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Original Research

Extensive molecular profiling of squamous cell anal carcinoma in a phase 2 trial population: Translational analyses of the "CARACAS" study

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KEYWORDS
Squamous cell anal carcinoma;
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Anti-PD-L1

Abstract ***Background:*** Molecular characteristics of squamous cell anal carcinoma (SCAC) are poorly explored. Immune checkpoint inhibitors showed limited activity in phase I/II trials, but predictive and prognostic biomarkers are lacking.
Patients and methods: In the phase II randomised trial CARACAS (NCT03944252), avelumab alone (Arm A) or with cetuximab (Arm B) was tested in pre-treated advanced SCAC , with overall response rate being the primary end-point. On pre-treatment tumour tissue samples, we assessed Human papillomavirus status, programmed-death ligand 1 (PD-L1) expression, mismatch repair proteins expression, tumour mutational burden (TMB) and comprehensive genomic profiling by FoundationOne CDx. Tumour-infiltrating lymphocytes were characterised on haematoxylin-eosine-stained samples. Primary objective was to describe response to immunotherapy in the CARACAS trial population according to molecular and histological characteristics. Secondary objectives were to assess progression-free survival (PFS) and overall survival (OS) according to molecular biomarkers.
Results: High PD-L1 (>40 with combined positive score) was significantly more frequent in patients with disease control (p = 0.0109). High TMB (>10 mutations per megabase) was related to better OS (hazard ratio (HR) = 0.09; 95%confidence interval (CI) 0.01−0.68; p = 0.019) and PFS (HR = 0.44; 95%CI = 0.15−1.27; p = 0.129). High expression of PD-L1 conferred longer OS (HR = 0.46; 95%CI = 0.19−1.08; p = 0.075) and PFS (HR = 0.42; 95%CI = 0.20−0.92; p = 0.03). Neither OS (HR = 1.30; 95%CI = 0.72 −2.36; p = 0.39) or PFS (HR = 1.31; 95%CI = 0.74−2.31; p = 0.357) was affected by high (>1.2) Tumour-infiltrating lymphocytes count. High TMB and PD-L1identified patients were with significantly better OS (HR = 0.33; 95%CI = 0.13−0.81; p = 0.015) and PFS (HR = 0.48; 95%CI = 0.23−1.00; p = 0.015).
Conclusions: To our knowledge, TranslaCARACAS is the first study to document prognostic role of TMB and PD-L1 in advanced SCAC patients treated with immune checkpoint inhibitors.

1. Introduction

Squamous cell anal carcinoma (SCAC) represents only 2% of all gastrointestinal tumours, but is characterised by high morbidity and mortality, with 5-year survival of 34% in a metastatic setting [1]. Standard treatments for advanced disease consist of platinum-based chemotherapy doublets, and have remained unchanged for 20 years; no targeted agents are currently approved.

Notably, more than 80% of patients are infected with Human papillomavirus (HPV), HPV DNA and p16 expression being related to better survival [2,3]. Less is known regarding other prognostic biomarkers in SCAC, despite a growing interest on molecular profiling has been raised in the last years.

Although programmed-death ligand 1 (PD-L1) blockade demonstrated activity in patients with advanced SCAC (aSCAC) [4,5], overall response rate is modest, and the occurrence of durable response is limited to a small subset of patients. The CARACAS study (NCT04944252) was an open-label, multicentre randomised phase 2 trial investigating anti PD-L1 avelumab alone or with anti-epidermal growth factor receptor cetuximab in pre-treated aSCAC [6]. Although the combination arm met the primary end-point with overall response rate of 17%, overall limited activity was confirmed for immunotherapy. Therefore, investigating predictive and prognostic biomarkers is useful to refine patients' selection, but few data are available in scientific literature [5]. In SCAC, PD-L1 is frequently expressed: in most cohorts, PD-L1 positive tumours represent more than 50% [7]. However, it is unlikely that responses may rely on a single biomarker since the immunogenicity of SCAC is based on a complex landscape related to the intrinsic characteristics of tumour cells, the immune microenvironment and the viral infection; thus, a comprehensive molecular and immune profiling may be the right path to investigate immune-resistance or immune-sensitivity.

The ancillary, prospective translational TranslaCARACAS study had the aim to provide a comprehensive molecular characterisation of aSCAC, and to prospectively describe correlations between molecular profile and overall survival, progression free survival and response pattern to immunotherapy alone or combined with anti-epidermal growth factor receptor drugs.

2. Materials and methods

2.1. Study design, participants and treatments

CARACAS (NCT03944252) was a multicentre, open-label, randomised phase 2 trial promoted by the Gruppo

Oncologico Nord Ovest (GONO) Foundation, and coordinated by Veneto Institute of Oncology IRCCS in Padua.

Patients treated with at least one previous line of treatment for metastatic disease, or experiencing progression of disease within six months after the completion of chemoradiotherapy for non-metastatic disease were eligible; HIV-positive patients were also eligible. Pre-treated advanced disease randomisation was conducted 1:1 between avelumab monotherapy 10 mg/kg intravenously q2w (arm A) or cetuximab 500 mg/m2 plus avelumab 10 mg/kg intravenously q2w (arm B).

Written informed consent for the study procedures and for molecular analyses was obtained from each patient before registration. Approval for the protocol was granted through the institutional Ethics Committee from each participating centre; the trial was conducted according to the Declaration of Helsinki.

2.2. Translational analyses

A formalin-fixed paraffin-embedded tumour block from primary tumour and/or metastases was mandatory for enrolment; HPV status was centrally assessed by means of polymerase chain reactionsingle step and Reverse Line Blot (Ampliquality HPV-type express v3.0; AB Analitica, Italy).

NGS was performed with FoundationOne CDx panel® (Roche Diagnostics, Switzerland), which encompassed microsatellite status and tumour mutational burden (TMB) quantification in mutations per Megabase (mut/Mb).

To validate NGS results, mutational status of genes of a particular interest was assessed through a hotspot multigene mutational custom panel, including hotspot regions of *PIK3CA*, *KRAS*, *NRAS* and *BRAF* using the Myriapod Colon status panel (Diatech Pharmacogenetics, Jesi) run on a MassARRAY Dx Analyser 4 (Agena Bioscience, Germany).

Immunohistochemical staining for mismatch repair (MMR) proteins (MLH1, PMS2, MSH2 and MSH6; Agilent, CA) was performed on a Leica Bond system (Bond-III; Leica Microsystems, Italy). Nuclear immunostaining was evaluated following the Italian Group of Gastrointestinal Pathologists (GIPAD-SIAPeC) criteria to identify MMR deficiency (MMRd) and MMR proficiency [8]. In order to describe immunogenicity of SCAC, PD-L1 expression was assessed by immunohistochemistry (clone 22C3; Agilent), and measured both with combined positive score (CPS) and tumour proportion score (TPS).

Tumour-infiltrating lymphocytes (TILs) were quantified on a pre-treatment and post-treatment tissue in haematoxylin-eosin. The number of TILs was defined as the mean value of five random observations and count at high-power fields (40×) of tumour-enriched areas composed of >60% of neoplastic cells. Only tumour epithelium-infiltrating lymphocytes were retained for scoring.

2.3. Statistical analyses

Primary objective was to search for possible correlations between molecular and histological characteristics of the tumour specimens analysed, and the response to experimental treatments received during the study in order to find predictive markers of response to immunotherapy.

Secondary objectives were to assess progression-free survival (PFS) and overall survival (OS) according to molecular characteristics to individuate new prognostic biomarkers in SCAC.

The distribution of categorical data was tested with Fisher's exact test. Differences in the distribution of numeric variables were calculated with Mann–Whitney U test. Univariate linear models were used to assess the correlation between continuous variables. Right-censored variables such as PFS and OS were modelled with univariate Cox regression. Maximisation of log-rank statistics was used to explore optimal cut-offs of continuous variables; given the exploratory nature of the study and the numerosity of the cohort, no methods for cut-offs validation were used. Data were imported and handled in R v4.1.2 using ggplot2, dplyr, survminer, survival, finalfit and ComplexHeatmap packages [9].

2.4. Data availability statement

The data generated in this study are available within the article and its supplementary data files.

3. Results

All the 60 patients enrolled in the CARACAS trial entered the final cohort for translational analyses. Potential predictive and prognostic factors were investigated in the overall population without distinction per arm since 100% of patients received immunotherapy.

As shown in Table 1, the main clinical, molecular and histological characteristics were equally distributed between the two arms. Tissue samples were from primary tumour in the majority of patients (48/60, 80%), but no substantial differences were observed regarding the feasibility and the results of the analyses between samples taken from primary versus metastatic tissue.

In our cohort, 20 (33%) tumours showed keratinising histology (Fig. 1).

TILs were assessable on pre-treatment samples from 51 patients (arm A/B: 24/27); median TILs were 1.6 (IQR: 0.6–3.1). TMB could be determined on pre-treatment samples of 40 patients (arm A/B: 21/19); median TMB was 4.00 mut/Mb (IQR: 2.52–6.3 mut/Mb). Only one tumour (2%) was dMMR and, expectedly, reported high TMB (63.04 mut/Mb). PD-L1 could be determined on pre-treatment samples of 52 patients

Table 1
Patients and clinical, histological and molecular characteristics according to the randomly allocated treatment arm.

	Levels	Ave (n = 30)	Ave + Cet (n = 30)	Total (n = 60)	*p*-values[a]
Histology	Non-keratinising	19 (63.3)	21 (70.0)	40 (66.7)	0.785
	Keratinising	11 (36.7)	9 (30.0)	20 (33.3)	
Median age (range), years	Median (range)	65 (35–84)	63 (39–77)	–	
Race, n (%)	Caucasian	29 (97)	30 (100)	59 (98.3)	1.000
	Asian	1 (3)	0 (0)	1 (1.7)	
Sex, n (%)	Female	24 (80)	17 (57)	41 (68.3)	0.095
	Male	6 (20)	13 (43)	19 (31.7)	
ECOG performance status, n (%)	0	16 (53)	18 (60)	34 (56.7)	0.0756
	1	12 (40)	11 (37)	23 (38.3)	
	2	2 (7)	1 (3)	3 (5)	
Synchronous versus metachronous disease	Synchronous	12 (40)	12 (40)	24 (40)	1.000
	Metachronous	18 (60)	18 (60)	36 (60)	
HIV status	Positive	3 (10)	1 (3)	4	0.612
	Negative	27 (90)	29 (97)	56	
HPV	Positive	25 (89.3)	27 (93.1)	52 (91.2)	0.67
	Negative	3 (10.7)	2 (6.9)	5 (8.8)	
	Missing	*2*	*1*	*3*	
HPV TYPE	16	13 (93%)	22 (96%)	35 (95%)	1.000
	Others[c]	1 (7%)	1 (4%)	2 (5%)	
	Not evaluable[b]	*11*	*4*	*15*	
TILs	Median (IQR)	1.8 (0.8–3.6)	1.6 (0.4–2.1)	1.6 (0.6–3.1)	0.260
PD-L1 (CPS)	Median (IQR)	1.0 (0.0–20.0)	10.0 (0.0–40.0)	5.0 (0.0–40.0)	0.476
PD-L1 (TPS)	Median (IQR)	0.0 (0.0–0.5)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.454
TMB	Median (IQR)	3.8 (2.5–6.3)	5.0 (2.5–7.6)	4.0 (2.5–6.3)	0.576
Mismatch repair proteins analysis	pMMR	29 (100.0)	26 (96.3)	55 (98.2)	0.482
	dMMR	0 (0.0)	1 (3.7)	1 (1.8)	
	Missing	*1*	*3*	*4*	
KRAS	WT	29 (100.0)	27 (93.1)	56 (96.6)	0.491
	MUT	0 (0.0)	2 (6.9)	2 (3.4)	
	Missing	*1*	*1*	*2*	
BRAF	WT	29 (100.0)	29 (100.0)	58 (100.0)	–
	Missing	*1*	*1*	*2*	
PIK3CA	WT	15 (51.7)	23 (79.3)	38 (65.5)	0.067
	MUT	12 (41.4)	5 (17.2)	17 (29.3)	
	AMPL	2 (6.9)	1 (3.4)	3 (5.2)	
	Missing	*1*	*1*	*2*	

[a] Mann–Whitney U was used for numeric variables, and Fisher test was used for categorical variables.
[b] Missing data were not included in the denominator for better comparison between groups with data.
[c] 1 HPV39-positive tumour in arm A, and 1 HPV6-positive tumour in arm B.

(arm A/B: 25/27); median CPS was 5 (IQRs: 0–40), while TPS was positive (>0) only in 12 (20%) of the patients (Table 1).

In 36 patients, simultaneous TILs, TMB and PD-L1 assessment was possible (Supplementary Fig. 1A). Enrichment in high TMb/CPS/TILs of these patients is showed in Supplementary Fig. 1B. Subsequent analyses with single biomarkers or their combinations refer to the cohort with corresponding available biomarkers.

As expected, the large majority of our patients were diagnosed with HPV infection (91%). HPV + cases were equally distributed between the two arms. In both arms, the most common HPV genotype found was 16 (Table 1). Only three patients, two in Arm A and one in Arm B (6.7% and 3.8%, respectively), were HIV positive. Even with the limitation of the small sample size, no substantial differences were observed in these patients compared to HIV negative patients regarding prognosis and molecular characteristics.

3.1. Survival analyses

We firstly investigated the best OS predictor between TPS and CPS in our cohort: CPS showed a better AUC for OS prediction at all time points (Supplementary Fig. 2). Therefore, CPS was used for all subsequent analyses. By means of maximisation of log-rank statistics, we then calculated optimal cut-offs for OS estimation for TILs, TMB and CPS, which were, respectively, 1.2, 10 mut/Mb and 40; these cut-offs were used for all subsequent analyses.

For patients with high TMB (N = 5), more than doubled PFS compared to patients with low TMB (N = 35) was observed, although the difference was not statistically significant (mPFS: 4.6 versus 2.0 months; hazard ratio (HR) = 0.44; 95%confidence interval (CI) = 0.15–1.27; $p = 0.129$) (Fig. 2A). Similarly, patients with high CPS (N = 10) showed improved PFS compared to patients with low CPS (N = 42) (mPFS:

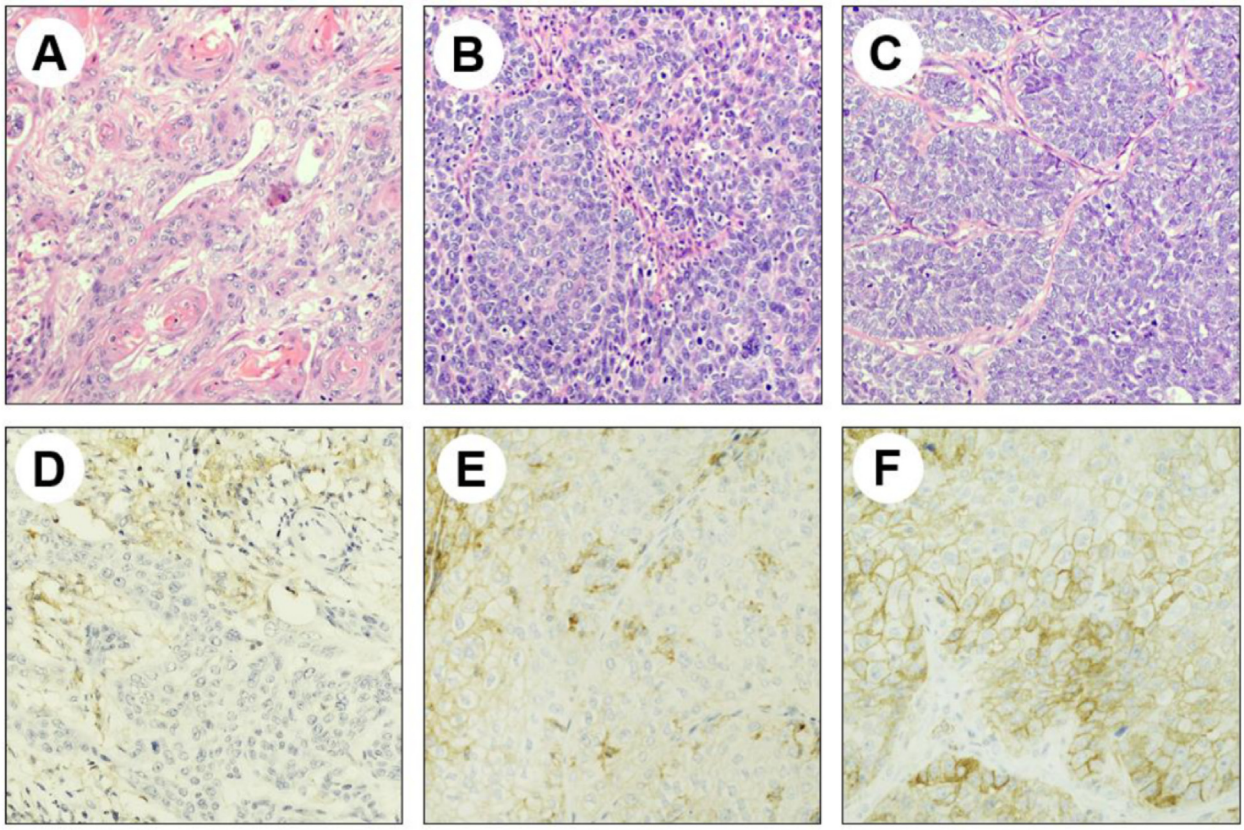

Fig. 1. HE stained sections of squamous cell anal carcinoma with keratinising (A) and non-keratinising (B, C) features, with abundant (B) or absent/mild (A, C) lymphocytic infiltrate. Representative images of squamous cell anal carcinoma samples stained with PD-L1 (22C3), showing no PD-L1 expression in the tumour cells, but in the inflammatory infiltration (D), PD-L1 expression in both tumour and inflammatory cells (E), and in tumour cells (F). Original magnifications 20x.

5.86 versus 2.05 months; HR = 0.42; 95% CI = 0.20–0.92; $p = 0.03$) (Fig. 2C). The presence of either high TMB or high CPS identified a group of patients (N = 12) with significantly longer PFS (mPFS 5.07 versus 1.86 months; HR = 0.48; 95% CI = 0.23–1.00; $p = 0.051$) (Fig. 2E). TILs did not have clear impact on PFS (mPFS 2.03 versus 4.35 months; HR = 1.31; 95%CI = 0.74–2.31; $p = 0.357$) (Supplementary Fig. 3A).

As observed for PFS, patients with high TMB had significantly higher OS compared to patients with low TMB (mOS 9.79 months versus not reached; HR = 0.09; 95%CI 0.01–0.68; $p = 0.019$ (Fig. 2B). Similarly, patients with high CPS showed borderline significant improvement of OS (mOS 14.39 versus 7.49 months; HR = 0.46; 95%CI = 0.19–1.08; $p = 0.075$) (Fig. 2D). The presence of high TMB or high CPS identified a group of patients with markedly improved OS (mOS 17.97 versus 8.03 months; HR = 0.33; 95% CI = 0.13–0,81; $p = 0.015$) (Fig. 2F). As observed for PFS, no differences in OS were found between patients with high TILs versus low TILs (mOS 9.79 versus 15.62 months; HR = 1.30; 95%CI = 0.72–2.36; $p = 0.39$) (Supplementary Fig. 2B).

As showed in the heatmap in Fig. 3A–B, none of the genes tested with NGS was found to be prognostic for survival.

3.2. Analyses of response

Comparing major histological and molecular characteristics between patients reaching disease control (stable disease or partial response) versus patients with progressive disease (PD) as best response per RECIST 1.1 [10], high PD-L1 was significantly more frequent in patients with disease control (p = 0.0109) (Table 2). All the other main histological and molecular characteristics were equally prevalent, as well as rarer genetic mutations (Table 2, Supplementary Table 1).

Depth of response is depicted in Fig. 4A; single patients' responses along with TMB or PD-L1 CPS status are reported in Fig. 4B.

None of the molecular characteristics explored showed predictive value. Given the low numerosity of the cohort, and the prevalence of genetic alterations, we only investigated predictive value of the two most frequently altered genes, *PIK3CA* and *MLL2*. None of them retained significant interaction with the treatment arm

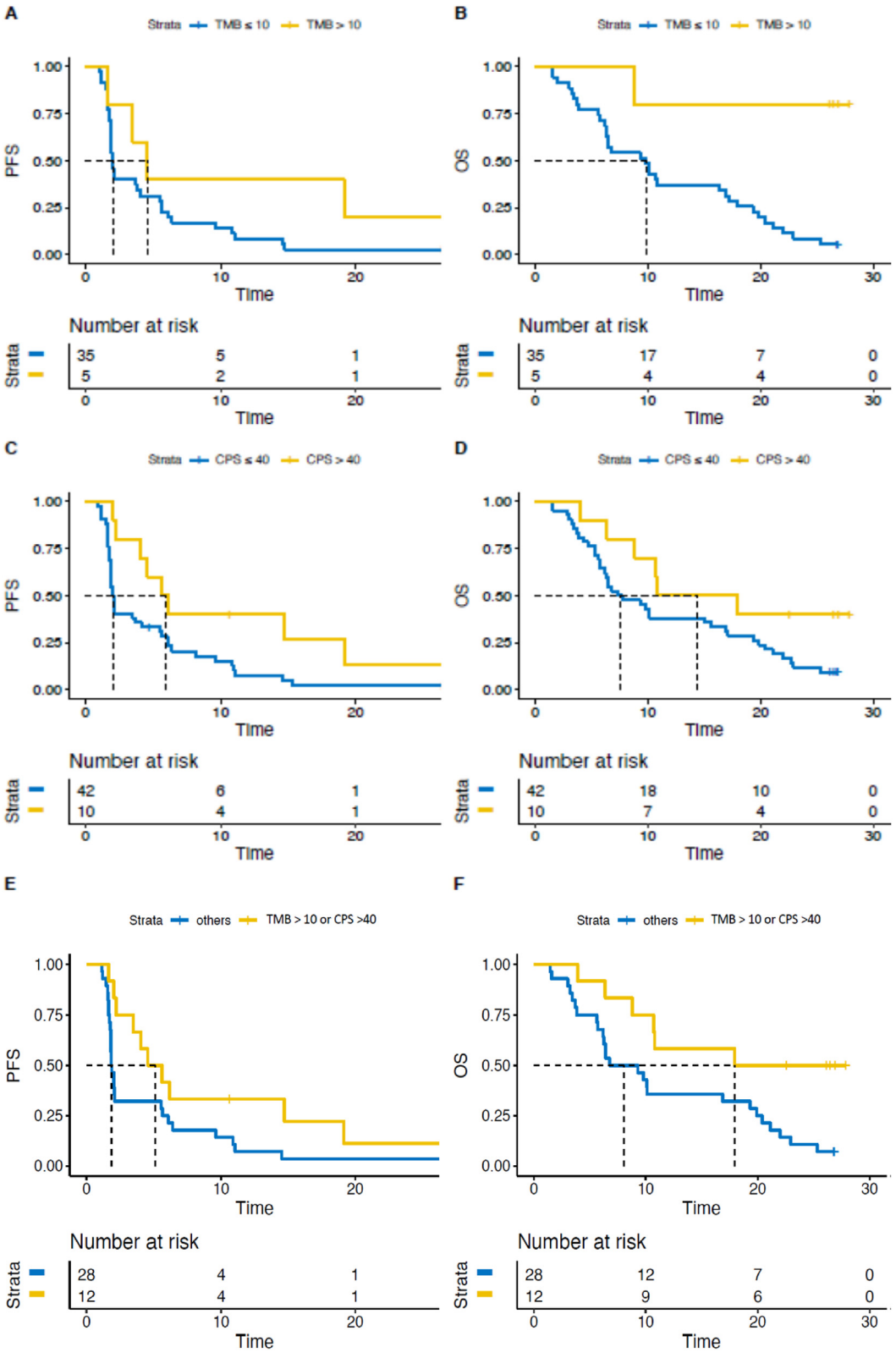

Fig. 2. Kaplan–Meier curves depicting overall survival and progression-free survivalaccording to tumour mutational burden (2A – 2B), PD-L1 expressed in combined positive score(2C-2D) and to either tumour mutational burden or PD-L1 (2E-2F).

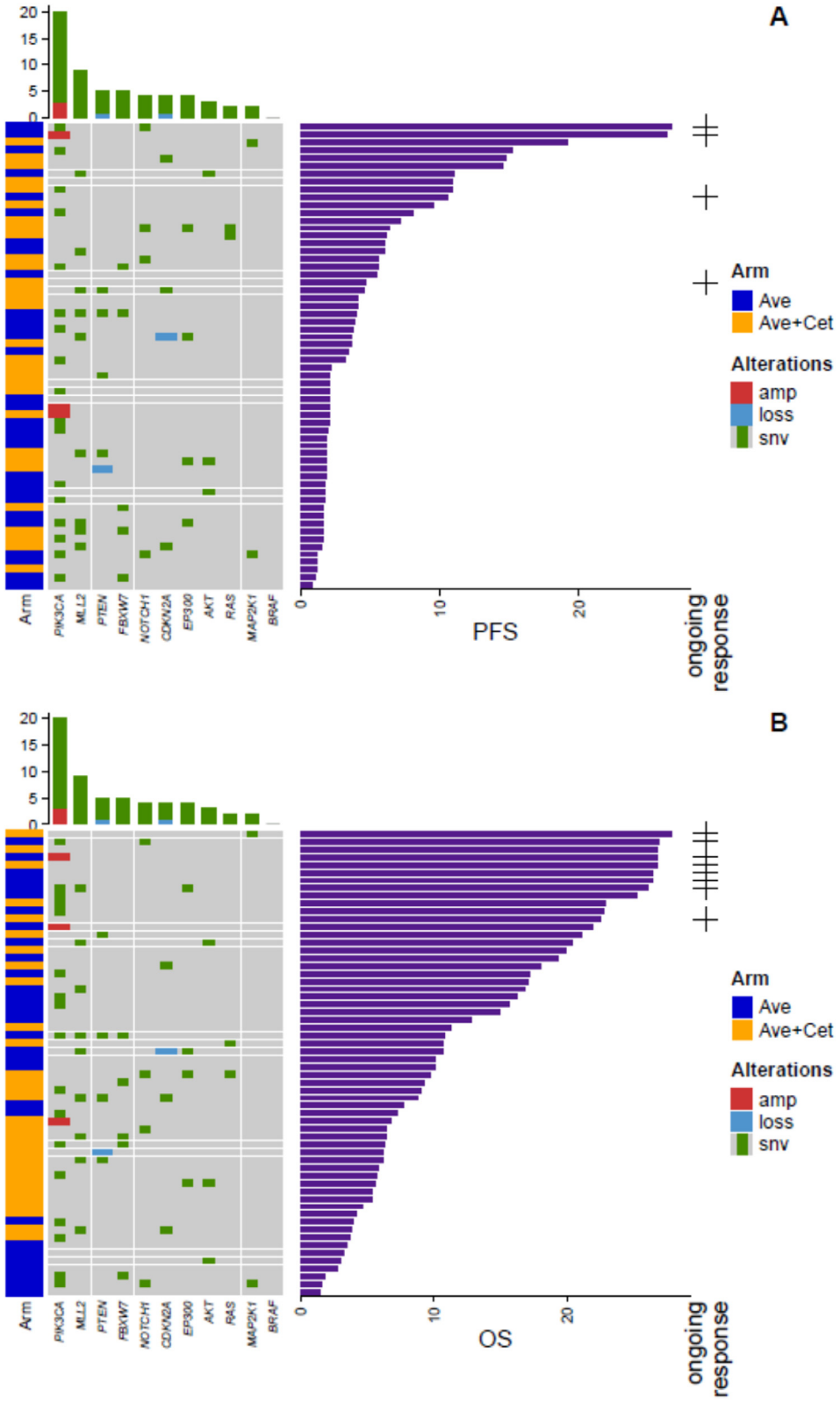

Fig. 3. Impact of single genes on progression-free survival(3A) and on overall survival (3B).

Table 2
Molecular and histological characteristics according to the best response. Missing data were not included in the denominator for better comparison between groups with data. P values were calculated with Fisher exact test.

	Best response SD or PR Tot = 32 n (%)	Best response PD Tot = 28 n (%)	*p* Value
Keratinising	11 (34)	9 (32)	$p = 1.0000$
Non-keratinising	21 (66)	19 (68)	
Not evaluable	*0*	*0*	–
TILs ≤ 1.2	15 (56)	9 (37)	$p = 0.2641$
TILS > 1.2	12 (44)	15 (63)	
Not evaluable	*5*	*4*	–
PD-L1 (CPS) ≤ 40	17 (65)	25 (96)	$p =$ **0.0109**
PD-L1 (CPS) > 40	9 (35)	1 (4)	
Not evaluable	*6*	*2*	–
TMB ≤ 10	14 (78)	21 (95)	$p = 0.1554$
TMB > 10	4 (22)	1 (5)	
Not evaluable	*14*	*6*	–
***PIK3CA* altered**	7 (50)	7 (47)	$p = 1.0000$
***PIK3CA* WT**	7 (50)	8 (53)	
Not evaluable	*1*	*0*	–
HPV+	27 (93)	25 (89)	$p = 0.6701$
HPV-	2 (7)	3 (11)	
Not evaluable	*3*	*0*	–

(p = 0.143 and p = 0.088, respectively). Similarly, the presence of high PD-L1 CPS was not linked with a significant interaction with the treatment arm (p = 0.929). The interaction of the arm with TMB could not be estimated due to the low numerosity of the subgroups.

4. Discussion

In the TranslaCARACAS study, extensive molecular characterisation of aSCAC treated with immune checkpoint inhibitors (ICI), with or without cetuximab, was provided. The most remarkable results of our work concern the prognostic role of TMB-high and PD-L1-positivity, associated with survival benefit in patients treated with ICI, with or without cetuximab.

TMB is gaining growing interest; however, although high TMB proved predictive value for ICI in several solid tumours (melanoma [11,12], lung cancer [13,14], bladder cancer [15] and dMMR tumours in general [16]), its influence in HPV-positive malignancies is still unclear. In head and neck squamous cell carcinoma, HPV infection was associated with increased benefit from PD1/PD-L1 blockade, irrespectively, of TMB; HPV-positive tumours also displayed significantly increased T-cell infiltration and T-cell-inflamed gene expression profile [17]. These findings suggest that, in HPV-positive malignancies, immunogenicity could be enhanced by viral infection more than by mutational load.

Consistently with other cohorts [18–20], in our study, high TMB (>10 mut/Mb) was found in a small

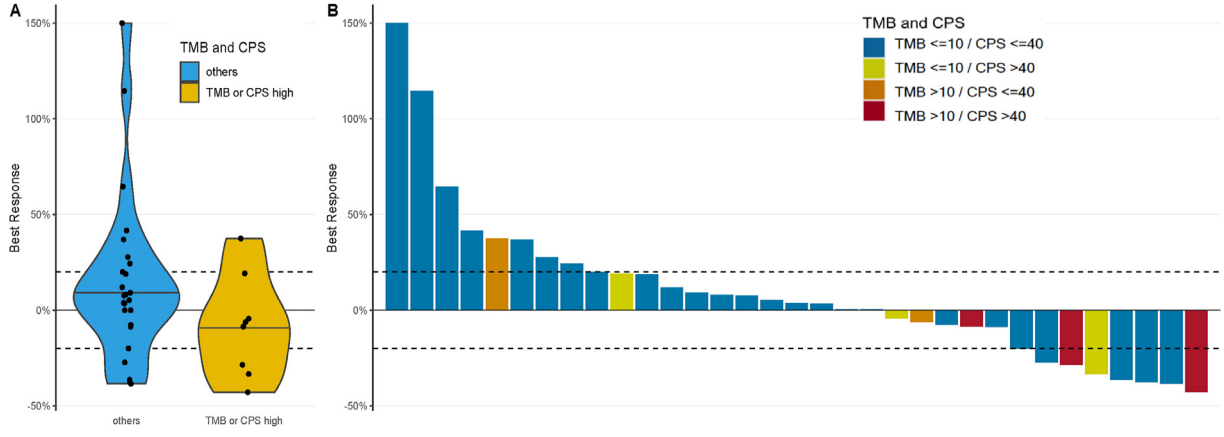

Fig. 4. Relation between response, tumour mutational burden (TMB) and combined positive score. In the Fig. 4A, a violin plot shows the distribution of patients according to best response reached and distinguished between patients with high TMB and/or combined positive score(yellow) and others (blue). In the waterfall plot (Fig. 4B), difference in deepness of response according to TMB and PD-L1 is depicted.

subgroup of patients (12%). Our results suggest predictive value of broad PD-L1 expression in therapy with ICI, especially when combined with high TMB. Unfortunately, the limited cohort size prevented us from exploring the prognostic value of TMB and CPS in a multivariate model; indeed, data regarding PD-L1 expression, TMB and TILs were simultaneously available only for 36 patients (60%), and overlapping of CPS>40 and TMB>10 mut/Mb occurred only in two patients (Supplementary Fig. 1A and 1B). The CARACAS study was the first to explore combination therapy of cetuximab and anti-PD-L1 in aSCAC; due to the small number of patients with high TMB, we cannot draw any conclusion about the predictive role of TMB and PD-L1 in each of the two therapeutic strategies separately. In our cohort, positive correlation between OS and high TMB was described, with similar trend in PFS; our trial was the first to document TMB prognostic value in a cohort of patients with SCAC. In scientific literature, few previous studies explored the prognostic value of TMB in SCAC: in these experiences, OS was not influenced by TMB. However, patients were treated with standard therapies, not ICI [20], thus supporting a potential predictive rather than prognostic weight of these markers with regard to the efficacy of ICI.

The prognostic role of PD-L1 has been explored in SCAC treated with standard chemoradiotherapy [7,21], and its predictive value has been formerly investigated in patients with aSCAC treated with ICI: in a phase II trial of Nivolumab in aSCAC in lines after the first, significant relationship was found between response and expression of CD8, Granzyme B, LAG-3, TIM-3 CD45 and PD-1 on T cells and PD-L1 on tumour cells, supporting the possible predictive role of tumour-infiltrating lymphocytes (TILs) and PD-L1 for ICI in aSCAC [5].

In SCAC at initial stages, PD-L1 was associated with survival benefit after treatment with standard chemoradiotherapy [7,21], but no data are available regarding its role in aSCAC treated with ICI. In our cohort of SCAC treated with ICI, with or without cetuximab, PD-L1 was related to higher PFS, with similar trend in OS. Nevertheless, in determining PD-L1 expression, some issues are still open: no validated cut-off exists in SCAC; in our study, cut-off of >40% for PD-L1 CPS was considered optimal, while in other studies $\geq$1 was used. These discrepancies might account for different outcomes across the studies.

In our trial, both primitive and metastatic tissues were accepted for determining PD-L1 and TMB. Preliminary studies on intratumour molecular heterogeneity indicate that TMB is a stable biomarker, and it is relatively independent of the location of acquisition [22], without significant variations between primary and metastatic sites. On the contrary, it is well established that PD-L1 expression rates may vary between primary tumours and different metastatic sites due to the influence of the tumour microenvironment [23]. Therefore, the inclusion of both primary and metastatic lesions in the analysis may represent a limitation for biomarker evaluation, particularly for PD-L1. However, no statistically significant difference in the predictive values of PD-L1 and TMB was found between primary and metastatic sites, also because the number of metastatic samples was limited in this study.

TILs did not show clear prognostic and predictive value: in our cohort, high TILs infiltrate seemed paradoxically related with worse survival. TILs have been studied mainly in localised SCAC, and were found to be predictive of response to standard chemoradiotherapy and prognostic of better survival outcomes [24,25]. In colorectal cancer , benefit in survival was confirmed in patients with high TILs infiltration [26]. Several factors might account for this controversial result: first of all, TILs were assessed on haematoxylin-eosin without further analysis in immunohistochemistry due to scarcity of available tissue, so a distinction between cytotoxic and regulator TILs was not feasible. Secondly, TILs were quantified on pre-treatment specimens, both primary tumour or metastases being accepted, this might account for differences of TILs concentrations.

Another factor deserving special consideration is HPV infection. It is well known that HPV infection shapes the tumour immune microenvironment [27]; furthermore, in head and neck squamous cell carcinoma, HPV infection has been related to increased immune effector cells infiltrate, but not to regulatory T cells (T-reg) [17]. These changes could provide a robust biological rationale for the employment of anti-PD(L)1 in HPV-related neoplasms.

In our trial, HPV had no predictive and prognostic value. On the other hand, in our cohort the large majority of patients were HPV+. Given the small sample size, our study might be underpowered to find differences in survival and response between HPV+ and HPV- patients.

In HPV + malignancies, several attempts to include ICI in combination therapies have been made. Atezolizumab plus bevacizumab reached an overall response rate of 10% (95% CI 9.5–20) in a cohort of 20 patients with pretreated aSCAC, 20% of them experiencing prolonged disease control [28]. Bintrafusp, a new fusion protein, consists in a bifunctional molecule composed of an extracellular TGF-β 'trap' domain fused to a human IgG1 mAb blocking PD-L1. Bintrafusp has been administered in 59 patients (6 affected by SCAC) with HPV + neoplasms in a phase I/II trial, reaching a total clinical response rate of 35.6% (95% CI, 23.6%–49.1%) [29]. Even with this small sample size, this study throws a new light on the possible treatment strategies in HPV + tumours.

5. Conclusions

To our knowledge, TranslaCARACAS was the first study to document prognostic role of TMB and PD-L1 in aSCAC treated with ICI. Undoubtedly, further investigation in larger cohorts is warranted to confirm our findings that remain exploratory. Considering the overall limited activity of ICI monotherapy in aSCAC, however, new biomarkers predictive of response are needed to select those patients who might benefit most from ICI. Moreover, testing new therapeutic associations or strategies comprising ICI could enhance their activity in order to give new therapeutic options in this rare and aggressive disease. As a matter of fact, our findings open new and interesting scenarios in the research for new prognostic biomarkers for SCAC treated with ICI, and are worth of further investigation in larger cohorts.

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Author contributions

AAP: conceptualisation, data curation, formal analysis, investigation and writing-original draft; PM: data curation, formal analysis, investigation and writing-original draft; MM, VF, GLF, MGZ, DCC, CO and MP: investigation, validation and visualisation; FB: conceptualisation, investigation, validation and visualisation; VA: formal analysis and validation; MS, MC, GM, MR and FM: investigation, validation and visualisation; VV: data curation, investigation and project administration; RS: formal analysis and validation; CR and GM: investigation, validation and visualisation; PDB: data curation and software; MM: formal analysis and validation; CC: conceptualisation, methodology, supervision and writing-review and editing; MF, FP and SL: conceptualisation, supervision and writing-review and editing.

Conflict of interest Statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interest.

SL (Sara Lonardi) was advisor and part of speakers' bureau of Amgen, Merck Serono, Lilly, Bristol-Myers Squibb, Servier, Roche, Pierre-Fabre and GSK; had advisory or consulting role for AstraZeneca, Incyte and Daiichi-Sankyo; and received research grant by Bayer. FM (Federica Morano) reported honoraria from Servier. VF (Vincenzo Formica) reported personal fees from Merck KGaA, Amgen, Servier and Sanofi. MS (Mario Scartozzi) reported personal fees from MSD, Merck, Eisai, Sanofi, Bayer and Servier. SM (Stefania Mosconi) reported personal fees from Merck Serono. MF (Matteo Fassan) reported personal fees (as speaker bureau or advisor) from Roche, MSD, GSK, Astellas Pharma, Pierre Fabre and Astra Zeneca; and received research grants from Astellas Pharma, QED therapeutics and Macrophage Pharma. CC (Chiara Cremolini) reported personal fees from Amgen, Bayer, Merck, MSD, Nordic Pharma, Pierre Fabre, Roche and Servier; and received research grants from Amgen, Merck, Bayer, Roche, and Servier. All of the above-mentioned financial relationships were outside this work. All remaining authors have declared no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2022.12.025.

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