performed in SAS V.9.4.

RESULTS

Investigational cohort

For the initial investigational cohort, a total of 104 tissue samples from five institutions were collected for analysis from 2015 to 2017, as previously reported.[4] Among them, 83% (86/104) of samples were sufficient for RNA analysis (demographics shown in table 1). Subtypes of RCC evaluated included clear cell (87%, 75/86), papillary (6%, 5/86), mixed subtypes not otherwise specified (NOS) (6%, 5/86), clear cell with sarcomatoid features (2%, 2/86), and spindle cell (1%, 1/86). Among the patients evaluated, 80% (69/86) were Caucasian, 5% (4/86) were African American, 3% (3/86) were Asian, and race was unknown in 12% (10/86), with a median age of all patients of 60 years (range 37–80). The majority of the patients received nivolumab 80% (69/86), followed by ipilimumab/nivolumab at 14% (12/86), and pembrolizumab at 6% (5/86). By RECIST V.1.1, three patients experienced CR (3%, 3/86), 12 patients had a PR (14%,

12/86), 32 patients had SD (37%, 32/86), and 38 patients had PD (44%, 38/86).

Evaluation of tumor PD-L1 (Dako 22C3) status and TMB is summarized in table 2. The majority of patients (70%, 60/86) had PD-L1 status of 0% and almost all patients had a TMB <10 mut/Mb (95%, 82/86). A summary of the gene expression data is provided in online supplemental table 1. For the responders, the 10 most highly expressed genes were TLR3, VEGFA, ITGB1, EGFR, NRP1, ITGA1, CD68, CSF1R, AXL, and LRP1, and the 10 genes with the lowest expression were MLANA, MAGEC2, MAGEA12, MAGEA3, BAGE, IL17A, IL17F, KIR2DL1, MAGEA1, and MAGEA4. For non-responders, the 10 most highly expressed genes were NRP1, TLR3, VEGFA, ID2, VCAM1, ITGA1, EGFR, AXL, TGFB1, ICOSLG and the 10 least expressed were IL17F, SSX2, IL17A, KIR2DL1, MAGEA3, MAGEA12, MAGEA4, MAGEC2, BAGE, and MAGEA1. The top 10 genes which were more highly expressed in responders compared with non-responders were FOXP3, CCR4, KLRK1, ITK, TIGIT, CD27, MYC, TP63, MS4A1, and IFITM1. The top 10 genes which were more highly expressed in non-responders compared with responders were ITGB2, SDHA, FUT4, LST1, HLA-DQB2, KLRF1, CEACAM1, POLR2A, HLA-DRB1, and GADD45GIP1.

A gene expression score was created using the top five genes which were most highly expressed in responders compared with non-responders (FOXP3, CCR4, KLRK1, ITK, TIGIT) (figure 1A). Using a cut-off point of 46% (the average gene expression of these five genes), 48 patients were classified as GE_{high}, and 38 patients were classified as GE_{low}. Of the patients who were classified as GE_{high}, the ORR was 29% (14/48), which was significantly different compared with ORR 3% (1/38) of GE_{low} patients (χ^2, p=0.001). For the continuum of 5-gene expression, RECIST responses were plotted by gene expression score (online supplemental figure 1). When using median gene expression for this panel, ORR in the GE_{low} group was 4.7% (2/43) and in the GE_{high} group was 30.2% (13/43). In the upper quartile of the combined expression, there were no patients who had progressive disease as their best response. PD-L1 expression also correlated with ORR in this cohort. Of the eight patients who were PD-L1 positive, the ORR was 50%. For patients who were PD-L1 negative, the ORR was 14% (table 1). TMB high status (≥10 mut/Mb), CD8 expression high (defined as >75% normalized RNA expression) and T_{eff} gene expression (from IMMotion150/151 analysis) did not correlate with ORR in this cohort. Figure 1B graphically represents patients stratified by radiographic response, PD-L1, TMB, CD8, the 5-gene expression panel, and the T_{eff} gene expression. Of the 48 patients categorized as GE_{high}, 36 (75%) were categorized as T_{eff}^{High} and 28 (58%) were CD8 high. Of the 38 patients categorized as GE_{low}, 31 (82%) were categorized as T_{eff}^{Low} and 36 (95%) were categorized as CD8 low.

Validation cohort

For the validation cohort, 69 patients with archival tumor specimens were initially identified and 62 passed

Table 1 Demographics for patients in investigational cohort and validation cohort

	Investigational cohort (n=86)	Validation cohort (n=62)
Age, median (range)	60 (37–80)	59 (34–83)
Sex		
Male, n (%)	63 (73)	47 (75.8)
Female, n (%)	23 (27)	15 (24.2)
Race		
Caucasian, n (%)	69 (80)	10 (16.1)
African American, n (%)	4 (5)	51 (82.3)
Asian, n (%)	3 (3)	0
Unknown, n (%)	10 (12)	1 (1.6)
IMDC risk group		
Favorable, n (%)	Not available	15 (24.2)
Intermediate, n (%)	Not available	40 (64.5)
Poor, n (%)	Not available	7 (11.3)
Checkpoint inhibitor		
Nivolumab, n (%)	69 (80)	0
Pembrolizumab, n (%)	5 (6)	0
Ipilimumab and nivolumab, n (%)	12 (14)	62 (100)
Line of therapy		
First, n (%)	Not available	47 (75.8)
Second, n (%)	Not available	10 (16.1)
Third or greater, n (%)	Not available	5 (8.1)
Histology		
Clear cell, n (%)	73 (85)	53 (85)
Clear cell with sarcomatoid features, n (%)	2 (2)	1 (1.6)
Papillary cell, n (%)	5 (6)	1 (1.6)
Spindle cell, n (%)	1 (1)	0
Mixed subtype NOS, n (%)	5 (6)	7 (11.3)
Site of tissue collection		
Kidney/primary	Not available	42 (67.7)
Metastatic site	Not available	20 (32.3)*
Objective response rate		
Complete response, n (%)	3 (3)	4 (6.5)
Partial response, n (%)	12 (14)	21 (33.9)
Stable disease, n (%)	32 (37)	19 (30.6)
Progressive disease, n (%)	38 (44)	18 (29.0)
Not evaluable, n (%)	1 (1)	0

*Metastatic site of origin (n): adrenal 1, bone 5, brain 2, chest wall 1, liver 3, lung/pleura 7, pancreas 1.
IMDC, International Metastatic RCC Database Consortium; NOS, not otherwise specified.

quality control for marker testing among patients treated with ipilimumab and nivolumab at Duke Cancer Institute (n=39) and Cleveland Clinic Taussig Cancer Center (n=23). Demographics are shown in table 1. The median age at diagnosis was 59 (range 34–83) and International Metastatic RCC Database Consortium (IMDC) risk groups were favorable, intermediate, or poor risk in 15 (24%), 40 (65%), and 7 (11%), respectively. Clear cell histology was documented in 53 (85%)

patients. The majority of patients were treated in the first-line setting (76%), and 26% of patients had bone metastases. A complete course of four doses of ipilimumab was administered in 39 (62.9%) patients. Median follow-up time was 581 days (range 51–1101 days) with 42 (67.7%) patients alive at the time of data cut-off. The median time on ipilimumab/nivolumab, including maintenance nivolumab, was 126 days (range 21–792). Transcriptomic sequencing was performed using the

Table 2 Clinical outcomes of patients in validation cohort treated with ipilimumab/nivolumab are evaluated by different gene signatures and by immune checkpoint status

Variable	Category	n	Comparison	ORR			OS			PFS		
				OR	CI low	CI high	HR	CI low	CI high	HR	CI low	CI high
Five gene panel (FOXP3, CCR4, KLRK1, ITK, TIGIT)	High	23	High vs not high	1.35	0.42	4.38	1.50	0.58	3.89	0.96	0.51	1.83
	Not high	39										
Cell proliferation	High	3	High vs poor	1.67	0.12	24.15	6.36	0.95	42.84	4.55	1.11	18.76
	Moderate	15	Moderate vs poor	1.26	0.34	4.64	2.90	1.05	7.99	1.49	0.72	3.08
	Poor	44	High vs moderate	1.32	0.08	21.21	2.20	0.36	13.27	3.05	0.74	12.60
TIGS (immunogenicity)	Strong	27	Strong vs weak	0.53	0.13	2.16	0.99	0.27	3.65	1.30	0.61	2.78
	Moderate	19	Moderate vs weak	0.43	0.09	2.04	3.28	0.90	11.96	1.27	0.55	2.97
	Weak	16	Strong vs moderate	1.21	0.31	4.73	0.30	0.10	0.92	1.02	0.48	2.15
IMDC risk group	Favorable	15	Favorable vs poor	0.36	0.05	2.70	0.12	0.02	0.68	0.54	0.19	1.51
	Intermediate	40	Intermediate vs poor	0.54	0.08	3.51	0.43	0.13	1.43	0.76	0.31	1.91
	Poor	7	Favorable vs intermediate	0.67	0.17	2.59	0.27	0.06	1.28	0.70	0.32	1.52
PD-L1 (IHC)	High	21	High vs not high	1.31	0.42	4.15	1.57	0.63	3.89	0.79	0.42	1.50
	Not high	41										
PD-L1 (RNA)	High	13	High vs not high	1.07	0.25	4.54	2.05	0.65	6.45	0.79	0.33	1.89
	Not high	49										
CTLA4 (RNA)	High	13	High vs not high	2.53	0.63	10.16	0.86	0.26	2.80	0.66	0.31	1.43
	Not high	49										
TMB (high=upper 20%)	High	13	High vs not high	0.97	0.22	4.28	1.01	0.28	3.62	1.39	0.65	3.00
	Not high	49										
Entire validation cohort		62										

Logistic regression objective response rate OR and Cox models for overall survival HR, and progression free survival HR for individual variables are shown. Logistic regression and Cox models a adjusted for sex, stage at diagnosis, ECOG (Eastern Cooperative Oncology Group) performance status at ipilimumab/nivolumab treatment, and time from diagnosis to ipilimumab/nivolumab of greater or less than 1 year.

CTLA-4, cytotoxic T-lymphocytes-associated protein 4; IHC, immunohistochemistry; IMDC, International Metastatic RCC Database Consortium; PD-L1, programmed death-ligand 1; TIGS, tumor immunogenic signature; TMB, tumor mutational burden.

A	Total Patients	Responders (CR+PR)	Non-Responders (SD + PD)	ORR	χ^2 test
All Patients	86	15	71	17%	
PD-L1 ≥1	8 (9%)	4	4	50%	
PD-L1<1	65 (76%)	9	56	14%	p=0.042
PD-L1 n/a	13 (15%)	1	12	8%	
CD8≥75%	30 (35%)	8	22	27%	p=0.176
CD8<75	56 (65%)	7	49	13%	
TMB ≥20	2 (2%)	0	2	0%	
TMB 10-20	2 (2%)	0	0	0%	p=1.0
TMB<10	82 (95%)	15	67	18%	
5-Gene High	48 (56%)	14	34	29%	p=0.001
5-Gene Low	38 (44%)	1	37	3%	
T_{eff} High	43 (50%)	10	33	23%	p=0.256
T_{eff} Low	43 (50%)	5	38	12%	
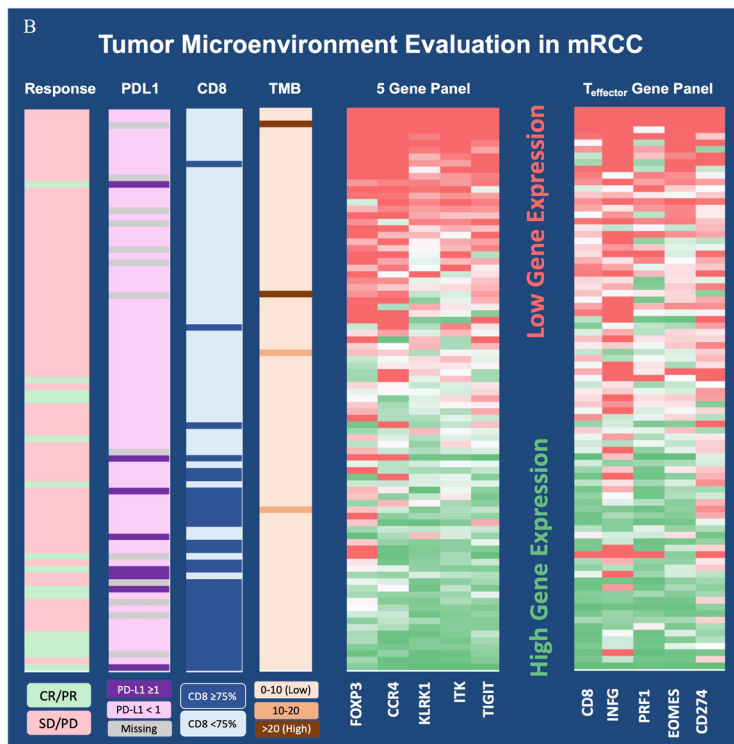
Figure 1 (A) Objective response rates (ORR) by tumor microenvironment factors evaluated in the investigation cohort of patients with metastatic renal cell carcinoma (mRCC) predominantly treated with single-agent programmed cell death protein-1 inhibitors. (B) Individual patient level data showing heat maps of the 5-gene panel and other tumor microenvironment factors. Every horizontal line across represents one patient. The 5-gene high and low cut-off point set as mean of 5-gene panel expression (46%). Green is higher gene expression and red is lower gene expression. CR, complete response; PR, partial response; PD, progressive disease; PD-L1, programmed death-ligand 1; SD, stable disease; T_{eff}, T-effector; TMB, tumor mutational burden.

CLIA-certified OmniSeq Immune Report Card in addition to IHC for PD-L1.

5-gene panel

We sought to validate the findings found in the investigational cohort that high expression of a 5-gene panel consisting of FOXP3, CCR4, KLRK1, ITK, and TIGIT (GE_{high}) was associated with a higher ORR compared with those with GE_{low} (figure 2A, table 2). In the validation cohort, 23 patients were found to be GE_{high} and 39 patients were GE_{low}. There was no difference in ORR between these two groups as patients with GE_{high} were found to have ORR 44% compared with ORR 38.5% in patients with GE_{low} (OR 1.4, 95% CI 0.41 to 5.04). PFS and OS were also not statistically different between the GE_{high} and GE_{low} groups (OS HR 1.50, 95% CI 0.58 to 3.89; PFS HR 0.96, 95% CI 0.51 to 1.83). Receiver operating characteristic curve was estimated from logistic regression to evaluate the performance of 5-gene panel as a classifier of ORR. Based on our result, area under the curve of 0.52 suggests no ability to predict treatment response (ORR) based on 5-gene panel.

Predictors of immune response to ipilimumab and nivolumab

CP is defined based on expression of 10 proliferation associated genes and was divided into one of three levels (high, moderate, and poor) as previously reported.[8] The majority of patients were categorized as having poor CP (44, 71.0%) with 15 patients categorized as moderate (24.1%) and 3 patients as high CP (4.8%). ORR across these three groups were 66.7%, 46.7%, and 36.4% for high, moderate, and poor CP, respectively. Kaplan-Meier (KM) analysis for OS and PFS based on CP score are shown in figure 2B. Notably, poor CP was associated with the best OS compared with moderate CP (HR 2.9, 95% CI 1.05 to 7.99). The high CP group only included three patients, and while it demonstrated a worse OS compared with the poor CP group (HR 6.36, 95% CI 0.95 to 42.84) interpretation is limited. Interestingly the ORR and OS outcomes are opposed with the poor CP group having a lower ORR but the best OS; while moderate and high CP had higher ORR, but inferior OS.

A three-level predefined TIGS score was assessed by RNA-seq and gene expression as previously defined,[7] with strong, moderate, or weak immunogenicity identified in 27 (43.5%), 19 (30.6%), and 16 (25.8%) tumors, respectively. ORR across these levels was 40.7%, 31.6%, and 50.0% for strong, moderate, and weak immunogenicity. PFS across these three levels was similar (see figure 2C, table 2). OS was shorter in the moderate immunogenicity group as compared with weak immunogenicity (HR 3.28, 95% CI 0.9 to 1.96) and OS was longer in the strong immunogenicity group as compared with the moderate immunogenicity group (HR 0.30, 95% CI 0.1 to 0.92).
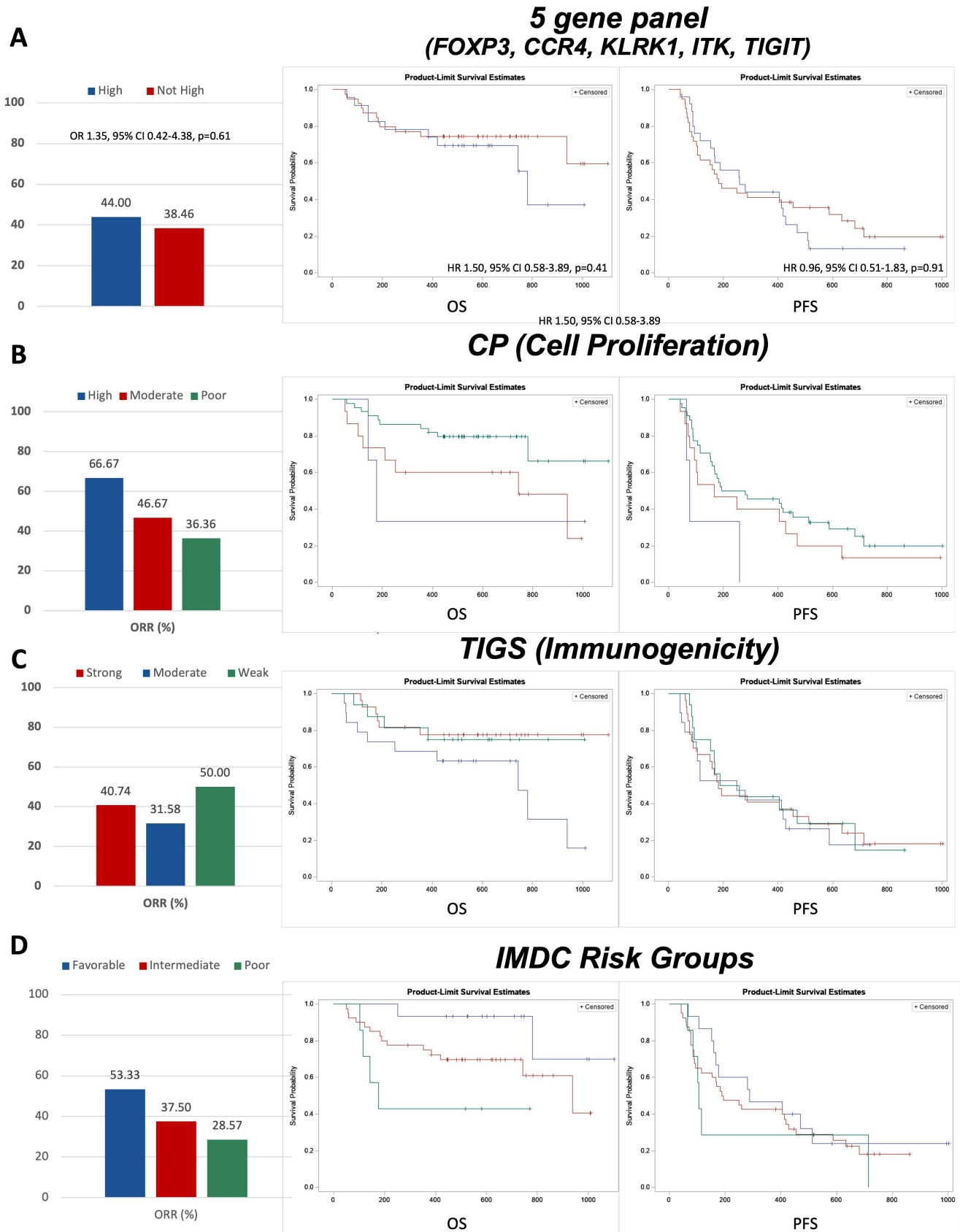
Figure 2 Validation cohort objective response rate (ORR), left, and Kaplan-Meier curves, right, for overall survival (OS) and progression free survival (PFS) for (A) 5-gene panel as developed in investigational cohort (GE_high vs GE_low); (B) CP: high, moderate, and poor; (C) Tumor immunogenicity signature (TIGS): strong, moderate, and weak; and, (D) International Metastatic RCC Database Consortium (IMDC) risk groups. Full OR and HR data including CIs can be found in table 2. GE_high, gene expression high; GE_low, gene expression low.

IMDC risk group was analyzed as a predictor of clinical outcomes. ORR for favorable (n=15), intermediate (n=40), and poor risk (n=7) patients were 53%, 37.5%, and 28.6%, though the difference was not statistically significant. IMDC risk group demonstrated a statistically significant difference in OS by the log-rank test (p=0.02) but not PFS (p=0.6) with median OS of NR, 31.3, and 5.9 months and median PFS of 9.6, 9.7, and 3.5 months, respectively, for favorable, intermediate, and poor risk groups. KM curves for OS and PFS by IMDC risk group are shown in figure 2D

In the validation cohort, other predictors of immunotherapy response were analyzed including high PD-L1 expression. High PD-L1 expression was detected by IHC (≥1%) and RNA expression (defined as ≥75% rank) in 13 (20.0%) and 21 (33.9%) patients, respectively. High PD-L1 by either IHC or RNA expression was not associated with a significantly different ORR, OS, or PFS (figure 3A–B, table 2). Notably high CTLA-4 expression by RNA-seq (defined as ≥75% rank) was detected in 13 (20.0%) patients and was associated with a numerically, but not statistically significantly higher ORR (64.5% vs 34.7%, OR 1.92, 95% CI 0.43 to 8.62, figure 3C, table 2). No statistical difference was detected in PFS or OS between CTLA-4 high versus low group, though PFS was numerically better in the CTLA-4 high group (PFS HR 0.66, 95% CI 0.31 to 1.43).

In the TMB analysis, two patients were found to have a high TMB defined as ≥10 mut/Mb, and neither patient experienced an objective response (online supplemental figure 2). When high TMB was defined as the top 20% (cut-off 7.0 mut/Mb, figure 3D), those with high TMB had a lower ORR compared with those with low TMB (30.8% vs 42.9%) and similar OS (HR 1.01, 95% CI 0.28 to 3.62) and PFS (HR 1.39, 95% CI 0.65 to 3.0).

DISCUSSION

At this time, there are no FDA-approved biomarkers for mRCC to select patients who may benefit from front-line combination immunotherapy (ipilimumab/nivolumab) versus ICI/tyrosine kinase inhibitor (TKI) or single-agent TKI therapy. Our study identified a gene expression panel consisting of five genes (FOXP3, CCR4, KLRK1, ITK, and TIGIT) which, when high, was found to be associated with a higher ORR among a cohort of patients primarily treated with single-agent nivolumab in the second-line or later mRCC setting. However, when evaluated in a subsequent validation cohort of patients treated predominantly in the front-line setting with combination ipilimumab/nivolumab, there was no difference in ORR, PFS, or OS observed between patients with high gene expression compared with those with low gene expression.

The 5-gene panel described above can be seen as a surrogate marker of infiltrating regulatory T-cells (T_{reg}) and natural killer (NK) cells. These genes include FOXP3 and CCR4 which are important for regulatory T-cells, and KLRK1, ITK, and TIGIT, which are highly expressed for NK cells. FOXP3, also known as scurfin, is a T_{reg} protein, involved in the establishment and maintenance of the T_{reg}.[9] CCR4, also known as CD194, is a protein receptor predominantly expressed by T helper 2 (Th_2) cells, whose primary task is to help orchestrate cell migration and homing of leukocytes during development, angiogenesis, and cancer metastases.[10] Killer cell lectin like receptor K1 (KLRK1), also known as NKG2D, belongs to a family of C-type lectin-like receptors which are expressed by all NK cells.[11] This activating receptor and its ligands are critical for the T-cell mediated immune response to tumors and are frequently detected on the surface of tumor cell lines and tumor tissues.[11] ITK is a critical member of the TEC-kinase family, which controls proximal T-cell receptor signaling and supports resting lymphocyte kinase (RLX).[12] ITK inhibition can diminish Th2 immunity and potentiate Th1 based immune responses.[12] The T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), also known as WUCAM, is a member of the PVR/nectin.[13]

Our investigational cohort consisted of patients primarily treated with single-agent PD-1 inhibitors. PD-1 inhibitors primarily act by blocking the inhibitory signaling between PD-1 and PD-L1 that occur between tumor cells and cytotoxic T-cells in the tumor microenvironment, but do not increase the immune infiltration. Thus, we hypothesize that patients who have gene expression profiles associated with higher rates of immune infiltration, possibly indicated by a high score on our 5-gene panel, may be more primed to respond to PD-1 inhibitors. However, the mechanism of CTLA-4 inhibitors occurs at the inhibitory interface between antigen presenting cells and T-cells, thus augmenting the immune cell infiltrate. CTLA-4 inhibition with ipilimumab may result in a more robust tumor inflammatory response, which could negate the predictive value of the above panel due to CTLA-4 modulated inflammatory infiltration. Additionally, our findings in the investigational cohort may not translate to the cohort of patients in the validation cohort who were primarily treated in the first-line setting as opposed to the later line setting.

A key secondary endpoint of this study was also to assess the utility of checkpoint expression and its correlation with clinical outcomes in ICI treated mRCC. High PD-L1 when measured by IHC or RNA expression was consistently not associated with a difference in ORR, OS, or PFS in both our investigational cohort and validation cohort. Notably, CTLA-4 expression in the ipilimumab/nivolumab treated validation cohort was associated with higher ORR, though statistical significance was not reached, and no differences in PFS and OS were observed. No data exists on CTLA-4 expression as a biomarker in mRCC but interestingly, prior studies in stage I–III non-small cell lung cancer have correlated high tumor CTLA-4 expression in tumor involved lymph nodes with worse clinical outcomes[14] and decreased CTLA-4 expression by RNA-seq has been associated with higher response rates among patients with melanoma.[15] Additional secondary goals were to evaluate
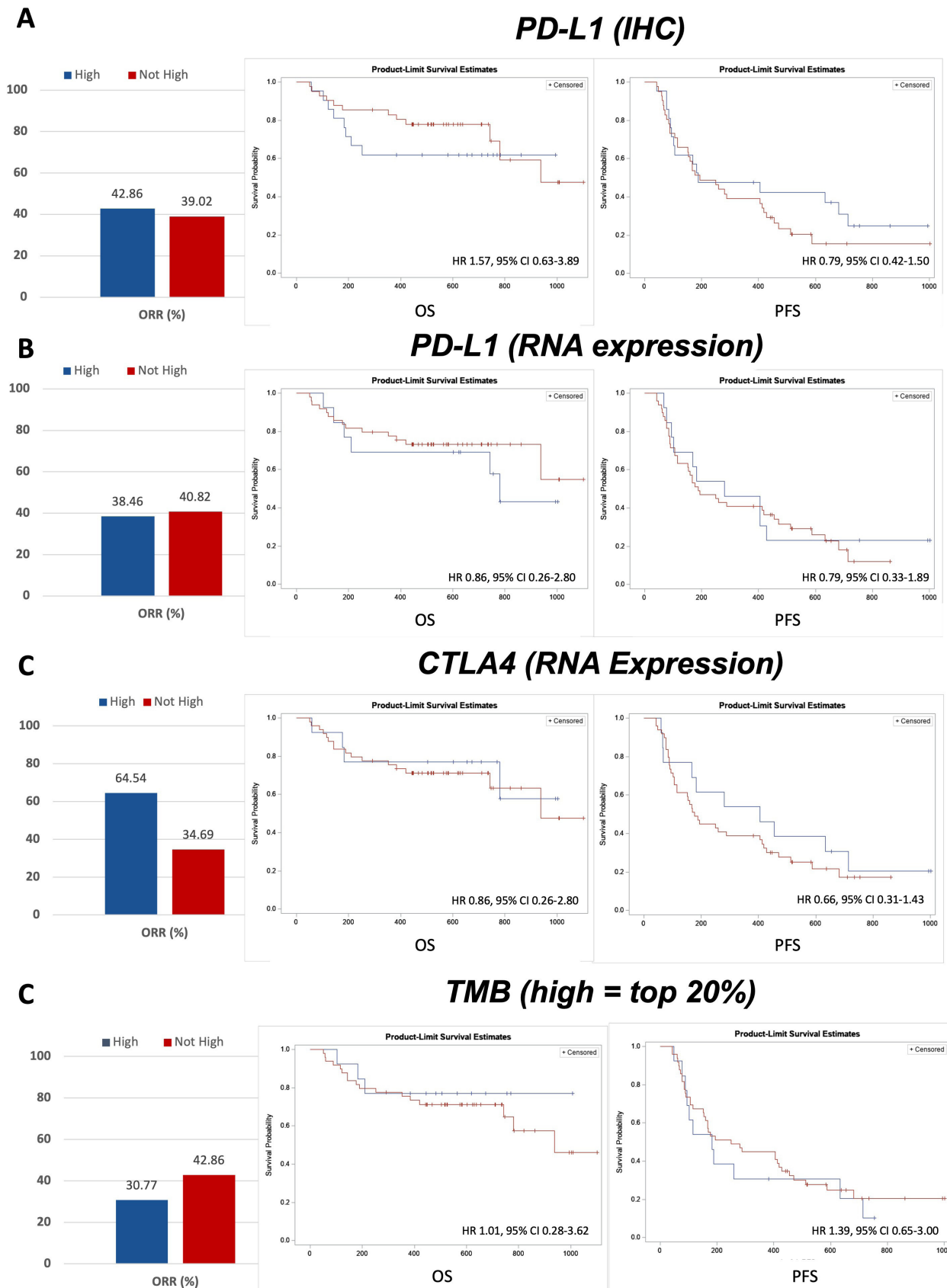
Figure 3 Validation cohort objective response rate (ORR), left, and Kaplan-Meier curves, right, for overall survival (OS) and progression free survival (PFS) as stratified by checkpoint expression: (A) programmed death-ligand 1 (PD-L1) high versus low by immunohistochemistry (IHC), (B) PD-L1 high versus low by RNA expression (high defined as ≥75% rank)), and (C) cytotoxic T-lymphocytes-associated protein 4 (CTLA-4) by RNA expression (high defined as ≥75% rank). Full OR and HR data including CIs can be found in table 2. TMB, tumor mutational burden.

previously defined gene panels developed by OmniSeq, including the TIGS score and CP score in our validation cohort. Though numbers are low and results are hypothesis generating, patients in the validation cohort treated with ipilimumab/nivolumab with poor CP experienced longer median OS, but poor CP was not predictive for median PFS when compared with those with moderate or high cell proliferation. We hypothesize that CP may be prognostic as a marker of more indolent disease biology rather than predictive of response to immunotherapy. This hypothesis is also supported by the poor CP group having lower ORR than either the moderate or high CP groups despite longer OS. Results evaluating the TIGS in the validation cohort demonstrate that the moderate immunogenicity group experienced worse OS and lower ORR, but similar PFS when compared with the weak and strong immunogenicity groups. These results warrant future study in additional validation cohorts.

Our results highlight that biomarkers may be specific to a treatment (such as single-agent PD-1 inhibition) and are not generalizable to other combinations (such as combination PD-1/CTLA-4 inhibition). In particular, transcriptome signatures in other phase 3 trials in mRCC have also demonstrated this principle, as the effector T-cell enriched and myeloid-enriched gene signatures found from the IMmotion150 trial did not predict for PFS to avelumab/axitinib-treated patients in the JAVELIN Renal 101 trial.[16] Most applicable to our study, RNA-seq data from a subset of patients treated with ipilimumab and nivolumab in Checkmate 214 was recently published did not show discrimination in outcomes when stratified by previously published gene expression signatures such as tumor inflammation, angiogenesis, T-effector, and the JAVELIN Renal 101 signature.[17] Multiple other clinical trials have been analyzed for predictive gene signatures relating to the tumor microenvironment[18] and angiogenic factors: IMmotion151,[19] JAVELIN Renal 100, JAVELIN Renal 101,[10] and Keynote 427.[20] Other predictive biomarkers that are promising include the baseline neutrophil to eosinophil ratio[21] and HLA typing.[22] Despite these results, predictive biomarkers in mRCC continue to have limited role in clinical practice and need further prospective testing for widespread clinical utility.

There are several limitations to our data. All data was assessed retrospectively, and clinical variables were abstracted based on chart review. Our targeted RNA-seq approach with 394 immune transcripts likely limits our scope. There was significant patient heterogeneity within our cohorts regarding line of therapy, and baseline variables that were not adjusted for. A limitation of our investigational cohort was the lack of OS and PFS, which were included in our validation cohort. Both the investigational and validation cohorts were composed of a relatively small number of patients, which also limits the interpretation of results. Additionally, our two cohorts were treated exclusively with ICIs, and these results likely do not apply to patients treated with ICI/TKI combinations. Lastly, our two cohorts were indeed different patient groups given the investigational cohort was primarily treated

in the second and later line whereas the validation cohort was primarily treated in the front-line setting, though this is a reflection of the real-world evolution in practice patterns based on available clinical evidence. A strength of our analysis is the presence of two independent cohorts to evaluate our gene expression panel. An additional strength is our inclusion of both an investigational cohort in which we identified a biomarker of interest in the 5-gene panel as well as a validation cohort to prospectively assess this biomarker. Finally, further prognostic markers such as cell proliferation and immunogenicity subgroups were further evaluated across the assessed cohorts. Together these data show use of molecular testing in mRCC and its application to patients treated with immunotherapies.

CONCLUSION

We identified a 5-gene expression panel that was associated with treatment response among patients with mRCC treated with single-agent PD-1 inhibitors. However, these results were not verified when this 5-gene panel was applied to a distinct cohort of patients treated with ipilimumab and nivolumab. Other results demonstrated that moderate tumor inflammation and high/moderate cell proliferation were negatively prognostic for OS. Predictive markers remain elusive for mRCC, and gene expression profiles that predict for response may vary by immunotherapy treatment regimen.

Author affiliations

[1]Levine Cancer Institute, Charlotte, North Carolina, USA

[2]Department of Medicine, Duke Cancer Institute, Durham, North Carolina, USA

[3]Department of Internal Medicine, Cleveland Clinic, Cleveland, Ohio, USA

[4]Bioinformatics, OmniSeq, Buffalo, New York, USA

[5]Department of Hematology and Medical Oncology, Taussig Cancer Institute, Cleveland, Ohio, USA

[6]Department of Medicine, Dartmouth Cancer Center, Lebanon, Pennsylvania, USA

[7]Department of Medicine, University of California-Irvine Health, Orange, California, USA

[8]Center for Personalized Medicine, Roswell Park Cancer Institute, Buffalo, New York, USA

[9]OmniSeq, Inc, Buffalo, New York, USA

[10]Department of Medicine, Roswell Park Cancer Institute, Buffalo, New York, USA

[11]Department of Hematology/Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA

[12]Hematology and Oncology, The University of Texas Southwestern Medical Center, Dallas, Texas, USA

declares that she has no conflicts of interest that might be relevant to the contents of this manuscript. CK declares that he has no conflicts of interest that might be relevant to the contents of this manuscript. YHL is/was an employee at OmniSeq. SP is/was an employee at OmniSeq. MKL declares that he has no conflicts of interest that might be relevant to the contents of this manuscript. JT declares that she has no conflicts of interest that might be relevant to the contents of this manuscript. KHD declares that he has no conflicts of interest that might be relevant to the contents of this manuscript. LJT declares that she has no conflicts of interest that might be relevant to the contents of this manuscript. 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ORCID iDs
Landon C Brown http://orcid.org/0000-0002-5231-4859
Sarabjot Pabla http://orcid.org/0000-0002-7746-9144
Matthew K Labriola http://orcid.org/0000-0003-0919-9864
Daniel J George http://orcid.org/0000-0002-0836-8542
Moshe C Ornstein http://orcid.org/0000-0003-0184-3467

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