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ORIGINAL RESEARCH

Clinical, molecular, and immune correlates of the *Immunotherapy Response Score* in patients with advanced urothelial carcinoma under atezolizumab monotherapy: analysis of the phase II IMvigor210 trial

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Background: In the advanced urothelial carcinoma (aUC) scenario there are no consistent immune checkpoint blockade predictive biomarkers. Recently a novel pan-tumor molecular tissue-based biomarker, the Immunotherapy Response Score (IRS), has been proposed. We conducted a retrospective study to validate the prognostic/predictive utility of the IRS in patients with aUC under atezolizumab monotherapy and to characterize its underlying molecular/immune features in the context of the IMvigor210 phase II trial.

Patients and methods: This is a *post hoc* pooled analysis of 261 patients with available clinical, molecular, and immune tumor data treated with atezolizumab monotherapy in the IMvigor210 phase II clinical trial. Efficacy endpoints were overall survival (OS), disease control rate (DCR), and overall response rate (ORR). Survival estimates were calculated by the Kaplan—Meier method, and groups were compared with the log-rank test. The Cox proportional hazards regression model was used to evaluate factors independently associated with OS. Factors associated with disease control (DC) and response were tested with logistic regression in univariable and multivariable analyses. Comparisons between patient and disease characteristics were carried out using chi-square or Fisher's exact tests. All P values were two-sided, and those <0.05 were considered statistically significant.

Results: High IRS was significantly associated with a better OS in univariable [hazard ratio (HR) $= 0.49$, $P < 0.001$] and multivariable (HR $= 0.60$, $P = 0.018$) analyses. DCR and ORR were significantly higher among high IRS patients (DCR for high IRS versus low IRS patients: 57% versus 32%, $P < 0.001$; ORR: 42% versus 10%, $P < 0.001$). High IRS patients presented a higher probability of DC and response in univariable [DC: odds ratio (OR) $= 2.72$, $P < 0.001$; response: OR $= 3.92$, $P < 0.001$] and multivariable (DC: OR $= 2.72$, $P < 0.001$; response: OR $= 3.92$, $P < 0.001$) analyses.

Conclusions: This study validates IRS as a strong independent prognostic and predictive biomarker for OS and DC/response in patients with aUC treated with atezolizumab monotherapy in the IMvigor210 phase II clinical trial.

Key words: atezolizumab, biomarker, bladder cancer, immunotherapy, urothelial carcinoma

INTRODUCTION

Bladder cancer is the 10th most commonly diagnosed cancer worldwide, with ~573 278 new cases and 212 536 estimated cancer deaths in 2020.[1] Although immunotherapy, particularly programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) blockers, has revolutionized cancer management in recent years, the combination platinum-based chemotherapy remains the standard of care for first-line treatment of advanced urothelial carcinoma (aUC).[2-4] Today, the use of avelumab, an anti-PD-L1 antibody, is indicated as first-line maintenance

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therapy if the disease has not progressed on platinum-based chemotherapy. Among those patients whose disease has progressed on a previous platinum-based strategy, pembrolizumab (an anti-PD-1 antibody) or atezolizumab (an anti-PD-L1 antibody) is the recommended treatment option.[3,4] Upfront therapy with single-agent immune checkpoint blockade, either pembrolizumab or atezolizumab, can be considered as a treatment option for those patients who are ineligible for any platinum-based regimen.[3,4] Moreover, on 3 April 2023, the combination of enfortumab vedotin-ejfv plus pembrolizumab was approved by the Food and Drug Administration (FDA) in this scenario, and therefore, presently, it could also be considered as an upfront therapy for patients not eligible for any platinum-based treatment.[3]

Over the past decade, a plethora of studies have evaluated the role of different prognostic and/or predictive biomarkers for immune checkpoint blockade in aUC. Numerous translational research initiatives have explored the role of different molecular markers, such as PD-L1,[5] tumor mutational burden (TMB),[6,7] copy number and single nucleotide variant counts,[8] alterations in DNA damage response and repair genes,[9] gene expression signatures,[10-13] peripheral blood T-cell receptor clonality,[14] and clinical variables.[15-18] Despite these huge efforts, to date, no consistent biomarker has been translated to the clinic. In this regard, Tomlins et al.[19] have recently developed and validated a novel pan-tumor tissue-based biomarker, the Immunotherapy Response Score (IRS), which integrates TMB and the expression of certain genes such as *PD-1*, *PD-L1*, *TOP2A*, and *ADAM12* in a Cox model, and identifies those patients who derived a higher benefit in terms of time to next therapy [which the authors defined as real-world progression-free survival (rwPFS)] and overall survival (OS) when treated with single-agent anti-PD-1 or anti-PD-L1 immunotherapy. However, the correlation of IRS with other important clinical outcomes such as disease control (DC) or response was not evaluated. Taking this into consideration, herein we conducted a retrospective study to validate the prognostic and predictive role of IRS in patients diagnosed with aUC treated with atezolizumab in the context of the IMvigor210 phase II clinical trial.[5,20] In addition, we explored the correlation of IRS with different molecular and immune tumor characteristics.

PATIENTS AND METHODS

Study design and patient population

The design and primary outcomes of the single-arm phase II study of atezolizumab in aUC (IMvigor210) were described in previous reports.[5,20] In brief, IMvigor210 was a multicenter, single-arm, two-cohort, phase II trial that investigated the efficacy and safety of atezolizumab in aUC. Cohort 1 enrolled cisplatin-ineligible patients without previous treatment for aUC.[20] Neoadjuvant or adjuvant chemotherapy or radiotherapy was permitted if $\geq$12 months had elapsed between treatment and recurrence. Cohort 2 enrolled patients whose disease had progressed after previous platinum-based chemotherapy (including previous neoadjuvant or adjuvant chemotherapy with first progression within $\leq$12 months).[5] This is a *post hoc* pooled analysis of 261 patients with available clinical, molecular, and immune tumor data from the IMvigor210 trial (70 and 191 patients from cohorts 1 and 2, respectively).[5,10,20] For the purpose of our analyses, our efficacy endpoints were OS, disease control rate (DCR), and overall response rate (ORR). Tumor responses were assessed according to RECIST version 1.1.

All clinical and molecular/immune tumor data (the latter generated from pretreatment tumor samples) used for this retrospective study have been previously deposited to the European Genome-Phenome Archive under accession number EGAS00001002556 and made freely available through the IMvigor210CoreBiologies R package (http://research-pub.gene.com/IMvigor210CoreBiologies; The R Foundation, Vienna, Austria).[10]

Individual patient IRS data were derived from the Cox model as previously described ($IRS = 0.273758 \times TMB + 0.112641 \times PD\text{-}1 + 0.061904 \times PD\text{-}L1 - 0.077011 \times TOP2A - 0.057991 \times ADAM12$) and considered as a binary predictor based on the previously defined cut-off threshold (high $\geq$0.873569 versus low $<$0.873569).[19] TMB was calculated as mutations per megabase (Mb) of genomic target territory of the FoundationOne panel.[10] TMB-high patients were defined as those with a TMB $\geq$10 mutations/Mb. Whole transcriptome profiles were generated using the TruSeq RNA Access technology (Illumina).[10] Raw count data for the genes of interest were transformed to $\log_2$-normalized reads per million, and values for each gene were median centered across a representative reference clinical population, The Cancer Genome Atlas Urothelial Bladder Carcinoma cohort. Nine samples with normalized reads per million equal to 0 for any of the four genes were removed. Differential gene expression analysis was carried out with the R package *DeSeq2* version 1.36.0. Only those genes with $>$10 counts in at least one-third of the samples were considered for this analysis. Gene set enrichment analysis (GSEA) was carried out with the R package *clusterProfiler* version 4.6.2.

Statements confirming compliance with ethical regulations, the committees that approved the IMvigor210 study protocol, and confirmation of informed consent from all study participants are included in the previous publications describing the IMvigor210 trial (NCT02108652 and NCT02951767).[5,10,20]

Statistical analysis

Survival estimates were calculated by the Kaplan–Meier method, and groups were compared with the log-rank test. The Cox proportional hazards regression model was used to evaluate factors independently associated with OS. Baseline variables included in the multivariable analysis were selected according to statistical significance in univariable analysis (cut-off, P value $<$0.05). The proportional hazard assumption was verified with the Schoenfeld residual method. Factors associated with DC and response were tested with logistic regression in univariable analyses. Variables included in the final multivariable model were selected according to their statistical significance in univariable analysis (cut-off, P value

Table 1. Distribution of the IRS according to patient and disease characteristics

Characteristics	Total (n = 261)	IRS high (n = 67, 26%)	IRS low (n = 194, 74%)	P value
Sex, *n* (%)				0.072
Female	53 (20)	8 (12)	45 (23)	
Male	208 (80)	59 (88)	149 (77)	
Previous intravesical BCG, *n* (%)				0.766
No	204 (78)	51 (76)	153 (79)	
Yes	57 (22)	16 (24)	41 (21)	
Tobacco smoking status, *n* (%)				0.093
Never	86 (33)	16 (24)	70 (36)	
Ever	175 (67)	51 (76)	124 (64)	
Previous platinum-based therapy, *n* (%)				0.865
No[a]	70 (27)	19 (28)	51 (26)	
Yes[b]	191 (73)	48 (78)	143 (74)	
AJCC stage at diagnosis, *n* (%)				0.838
I-II	155 (59)	41 (61)	114 (59)	
III-IV	106 (41)	26 (39)	80 (41)	
Site of metastases, *n* (%)				*0.024*
Liver	70 (27)	11 (16)	59 (30)	
Lymph node only	43 (16)	16 (24)	27 (14)	
Visceral[c]	123 (47)	30 (45)	93 (48)	
NA	25 (10)	10 (15)	15 (8)	
IC PD-L1 level, *n* (%)				*<0.001*
IC0/1	159 (61)	27 (40)	132 (68)	
IC2/3	102 (39)	40 (60)	62 (32)	
Immune phenotype, *n* (%)				*<0.001*
Inflamed	170 (65)	55 (82)	115 (59)	
Non-inflamed	57 (22)	3 (4)	54 (28)	
NA	34 (13)	9 (13)	25 (13)	
TC PD-L1 level, *n* (%)				*0.040*
TC0/1	221 (85)	51 (76)	170 (88)	
TC2/3	40 (15)	16 (24)	24 (12)	
ECOG-PS, *n* (%)				0.285
0	102 (39)	22 (33)	80 (41)	
1	159 (61)	45 (67)	114 (59)	
TMB, *n* (%)				0.069
High	10 (4)	67 (100)	184 (95)	
Low	251 (96)	0 (0)	10 (5)	
Lund taxonomy, *n* (%)				*0.002*
Genomically unstable	59 (23)	25 (37)	34 (18)	
Non-genomically unstable	202 (77)	42 (63)	160 (82)	

Italicized numbers indicate statistically significant values.
AJCC, American Joint Committee on Cancer; BCG, bacillus Calmette-Guérin; ECOG-PS, Eastern Cooperative Oncology Group performance status; IC, tumor-infiltrating immune cell; IRS, Immunotherapy Response Score; NA, not available; PD-L1, programmed death-ligand 1; TC, tumor cell; TMB, tumor mutational burden.
[a]Patients from cohort 1 of the IMvigor210 trial.
[b]Patients from cohort 2 of the IMvigor210 trial.
[c]Visceral metastasis defined as lung, bone, or any non-lymph node/non-liver or soft tissue metastasis.

<0.05). Comparisons between patient and disease characteristics were carried out using chi-square or Fisher's exact tests. All P values were two-sided, and those <0.05 were considered statistically significant. The Benjamini—Hochberg procedure was used to control the false discovery rate in case of multiple comparisons. All statistical analyses were carried out using R version 4.2.2.

RESULTS

Patient population

From 348 patients enrolled in the IMvigor210 trial and treated with atezolizumab, 261 had all the clinical, molecular, and immune tumor data to be included in this retrospective study (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2023.101611). Baseline patient and disease characteristics are summarized in Table 1. About 26% (n = 67) of patients had a high IRS, while 74% (n = 194) had low IRS; 27% (n = 70) of patients were from cohort 1 and 73% (n = 191) from cohort 2. The distribution of different patient and disease characteristics according to IRS and cohort of origin is shown in Table 1 and Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2023.101611. To note, in the IRS high group there was a higher proportion of patients with genomically unstable Lund taxonomy subtype (P = 0.002), PD-L1 expression on tumor-infiltrating immune cells (ICs) ≥5% (IC2/3) (P < 0.001), PD-L1 expression on tumor cells (TCs) ≥5% (TC2/3; P = 0.040), and immune-inflamed phenotype (P < 0.001). Importantly, there were no statistically significant differences in TMB (high versus low) distribution among IRS high and IRS low cases.

Clinical significance of the IRS

OS. Among 261 cases included in this retrospective study, median OS was 8.90 months [95% confidence interval (CI)

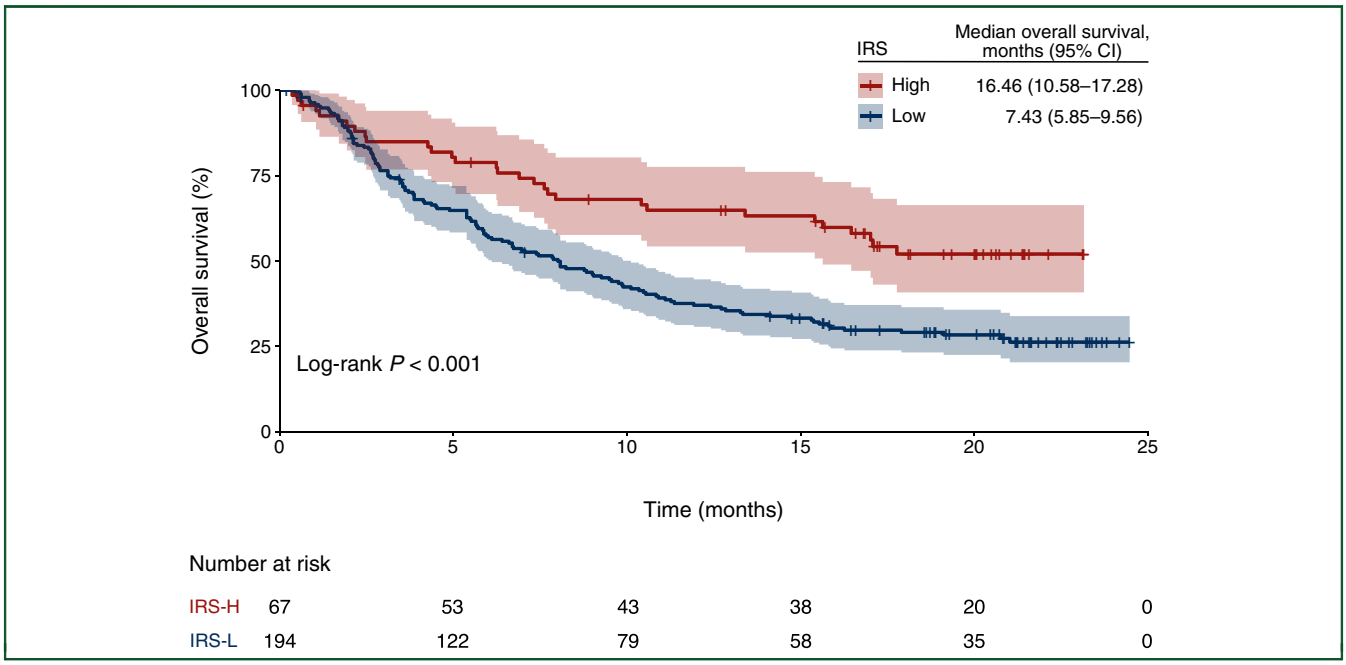

Figure 1. Kaplan–Meier overall survival estimates according to the Immunotherapy Response Score (IRS).
CI, confidence interval; IRS-H, IRS high; IRS-L, IRS low.

7.06-10.91; Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2023.101611]. Median OS for high and low IRS patients was 16.46 months (95% CI 10.58-17.28) and 7.43 months (95% CI 5.85-9.56) ($P < 0.001$), respectively (Figure 1). High IRS was significantly associated with a better OS in univariable [hazard ratio (HR) = 0.49, 95% CI 0.33-0.74, $P < 0.001$] and multivariable (HR = 0.57, 95% CI 0.37-0.86, $P = 0.007$) analyses (Table 2). Other baseline variables independently associated with a better OS in multivariable analysis were Eastern Cooperative Oncology Group performance status (ECOG-PS) 0 (HR = 0.42, 95% CI 0.30-0.60, $P < 0.001$), genomically unstable Lund taxonomy subtype (HR = 0.47, 95% CI 0.30-0.72, $P < 0.001$), PD-L1 expression on ICs ≥5% (IC2/3; HR = 0.64, 95% CI 0.46-0.90, $P = 0.011$), and presence of liver metastases (HR = 1.53, 95% CI 1.10-2.14, $P = 0.013$) (Table 2). High TMB was not associated with an improved OS (Table 2). By contrast, high IRS maintains its prognostic significance when evaluated in cohorts 1 (HR = 0.40, 95% CI 0.17-0.95, $P = 0.038$) and 2 (HR = 0.53, 95% CI 0.34-0.83, $P = 0.006$) separately (Supplementary Figure S2A and B, available at https://doi.org/10.1016/j.esmoop.2023.101611).

DC and response. Among 261 cases included in this retrospective study, DCR and ORR were 38.70% (95% CI 32.75% to 44.90%) and 22.22% (95% CI 17.33% to 27.56%) respectively, including 21 (8.05%) complete responses (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2023.101611). DCR and ORR were

Table 2. Univariable and multivariable Cox regression analyses for overall survival

Characteristics	Univariable analysis HR (95% CI)	*P* value	Multivariable analysis HR (95% CI)	*P* value
Sex (male versus female)	0.95 (0.66-1.38)	0.792		
Previous intravesical BCG (yes versus no)	1.03 (0.72-1.48)	0.884		
Tobacco smoking status (ever versus never)	0.91 (0.66-1.25)	0.550		
Previous platinum-based therapy (yes versus no)	1.21 (0.84-1.73)	0.300		
AJCC stage at diagnosis (III-IV versus I-II)	1.21 (0.89-1.64)	0.232		
Site of metastases (liver versus non-liver/NA)	1.80 (1.30-2.50)	*<0.001*	1.53 (1.10-2.14)	*0.013*
IC PD-L1 level (IC2/3 versus IC0/1)	0.59 (0.42-0.81)	*0.001*	0.64 (0.46-0.90)	*0.011*
Immune phenotype (inflamed versus non-inflamed/NA)	1.18 (0.86-1.62)	0.295		
TC PD-L1 level (TC2/3 versus TC0/1)	0.98 (0.65-1.49)	0.927		
ECOG-PS (0 versus 1)	0.46 (0.33-0.64)	*<0.001*	0.42 (0.30-0.60)	*<0.001*
TMB (high versus low)	0.94 (0.44-2.00)	0.878		
Lund taxonomy (genomically unstable versus non-genomically unstable)	0.47 (0.31-0.72)	*<0.001*	0.47 (0.30-0.72)	*<0.001*
IRS (high versus low)	0.49 (0.33-0.74)	*<0.001*	0.60 (0.39-0.92)	*0.018*

Italicized values indicate statistically significant values.
AJCC, American Joint Committee on Cancer; BCG, bacillus Calmette-Guérin; CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; IC, tumor-infiltrating immune cell; IRS, Immunotherapy Response Score; NA, not available; PD-L1, programmed death-ligand 1; TC, tumor cell; TMB, tumor mutational burden.

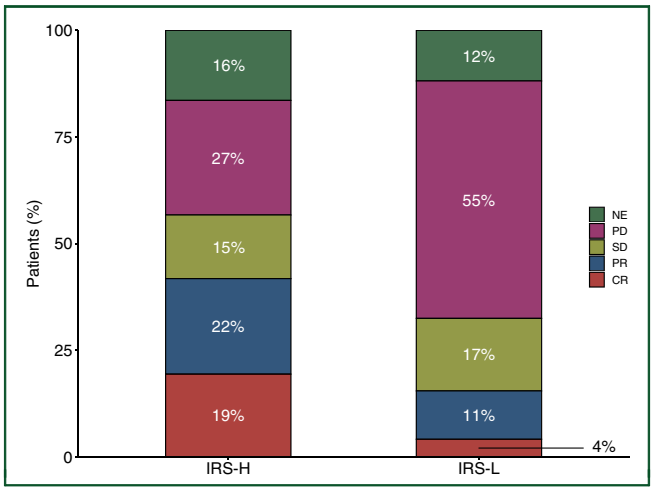

Figure 2. Atezolizumab response distribution by the Immunotherapy Response Score (IRS).
CR, complete response; H, IRS high; IRS-L, IRS low; NE, not evaluable; PD, progressive disease; PR, partial response; SD, stable disease.

significantly higher among high IRS patients (DCR for high IRS versus low IRS patients: 57% versus 32%, $P < 0.001$; ORR for high IRS versus low IRS patients: 42% versus 10%, $P < 0.001$; Figure 2). High IRS patients presented a higher probability of DC and response in univariable analysis [DC: odds ratio (OR) = 2.72, 95% CI 1.54−4.81, $P < 0.001$; response: OR = 3.92, 95% CI 2.11-7.31, $P < 0.001$]. Other variables associated with a higher probability of DC and response in univariable analysis were ECOG-PS 0 (DC: OR = 2.04, 95% CI 1.22-3.40, $P = 0.006$; response: OR = 1.95, 95% CI 1.08-3.52, $P = 0.027$), PD-L1 expression on tumor-infiltrating IC2/3 (DC: OR = 2.04, 95% CI 1.22-3.40, $P = 0.006$; response: OR = 2.13, 95% CI 1.18-3.86, $P = 0.012$), and genomically unstable Lund taxonomy subtype (DC: OR = 2.50, 95% CI 1.39-4.52, $P = 0.002$; response: OR = 3.39, 95% CI 1.79-6.41, $P < 0.001$; Tables 3 and 4). Presence of liver metastases was associated with a lower probability of DC and response (DC: OR = 0.41, 95% CI 0.22-0.76, $P = 0.004$; response: OR = 0.36, 95% CI 0.16-0.81, $P = 0.014$; Tables 3 and 4). When these variables were evaluated in multivariable analysis, IRS, ECOG-PS, and Lund taxonomy were independently associated with a higher probability of DC (high IRS: OR = 2.09, 95% CI 1.11-3.95, $P = 0.023$; ECOG-PS 0: OR = 2.17, 95% CI 1.24-3.79, $P = 0.007$; and genomically unstable Lund taxonomy subtype: OR = 2.53, 95% CI 1.33-4.84, $P = 0.005$) and response (high IRS: OR = 2.95, 95% CI 1.46-5.95, $P = 0.003$; ECOG-PS 0: OR = 2.20, 95% CI 1.13-4.29, $P = 0.020$; and genomically unstable Lund taxonomy subtype: OR = 3.25, 95% CI 1.60-6.58, $P = 0.001$; Tables 3 and 4). By contrast, presence of liver metastases was correlated with a lower probability of DC (OR = 0.46, 95% CI 0.24-0.88, $P = 0.020$). When cohorts 1 and cohort 2 were separately evaluated, DCR and ORR were again higher among high IRS patients (DCR for high IRS versus low IRS patients: cohort 1, 63% versus 37%, $P = 0.063$; cohort 2, 54% versus 31%, $P < 0.001$; ORR for high IRS versus low IRS patients: cohort 1, 47% versus 18%, $P = 0.026$; cohort 2, 40% versus 15%, $P < 0.001$). Similarly, high IRS patients presented a higher probability of DC (cohort 1: OR = 2.89, 95% CI 0.97-8.60, $P = 0.057$; cohort 2: OR = 2.66, 95% CI 1.36-5.19, $P = 0.004$) and response (cohort 1: OR = 4.20, 95% CI 1.33-13.30, $P = 0.015$; cohort 2: OR = 3.81, 95% CI 1.81-7.99, $P < 0.001$) in both cohorts.

Biological significance of the IRS

To fully characterize the IRS from a biological viewpoint, we carried out a differential gene expression analysis (Figure 3A) followed by a GSEA (Figure 3B, Supplementary Figure S3A and B and Supplementary Table S3A-C, available at https://doi.org/10.1016/j.esmoop.2023.101611). As expected, this analysis revealed an enrichment of important biological processes associated with immune system activation such as natural killer cell-mediated immunity (adjusted P value <0.001), lymphocyte-mediated immunity (adjusted P value <0.001), lymphocyte migration (adjusted

Table 3. Univariable and multivariable logistic regression analyses for disease control

Characteristics	Univariable analysis OR (95% CI)	*P* value	Multivariable analysis OR (95% CI)	*P* value
Sex (male versus female)	0.86 (0.47-1.59)	0.638		
Previous intravesical BCG (yes versus no)	1.00 (0.54-1.82)	0.986		
Tobacco smoking status (ever versus never)	1.48 (0.86-2.55)	0.154		
Previous platinum-based therapy (yes versus no)	0.73 (0.42-1.27)	0.263		
AJCC stage at diagnosis (III-IV versus I-II)	0.87 (0.52-1.45)	0.601		
Site of metastases (liver versus non-liver/NA)	0.41 (0.22-0.76)	*0.004*	0.46 (0.24-0.88)	*0.020*
IC PD-L1 level (IC2/3 versus IC0/1)	2.04 (1.22-3.40)	*0.006*	1.72 (0.98-3.01)	0.060
Immune phenotype (inflamed versus non-inflamed/NA)	0.59 (0.34-1.01)	0.055		
TC PD-L1 level (TC2/3 versus TC0/1)	1.36 (0.69-2.68)	0.375		
ECOG-PS (0 versus 1)	2.04 (1.22-3.40)	*0.006*	2.17 (1.24-3.79)	*0.007*
TMB (high versus low)	0.94 (0.26-3.43)	0.931		
Lund taxonomy (genomically unstable versus non-genomically unstable)	2.50 (1.39-4.52)	*0.002*	2.53 (1.33-4.84)	*0.005*
IRS (high versus low)	2.72 (1.54-4.81)	*<0.001*	2.09 (1.11-3.95)	*0.023*

Italicized numbers indicate statistically significant values.
AJCC, American Joint Committee on Cancer; BCG, bacillus Calmette-Guérin; CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group performance status; IC, tumor-infiltrating immune cell; IRS, Immunotherapy Response Score; NA, not available; OR, odds ratio; PD-L1, programmed death-ligand 1; TC, tumor cell; TMB, tumor mutational burden.

Table 4. Univariable and multivariable logistic regression analyses for response

Characteristics	Univariable analysis OR (95% CI)	*P* value	Multivariable analysis OR (95% CI)	*P* value
Sex (male versus female)	1.11 (0.53-2.33)	0.774		
Previous intravesical BCG (yes versus no)	1.18 (0.59-2.36)	0.631		
Tobacco smoking status (ever versus never)	1.24 (0.66-2.34)	0.504		
Previous platinum-based therapy (yes versus no)	0.77 (0.40-1.45)	0.412		
AJCC stage at diagnosis (III-IV versus I-II)	0.72 (0.39-1.32)	0.282		
Site of metastases (liver versus non-liver/NA)	0.36 (0.16-0.81)	*0.014*	0.43 (0.18-1.01)	0.053
IC PD-L1 level (IC2/3 versus IC0/1)	2.13 (1.18-3.86)	*0.012*	1.64 (0.84-3.19)	0.147
Immune phenotype (inflamed versus non-inflamed/NA)	0.65 (0.34-1.24)	0.189		
TC PD-L1 level (TC2/3 versus TC0/1)	0.86 (0.37-1.97)	0.714		
ECOG-PS (0 versus 1)	1.95 (1.08-3.52)	*0.027*	2.20 (1.13-4.29)	*0.020*
TMB (high versus low)	2.64 (0.33-21.31)	0.361		
Lund taxonomy (genomically unstable versus non-genomically unstable)	3.39 (1.79-6.41)	*<0.001*	3.25 (1.60-6.58)	*0.001*
IRS (high versus low)	3.92 (2.11-7.31)	*<0.001*	2.95 (1.46-5.95)	*0.003*

Italicized numbers indicate statistically significant values.
AJCC, American Joint Committee on Cancer; BCG, bacillus Calmette-Guérin; CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group performance status; IC, tumor-infiltrating immune cell; IRS, Immunotherapy Response Score; NA, not available; OR, odds ratio; PD-L1, programmed death-ligand 1; TC, tumor cell; TMB, tumor mutational burden.

P value <0.001), and response to interferon-γ (adjusted *P* value <0.001) in IRS high cases. By contrast, processes associated with stroma such as extracellular matrix organization (adjusted *P* value <0.001) and extracellular structure organization (adjusted *P* value <0.001) were upregulated in IRS low cases (Figure 3B). Other statistically significantly enriched Gene Ontology components (Molecular Function and Cellular Component) are described in Supplementary Figure S3A and B and Supplementary Table S3A-C, available at https://doi.org/10.1016/j.esmoop.2023.101611.

DISCUSSION

Multiple studies have been carried out to discover predictive biomarkers for cancer immunotherapy, but to date, only microsatellite instability has been adopted in the clinic as the first tissue/site-agnostic predictive biomarker for the anti-PD-1 antibody pembrolizumab. Although TMB has been also approved by the FDA as a predictive biomarker for the same drug in a tissue/site-agnostic cancer indication, its utility in the daily clinical practice remains debatable. Taking this into consideration, Tomlins et al.[19] have developed a new pan-solid tumor prognostic/predictive biomarker, the IRS, which integrates TMB and the normalized expression of *PD-1*, *PD-L1*, *TOP2A*, and *ADAM12* genes in a Cox model, and identifies those patients who derived a higher benefit in terms of rwPFS and OS when treated with single-agent anti-PD-1 or anti-PD-L1 immunotherapy. Considering the importance of validating biomarkers in prospective cohorts, herein we conducted a retrospective study to validate the prognostic and predictive role of IRS in patients diagnosed with aUC treated with atezolizumab in the IMvigor210 phase II clinical trial.[5]

First, according to the clinicopathological and tumor molecular features, we found an enrichment of different characteristics classically correlated with more immunogenic tumors in IRS high cases such as the genomically unstable Lund taxonomy subtype, the expression of PD-L1 on tumor-infiltrating IC2/3, the expression of PD-L1 on TC2/3, and the immune inflamed phenotype.[21,22] Interestingly and in line with the work of Tomlins et al.,[19] we did not find any statistically significant difference in the TMB status distribution among IRS groups. Moreover, we found a higher proportion of patients with metastatic liver disease among those with a low IRS. This finding is congruent with a detrimental effect of systemic immunotherapy reported in 2021 by Yu et al.[23] among preclinical mouse models and patients with liver metastases. The authors found that patients with liver metastases present a reduced number of peripheral T cells and tumoral T-cell diversity and function, which means a limited benefit from immunotherapy independent of many other well-established predictive factors. Moreover, in preclinical models, activated $CD8^+$ T cells underwent apoptosis following their interaction with $FasL^+CD11b^+F4/80^+$ monocyte-derived macrophages presented in the liver.[23]

Second, we evaluated the correlation of IRS with OS of patients with aUC treated with atezolizumab. As expected, the IRS demonstrated a strong independent prognostic significance, with IRS high cases presenting a 51% reduction in the risk of death compared with IRS low cases. These results are in line with those reported by Tomlins et al.,[19] who found a risk of death reduction of 48% and 51% in the discovery and validation pan-tumor cohorts of their study, respectively. It is important to highlight that our study validates for the first time the prognostic utility of IRS in a prospective cohort of 261 patients with aUC treated with atezolizumab. This represents an important step in the aUC clinical scenario, taking into consideration that the original study[19] was not specifically designed to address this question in this specific tumor type, and only included 62 bladder cancer cases treated with different immune checkpoint inhibitors, either pembrolizumab monotherapy (45 patients) in the discovery cohort, or an alternative anti-PD-1/PD-L1 monotherapy (17 patients: 12 treated with atezolizumab, 3 with nivolumab, and 2 with avelumab) in the validation cohort.

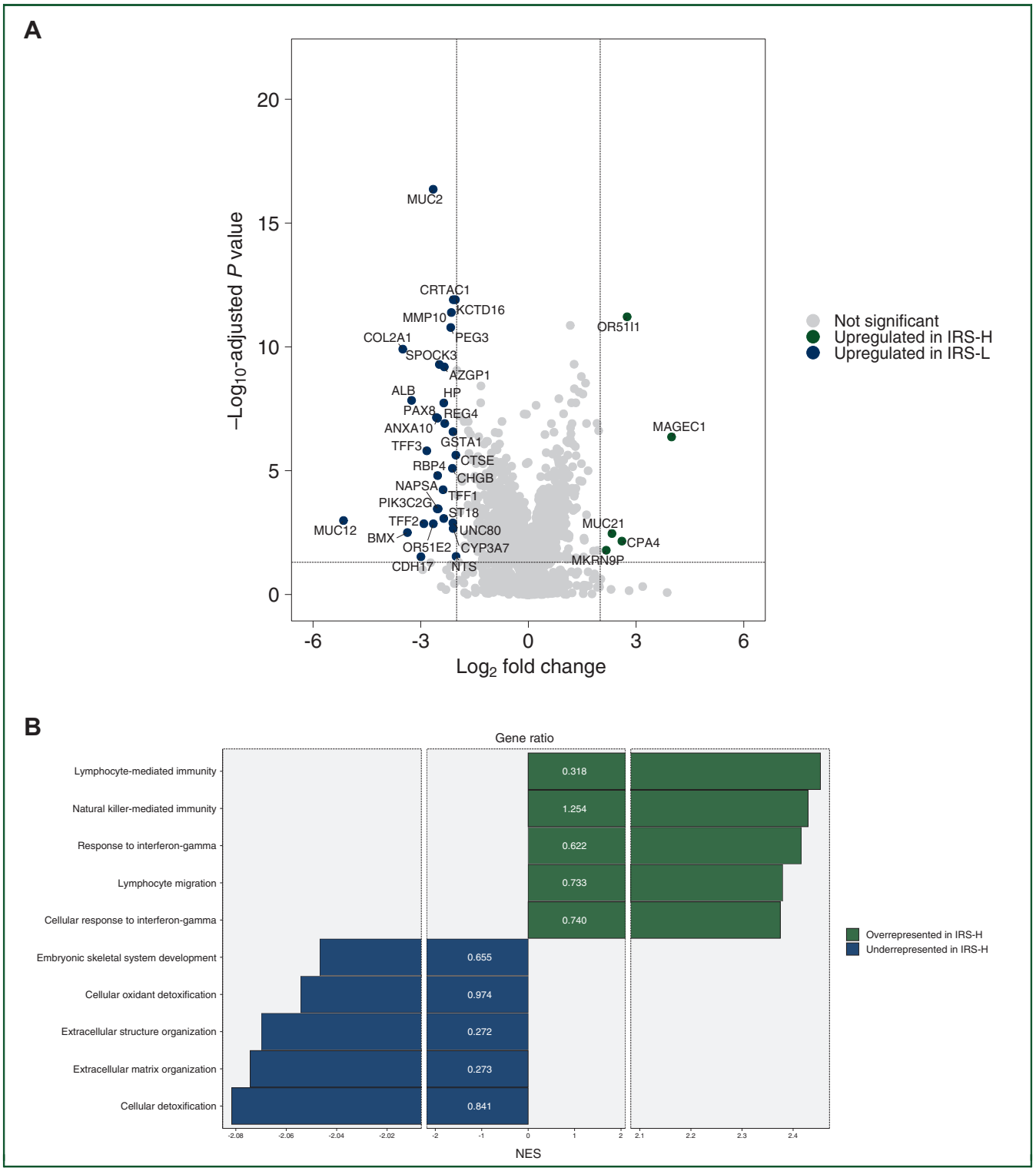

Figure 3. (A) Volcano plot representing gene expression differences between Immunotherapy Response Score (IRS) high (IRS-H) and IRS low (IRS-L) cases. (B) Gene set enrichment analysis showing statistically significantly overrepresented or underrepresented Gene Ontology Biological Processes in IRS-H cases compared with IRS-L cases.

For simplicity, biological processes natural killer cell-mediated cytotoxicity, natural killer cell-mediated immunity, natural killer cell activation, and regulation of natural killer cell-mediated cytotoxicity were represented together under the term natural killer cell-mediated immunity. In this particular case, represented normalized enrichment score (NES) and gene ratio are the median of these four biological processes.

Third, while Tomlins et al.[19] validated the predictive nature of IRS using various indirect approaches involving rwPFS, herein we directly demonstrated its ability to predict DC and response. In our study, IRS high cases not only had a higher DCR and ORR, but also had an increased probability of DC and response compared with IRS low cases.

Finally, in an attempt to fully characterize the IRS from a biological viewpoint, we carried out a GSEA, which, as

expected, revealed an enrichment of important biological processes associated with immune system activation in IRS high cases. By contrast, IRS low cases were enriched in biological processes associated with stroma, which agree with previous findings associating a lack of response to atezolizumab in those patients with aUC with an immune-excluded tumor microenvironment with a high pan-fibroblast transforming growth factor-beta response signature.[10]

Our study has two main limitations. The first one is the use of a prospective cohort from a single-arm phase II clinical trial. Although we could evaluate the correlation of IRS with DC and response to atezolizumab, due to the lack of a comparator arm of patients treated with an alternative drug, we were limited to carry out a test of interaction to definitively demonstrate the predictive value of IRS in this particular clinical scenario. This point could be clarified when validating our results in a prospective cohort from a randomized phase III clinical trial. The second limitation is the use of different molecular platforms to estimate the TMB and the expression of genes comprising the IRS. Although originally the IRS includes TMB from a StrataNGS comprehensive genomic profiling test, and expression of *PD-1*, *PD-L1*, *TOP2A*, and *ADAM12* genes from a multiplex PCR-based quantitative transcriptional profiling test, in our study TMB and gene expression were evaluated using a FoundationOne panel and whole transcriptome sequencing, respectively. However, from a pragmatic viewpoint and considering the high concordance demonstrated in a previous validation study using TMB estimated with either the StrataNGS comprehensive genomic profiling assay or the FoundationOne panel,[24] we feel confident about the robustness and interchangeability of our results.

Although it is out of the scope of this study, one can think about the advantages of IRS over more simple and inexpensive clinical scores already developed and with potential clinical utility such as those developed by Ruiz-Bañobre et al.[15] [the three-risk category prognostic model, which includes ECOG-PS, proton-pump inhibitor use, albumin level, presence of liver metastases, and presence of peritoneal metastases], Sonpavde et al.[16] (the five-factor prognostic model, which integrates ECOG-PS, liver metastases, platelet count, neutrophil-to-lymphocyte ratio, and lactate dehydrogenase), Khaki et al.[17] (the four-factor prognostic model, which integrates ECOG-PS, albumin, neutrophil-to-lymphocyte ratio, and presence of liver metastases), and Bamias et al.[18] (the four-risk category prognostic model, which includes ECOG-PS, alkaline phosphatase, hemoglobin, neutrophil-to-lymphocyte ratio, presence of liver metastases, presence of bone metastases, and time from last chemotherapy). However, some questions remain: Will the IRS be much better than these clinical tools? Can these scores be combined in a unique integrative clinical and molecular algorithm? To respond these questions further research efforts benchmarking the different available predictors and exploring their single and combined capacities are needed.

Today, either in daily clinical practice or in a clinical trial scenario, there are available different treatment options for the management of patients with aUC. In this context, the development of tools to help in the decision-making process is mandatory. In this study, in addition to demonstrating the prognostic and predictive utility of the IRS in patients with aUC under atezolizumab monotherapy, we characterized its underline molecular and immune features. If the results of this study are definitively validated, the IRS will represent a valuable tool for therapy selection in this setting: immunotherapy yes or not, alone or in combination.

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DISCLOSURE

UA-H reports travel, accommodations, and expenses covered by Ipsen, Bayer, Merck, and Pfizer; honoraria for educational activities from Advanced Accelerator Applications—Novartis, Bayer, Ipsen, MSD, AstraZeneca, Merck, Eisai, Bristol-Myers Squibb, Kyowa Kirin, ROVI, GlaxoSmithKline, and LEO Pharma; honoraria for consultancies from Advanced Accelerator Applications—Novartis, Ipsen, AstraZeneca, Merck, Pfizer, Astellas, and Bayer. VC-L reports travel, accommodations, and expenses covered by AstraZeneca, Bristol-Myers Squibb, Eisai, Ipsen, Kyowa Kirin, Merck, Novartis, Pfizer, PharmaMar, Pierre-Fabre, Roche, and Sanofi; honoraria for educational activities from AstraZeneca and PharmaMar. LL-M reports travel, accommodations, and expenses covered by Bristol-Myers Squibb, Lilly, MSD, and Roche; honoraria for educational activities from AstraZeneca, Boehringer Ingelheim, Novartis, Jansen, Astellas, and Sanofi; honoraria for consultancies from AstraZeneca, Boehringer Ingelheim, Novartis, Jansen, Astellas, and Sanofi. JG-G reports travel, accommodations, and expenses covered by AstraZeneca, Bristol-Myers Squibb, MSD, Roche, Sanofi, and Takeda; honoraria for educational activities from AstraZeneca, Bristol-Myers Squibb, MSD, Novartis, Pierre-Fabre, Roche, Sanofi, and Takeda; honoraria for consultancies from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, MSD, Novartis, Roche, Sanofi, and Takeda. NF-D reports travel, accommodations, and expenses covered by GlaxoSmithKline and Sanofi. RL-L reports travel, accommodations, and expenses covered by Lilly, Novartis, Pfizer, Merck, Roche, and Bristol-Myers Squibb; honoraria for educational activities from

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DATA SHARING

All clinical and molecular/immune tumor data from the IMvigor210 trial used for this retrospective study have been previously deposited to the European Genome-Phenome Archive under accession number EGAS00001002556 and made freely available through the IMvigor210CoreBiologies R package (http://research-pub.gene.com/IMvigor210CoreBiologies).[10]

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