ORIGINAL ARTICLE

MITO END-3: efficacy of avelumab immunotherapy according to molecular profiling in first-line endometrial cancer therapy

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Background: Immunotherapy combined with chemotherapy significantly improves progression-free survival (PFS) compared to first-line chemotherapy alone in advanced endometrial cancer (EC), with a much larger effect size in microsatellite instability-high (MSI-H) cases. New biomarkers might help to select patients who may have benefit among those with a microsatellite-stable (MSS) tumor.

Patients and methods: In a pre-planned translational analysis of the MITO END-3 trial, we assessed the significance of genomic abnormalities in patients randomized to standard carboplatin/paclitaxel without or with avelumab.

Results: Out of 125 randomized patients, 109 had samples eligible for next-generation sequencing analysis, and 102 had MSI tested. According to The Cancer Genome Atlas (TCGA), there were 29 cases with MSI-H, 26 with MSS *TP53* wild type (wt), 47 with MSS *TP53* mutated (mut), and 1 case with *POLE* mutation. Four mutated genes were present in >30% of cases: *TP53*, *PIK3CA*, *ARID1A*, and *PTEN*. Eleven patients (10%) had a *BRCA1/2* mutation (five in MSI-H and six in MSS). High tumor mutational burden ($\geq$10 muts/Mb) was observed in all MSI-H patients, in 4 out of 47 MSS/*TP53* mut, and no case in the MSS/*TP53* wt category. The effect of avelumab on PFS significantly varied according to TCGA categories, being favorable in MSI-H and worst in MSS/*TP53* mut (*P* interaction = 0.003); a similar non-significant trend was seen in survival analysis. *ARID1A* and *PTEN* also showed a statistically significant interaction with treatment effect, which was better in the presence of the mutation (*ARID1A P* interaction = 0.01; *PTEN P* interaction = 0.002).

Conclusion: The MITO END-3 trial results suggest that *TP53* mutation is associated with a poor effect of avelumab, while mutations of *PTEN* and *ARID1A* are related to a positive effect of the drug in patients with advanced EC.

Key words: endometrial cancer, chemotherapy, avelumab, next-generation sequencing, The Cancer Genome Atlas

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INTRODUCTION

Endometrial cancer (EC) is the most frequent gynecological malignancy.[1] Women with recurrent or advanced disease with International Federation of Gynecology and Obstetrics (FIGO) classification III or IV deserve 5-year overall survival (OS) rates of 50% and 20%, respectively.[2]

To date, for these patients, the first-line medical treatment consists of chemotherapy, with carboplatin plus paclitaxel, or hormonal therapy, depending on clinical features.[3] The majority of the patients progress or are primarily resistant to therapy and have a poor prognosis. New and more effective treatments are urgently needed.

Traditionally, EC was classified into two subtypes—type I and II—on the basis of clinical, histological, and endocrine features.[4] However, in the past few years, the complexity and heterogeneity of EC have been recognized in the analysis of The Cancer Genome Atlas (TCGA), which has resulted in a molecular categorization of EC into four different subgroups: *POLE* ultramutated, microsatellite instability (MSI) hypermutated, copy number low, and copy number high.[5] Even this molecular classification suffers from a certain degree of overlapping mutations, and probably more molecular subtypes could be identified in order to target cancer within a precision medicine strategy.

Immunotherapy has been recognized as an effective treatment in recurrent EC with dostarlimab, pembrolizumab, and pembrolizumab/lenvatinib approved by the Food and Drug Administration and European Medicines Agency.[6-8]

The activity is higher in patients with high mutational load, as occurs in the *POLE*-mutated and MSI-high (MSI-H) EC subtypes, which constitute only 25% of the cases.[9-12]

In a recent phase II randomized trial, we showed that the combination of chemotherapy (carboplatin plus paclitaxel) and avelumab (given concurrently and as maintenance) in the first-line treatment of EC has been able to prolong progression-free survival (PFS), with significant interaction with the mismatch repair (MMR) status.[13] The improvement in PFS and OS produced by avelumab has been observed in a pre-planned analysis almost exclusively in patients with MMR-deficient (dMMR) tumors.[13] The results of the phase III RUBY trial exploring the role of dostarlimab in combination with carboplatin and paclitaxel in the same setting have met the primary endpoint of prolonging PFS in the pre-specified dMMR/MSI-H patient subgroup [hazard ratio (HR) 0.28, 95% confidence interval (CI) 0.16-0.50, $P < 0.001$] and the overall population (HR 0.64, 95% CI 0.51-0.80, $P < 0.001$).[14]

Positive results have also been obtained by combining pembrolizumab with chemotherapy. The phase III NRG-GY018 trial demonstrated improved PFS with pembrolizumab both in the MSI-H (HR 0.30, 95% CI 0.19-0.48, $P < 0.001$) and in the MSS cases (HR 0.54, 95% CI 0.41-0.71, $P < 0.001$).[15] Other phase III trials for EC are ongoing in the first line with pembrolizumab (NCT05173987), atezolizumab (NCT03603184), durvalumab (NCT04269200), and pembrolizumab/lenvatinib (NCT03884101).

Although the effect of immunotherapy has been changing in the MSI-H patients, more data are needed for MSS cases that represent a more heterogeneous disease. In particular, no data on the efficacy of checkpoint inhibitors are available, both in first line and recurrence, according to the molecular profile and main biological features of the tumors.

Here, we report the results of a translational analysis of the randomized phase II MITO END-3 trial comparing carboplatin/paclitaxel with carboplatin/paclitaxel plus avelumab according to the main molecular biomarkers.

PATIENTS AND METHODS

Study design and participants

MITO END-3 is an academic, multicenter, randomized, phase II trial of avelumab plus carboplatin/paclitaxel versus carboplatin/paclitaxel as first-line treatment of patients with advanced (FIGO stage III-IV) or recurrent EC. The trial was promoted by the Istituto Nazionale per lo Studio e la Cura dei Tumori—IRCCS Fondazione G. Pascale (Naples, Italy) and involved 31 centers in Italy. The protocol was approved by ethics committees at each participating institution and is available online. The study was carried out in accordance with the ethical principles of the Declaration of Helsinki, which are consistent with the International Conference on Harmonisation/Good Clinical Practice and applicable regulatory requirements. An independent data monitoring committee oversaw the study. Molecular characterization of formalin-fixed paraffin-embedded (FFPE) tumor samples from primary surgery, according to TCGA classification, detected by next-generation sequencing (NGS), was added by a protocol amendment approved as of May 2022.

All patients provided informed consent before the initiation of trial procedures. The trial is registered as NCT03503786 and EudraCT 2016-004403-31.

Eligible patients were at least 18 years old with histologically confirmed advanced (stage III-IV) or recurrent EC, an Eastern Cooperative Oncology Group performance status (PS) of 0-1, and no previous systemic anticancer therapy as primary treatment for advanced or metastatic disease.

Patients were required to have measurable disease as per the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) and availability of an FFPE tumor tissue sample for biomarker analyses.

The main exclusion criteria were sarcoma and carcinosarcoma histology, an active autoimmune disease that might deteriorate when receiving an immune-stimulatory agent (excluding diabetes type I, vitiligo, psoriasis, hypothyroidism or hyperthyroidism not requiring immunosuppressive treatment), major organ dysfunction, and concomitant pathologies or medications that prevented or contraindicated the use of study drugs.

In the standard arm, patients received carboplatin (area under the concentration—time curve 5 mg·min/ml) plus paclitaxel (175 mg/m^2) intravenously every 3 weeks for six to eight cycles. In the experimental arm, avelumab (10 mg/kg intravenously) was added to each administration of the same chemotherapy planned in the standard arm and was given every 2 weeks as a single agent as maintenance treatment after the end of chemotherapy until disease progression or unacceptable toxicity.

Archival FFPE tumor tissue samples were collected before randomization. A 5-μm section was cut from each

FFPE block, stained with hematoxylin—eosin, and quality control was carried out at the National Cancer Institute of Naples.

The adequate tumor blocks were tested with the NGS platform FoundationOne CDX 150 (Foundation Medicine®, Cambridge, MA). Pathogenic alterations and variants of unknown significance (VUS), tumor mutational burden (TMB) high versus low, and MSI status were analyzed. If the FoundationOne test failed to detect MSI status, Idylla™ MSI Test CE-IVD (Biocartis, Mechelen, Belgium) was carried out according to the manufacturer's protocol at the National Cancer Institute of Naples.

Programmed death-ligand 1(PD-L1) expression was determined by combined positive score (CPS) in a central laboratory using the analytically and clinically validated approved immunohistochemical (IHC) 22C3 PharmaDx assay (Agilent, Milan, Italy). Cases with CPS $\geq$1% were defined as positive.

In addition, 100 FFPE endometrial tissue samples were analyzed in IHC to determine lymphocyte infiltration. Using prediluted monoclonal antibodies against CD8 (rabbit, clone SP57, Roche Tissue Diagnostics, Oro Valley, AZ) and CD3 (rabbit, clone 2GV6, Roche Tissue Diagnostics, Oro Valley, AZ), an automated IHC analysis was carried out using the BenchMark XT (Roche Tissue Diagnostics, Oro Valley, AZ). The slides were stained following the Ventana protocol using the OptiView DAB IHC Detection Kit (Roche Tissue Diagnostics, Oro Valley, AZ). The stained slides were observed by light microscopy (Leica ICC50W) and scored by two expert pathologists twice with a washout period of 3 days. For tumor-infiltrating lymphocyte (TIL) evaluation, at the moment, only breast cancer is available as a consensus recommendation, and we refer to it.[16] The degree of lymphocytic infiltration was reported as the proportion of positively stained cells in the slides; large areas of central necrosis or fibrosis were not included in the evaluation. Specifically, we separated the evaluation of the TILs (intratumoral areas) from the lymphocytes of peritumoral areas (PTLs) within the tumor border (peritumoral areas).

Outcomes

PFS was defined as the time from randomization to the first occurrence of either disease progression or death from any cause.

OS was defined as the time from randomization to death or the last follow-up date for patients who were alive.

Translational analyses aimed to describe the prognostic effect and the interactions with the treatment of MSI status and NGS-defined molecular subtypes in terms of PFS and OS.

Statistical analysis

Analyses were carried out with data available as of 25 March 2022. Patients' characteristics at baseline were compared between treatment arms using chi-square or Fisher's exact test or Wilcoxon rank test as appropriate.

Microsatellite (MS) status, gene mutations, TMB, TILs, and PTLs were tested for their association with baseline patients' characteristics and TCGA categories using chi-square test for categorical variables and Wilcoxon rank test for continuous variables.

The association between the objective response and the same variables was tested within logistic regression models, incorporating histology (papillary serous carcinoma and clear-cell carcinoma versus others), disease stage (advanced versus recurrent), and other biomarkers eventually found to be associated with univariate analysis as covariates. Interactions between biomarkers and the treatment arm on the objective response were tested, and forest plots were used to show the results.

The visualization and summarization of pathogenic mutations, as well as the analysis of variants by NGS, were carried out by using waterfall plots for those patients for whom allelic frequencies were available.

Prognostic and predictive effects on both PFS and OS were tested for gene alterations that were found at NGS in at least 30% of cases.

PFS and OS curves were estimated with the Kaplan—Meier method. Curves were compared using the log-rank test. Medians are reported with 95% CIs, and the significant threshold for a two-sided P value is 0.05.

HRs were estimated with a Cox regression model stratified by histology (papillary serous carcinoma and clear-cell carcinoma versus others) and stage of disease (advanced versus recurrent) according to study protocol. The Schoenfeld residuals test was used to verify the proportional-hazard assumption.

First-order interaction between the treatment arm and MSI status was tested by the likelihood ratio test of the two nested models, with and without interaction. The heterogeneity of treatment effect across molecular subgroups was described in a forest plot for both PFS and OS.

In a similar way, interactions between the treatment arm and the more frequent gene alterations were tested, both in the overall population and in the MSS subgroup.

All the analyses were carried out with STATA 14 MP (StataCorp 2015, Stata Statistical Software: Release 14, StataCorp LP, College Station, TX) and using R version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org).

RESULTS

Characteristics of patients and samples

As reported in Figure 1, out of 166 patients enrolled and 125 analyzed in the primary analysis, 109 had a block available for NGS analysis and were successfully analyzed for mutations. In 15 of these cases, MS status was not reported because of insufficient material or other technical reasons. In 8 of these 15 patients, the MS status was determined with the Idylla™ MSI Test. Therefore, MS status was available for 102 patients.

Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2024.04.007, shows the main characteristics of the 109 patients in the NGS analysis compared to the primary analysis. Treatment groups were balanced for
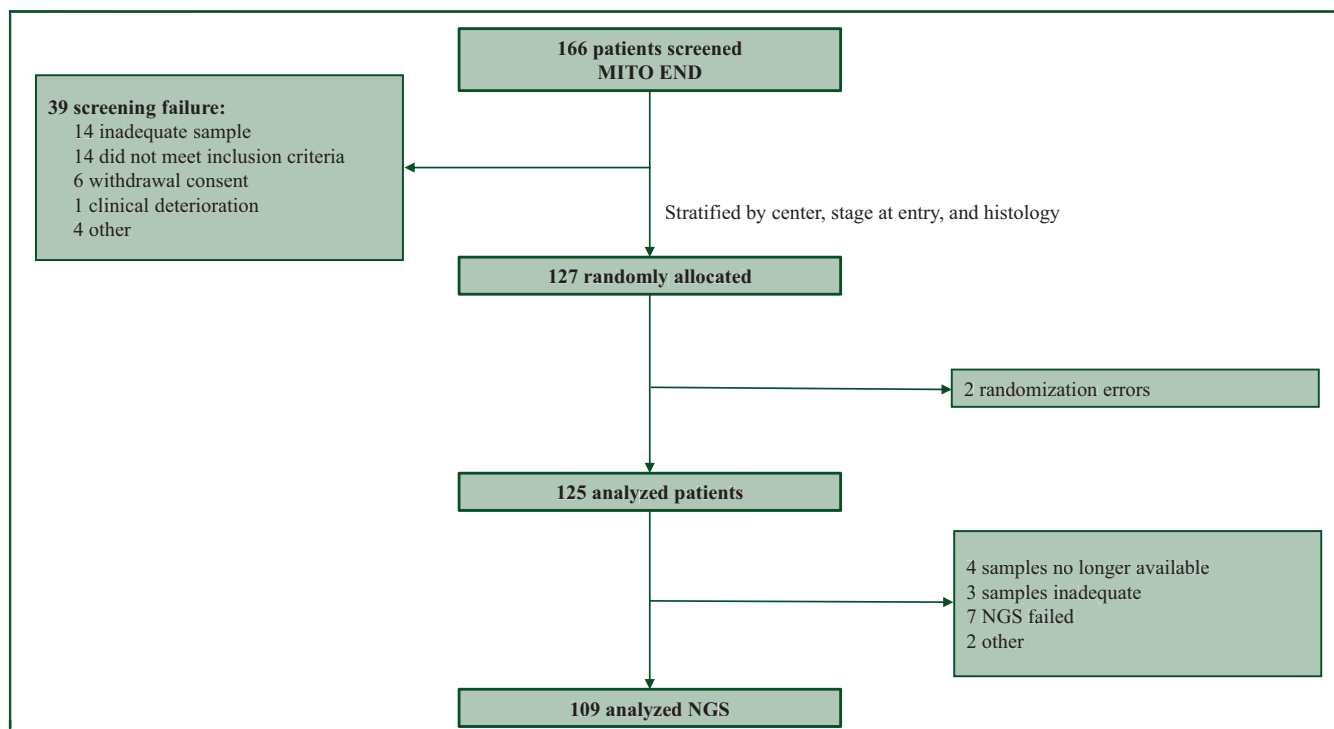
Figure 1. Flow chart of the study population.
NGS, next-generation sequencing.

known prognostic factors, although small differences were observed for grading and PS.

Molecular subtypes and genomic alterations

Patients with MS status available were categorized into TCGA molecular subtypes according to the pathogenic mutations found: 1 patient showed a *POLE* mutation (*POLE*, ultramutated); 29 patients showed MSI (MSI-H, unstable/hypermutated); 26 patients were MS stable and *TP53* wild type (MSS/*TP53* wt, copy number low); and 47 patients were MS stable *TP53* mutated (MSS/*TP53* mut, copy number high).

In the 109 patients analyzed by NGS, pathogenic mutations were found in 169 genes. Figure 2 shows the overall molecular findings according to the NGS analysis considering only the pathogenic mutations. Supplementary Figure S1, available at https://doi.org/10.1016/j.annonc.2024.04.007, shows pathogenic mutations and VUS (both figures include 107 patients for whom allelic frequencies were available).

The four most frequently mutated genes, present in >30% of cases, were *TP53*, *PIK3CA*, *ARID1A*, and *PTEN*. Of note, 11 out of 109 patients (10%) were found with a *BRCA1* or *2* mutation (5 in MSI-H and 6 in MSS). The distribution of such mutations by baseline characteristics of patients is presented in Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2024.04.007.

As shown in Table 1, mutations and positive CPS scores for PD-L1 expression were observed in all three TCGA categories. A higher number of intratumoral- and peritumoral-infiltrating lymphocytes were observed in MSI-H compared to the other categories. TMB ($\geq$10 muts/Mb) was observed

in all MSI-H patients, in 4 out of 47 MSS/*TP53* mut, and in no case in the MSS/*TP53* wt category.

Regarding *TP53*, the majority (65%) of *TP53* mutations were missense mutations: 34 patients had *TP53* missense mutations, 7 patients had nonsense mutations, 3 patients had a deletion, 3 patients had a splice site mutation, and 6 patients had a frameshift mutation. Of note, more than one pathogenic mutation was found in four patients. The overall details of the *TP53* mutations found are shown in Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2024.04.007. The distribution of TMB, MS, TILs, and PTLs by baseline characteristics of patients is presented in Supplementary Tables S4 and S5, available at https://doi.org/10.1016/j.annonc.2024.04.007, respectively.

Prognostic value

The distribution of the most frequent mutation, TMB, MS, TILs, and PTLs according to the best response by the treatment arm is presented in Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2024.04.007. As shown in Supplementary Table S7, available at https://doi.org/10.1016/j.annonc.2024.04.007, no associations were found between response and mutational status, TMB, MSI, TILs, and PTLs. The only interaction between the treatment arm and the response was found for TMB ($P =$ 0.030, Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2024.04.007).

As shown in Table 2, *TP53* (mutated versus wt; HR 2.16, 95% CI 1.34-3.47, $P =$ 0.002) and *PTEN* (mutated versus wt; HR 0.61, 95% CI 0.38-0.97, $P =$ 0.038) were prognostic in terms of PFS and only *TP53* in OS (HR 2.32, 95% CI 1.14-4.71, $P =$ 0.02). According to TCGA classification, as
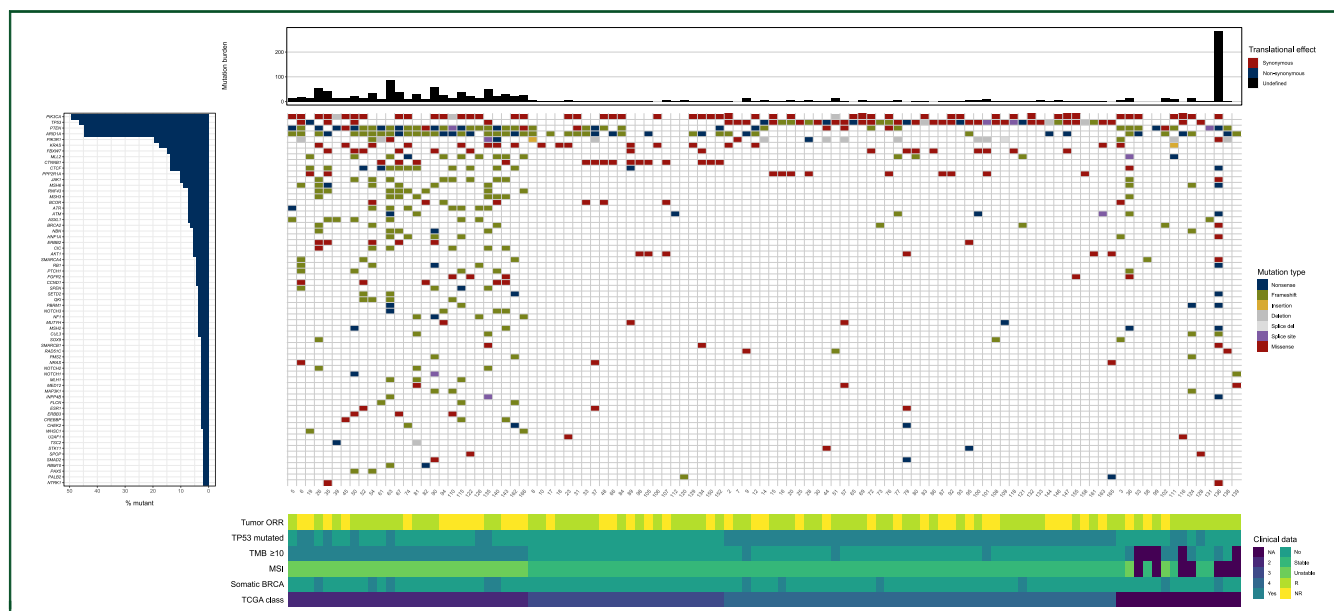
Figure 2. Waterfall plot of pathogenetic mutations found in 107 patients analyzed by next-generation sequencing. The figure shows the type and frequency of all pathogenetic mutations found. Furthermore, each patient is represented by TMB (upward in the figure), tumor ORR, *TP53* mutated, TMB ≥10 muts/Mb, MSI, somatic *BRCA*, TCGA class (down in the figure).
MSI, microsatellite instability; NA, not available; NGS, next-generation sequencing; NR, non-responders; ORR, objective response rate; R, responders; TCGA, The Cancer Genome Atlas; TMB, tumor mutational burden.
1: *POLE* (DNA Polymerase Epsilon, Catalytic Subunit) = ultramutated; 2: MSI-H = microsatellite instability-high; 3: MSS/*TP53* wt = microsatellite stable and *TP53* wild characteristics type; 4: MSS/*TP53* mut = microsatellite stable and *TP53* mutated.

expected, the MSI-H subgroup had the best prognosis compared to the other two categories although it was not statistically significant.

Predictive value

The interaction of TCGA categories with the effect of avelumab was tested in 102 out of 103 patients with TCGA classification, excluding the case with *POLE* mutation.

Kaplan–Meier PFS and OS curves according to TCGA categories are shown in Figure 3; avelumab effect was only evident in the MSI-H subgroup, while a worse outcome was observed in the MSS/*TP53* mut subgroup. Figure 4 shows a statistically significant interaction with treatment for PFS (*P* interaction = 0.003) and a non-significant interaction for OS in the 102 patients analyzed by TCGA categories (*P* interaction = 0.069).

The interaction of the four more frequent mutations (*TP53*, *PIK3CA*, *PTEN*, *ARID1A*) with treatment effect was analyzed in 109 patients. Figure 4 shows that *ARID1A* and *PTEN* had a statistically significant interaction with the treatment effect. Avelumab improved PFS in the presence of the mutation of *ARID1A* and *PTEN* (*P* interaction = 0.01 and *P* interaction = 0.002, respectively), while a significant interaction was found only in *PTEN* for OS (*P* interaction = 0.026). Kaplan–Meier PFS and OS curves showing treatment effect according to *ARID1A*, *PTEN*, and *PIK3CA* mutation are reported in Supplementary Figures S3-S8, available at https://doi.org/10.1016/j.annonc.2024.04.007, respectively.

An exploratory analysis was carried out in 73 patients with MSS to check whether any molecular variable was associated with the treatment effect. As shown in

Supplementary Figure S9, available at https://doi.org/10.1016/j.annonc.2024.04.007, an interaction with treatment effect in relation to PFS was observed only for *TP53* (*P* interaction = 0.041), with no statistically significant findings for OS.

DISCUSSION

This pre-specified analysis of the MITO END-3 trial shows significant interactions of avelumab efficacy with the molecular characteristics of the collected tumor samples. Patients with MSI-H largely benefit from the addition of avelumab to chemotherapy (concomitant and then as maintenance), while in patients with MSS tumors, the presence of *TP53* mutation is not associated with a benefit from the experimental treatment.

Immunotherapy with avelumab,[13] dostarlimab,[14] and pembrolizumab[15] has recently been shown to prolong PFS when added to first-line chemotherapy in advanced or recurrent EC. These results are going to change the standard of care for this disease. While avelumab was effective only in MSI-H patients,[13] dostarlimab and pembrolizumab also showed efficacy in the MSS population.[14,15] However, the results of these trials indicate that contrary to what has been observed in MSI-H, the results in the MSS population are still far to be considered optimal and new biomarkers need to be investigated to better select the patients who would benefit from immunotherapy.

MSS patients are a heterogeneous subgroup of patients: single-agent immunotherapy trials reported up to 15% response rate in MSS patients, thus supporting the necessity to identify predictive biomarkers of response/resistance in this setting.[6]

Table 1. Microsatellite instability, tumor mutational burden, combined positive score for PD-L1 expression, the four more frequently mutated genes, and TILs and PTLs according to TCGA classification

	TCGA (n = 102), n (%)/median (IQR)		
	MSI-H (n = 29)	MSS/TP53 wild type (n = 26)	MSS/TP53 mutant (n = 47)
TMB ≥10	29 (100)	0 (0)	4 (9)
Missing	0 (0)	2 (8)	0 (0)
PD-L1 CPS			
<1	16 (55)	13 (50)	32 (68)
≥1	13 (45)	11 (42)	14 (30)
Missing	0 (0)	2 (8)	1 (2)
Type of TP53 mutation[a]			
Missense	5 (17)	0 (0)	29 (62)
Nonsense	1 (3)	0 (0	6 (13)
Deletion	0 (0)	0 (0%)	3 (6)
Splice site	0 (0)	0 (0)	3 (6)
Frameshift	0 (0)	0 (0)	6 (13)
PIK3CA mutation[b]	22 (76)	11 (42)	21 (45)
ARID1A mutation[c]	26 (90)	12 (4)	7 (15)
PTEN mutation[d]	27 (93)	9 (35)	10 (21)
TILs CD8	17.5 (10-30)	5% (5-10)	5% (5-10)
PTLs CD8	20 (10-20)	10% (5-20)	10% (5-15)
TILs CD3	20 (10-30)	10% (10-20)	15% (10-20)
PTLs CD3	20 (15-35)	20% (12.5-30)	15% (10-20)

The median degree of lymphocytic infiltration is expressed as a percentage.
CPS, combined positive score; IQR, interquartile range; MSI-H, microsatellite instability-high; MSS, microsatellite stable; PD-L1, programmed death-ligand 1; PTLs, peritumoral lymphocytes; TCGA, The Cancer Genome Atlas; TILs, tumor-infiltrating lymphocytes; TMB, tumor mutational burden.
[a]Two different TP53 mutations were found in the same patient in four cases: three patients had two missense mutations, one patient had both one deletion and one splice site mutation. One case with a TP53 mutation is not included in the table because MSI was not available.
[b]More than one PIK3CA mutation was found in 12 patients.
[c]More than one ARID1A mutation was found in 23 patients.
[d]More than one PTEN mutation was found in 20 patients.

The new classification of EC according to TCGA has significantly changed the treatment of the disease in the early setting. The new classification has been incorporated in the risk classification after surgery being part of the decisional algorithm.[3] In particular, the presence of a POLE mutation downgrades the risk of recurrence, while the mutation of TP53 upgrades the risk and prompts a more aggressive adjuvant therapy.[3] In the copy number-high subgroup, according to TCGA, a high frequency of TP53 mutation (85%), serous morphology (73.3%), older age, a more advanced stage, and poor prognosis have been observed.[16,17] A p53-abnormal signature is also present in the vast majority of carcinosarcomas (73.9%),[18] and almost half of clear-cell EC (42.5%).[19,20]

In advanced EC, few data are available on the response to treatment according to molecular data, particularly when immunotherapy is used. A preliminary result of the GARNET study in the second-line setting, with dostarlimab, showed fewer responses to the drug in patients carrying TP53 mutation.[21] To our knowledge, no data are available in the first line with regard to immunotherapy efficacy.

In our trial, we found that TP53, PIK3CA, ARID1A, and PTEN were the mutations more frequently identified. PIK3CA, ARID1A, and PTEN mutations were observed in all TCGA categories. Furthermore, TP53, PIK3CA, and PTEN were prognostic for PFS when considering the entire population.

We found a statistically significant interaction of TP53 mutation with avelumab treatment, suggesting that TP53 mutations can be associated with resistance to immunotherapy. Apparently, a detrimental effect was observed in this study, even if data need to be taken with caution because of the small number of patients. Some mechanisms that might explain the latter finding can be hypothesized but need to be verified. Kim et al.[22] recently showed that some TP53 mutations seem to be related to a worse prognosis and shorter PFS and OS in patients with different types of metastatic solid tumors and treated with both chemotherapy and immunotherapy. One described mechanism of primary resistance to immunotherapy, called hyperprogression, has been potentially related to the p53 pathway.[23] In fact, existing studies suggest that amplification of the murine double minute (MDM) 2/4 gene is associated with the development of hyperprogression.[23-25] Overexpression of MDM2, as a ubiquitin ligase, can perturb the regulation of TP53 wt, impede the transcriptional activation domain of a TP53 gene, and lead to TP53 inactivation by reducing of ubiquitin-dependent p53 protein by proteases.[24,25]

Also, some studies indicate that loss of p53 function in cancer cells has effects on the tumor immune microenvironment, allowing them to escape from the immune attack.[26,27] The pathway of p53 also has a crucial role in

Table 2. Prognostic value of The Cancer Genome Atlas, microsatellite instability, and the more frequently mutated genes on progression-free survival and overall survival

	PFS (events: 79/109)			OS (events: 39/109)		
	HR	(95% CI)	P value	HR	(95% CI)	P value
TCGA (MSS/TP53 wt versus MSI-H)	1.26	(0.65-2.47)	0.493	0.60	(0.20-1.78)	0.358
TCGA (MSS/TP53 mut versus MSI-H)	1.81	(0.98-3.34)	0.058	2.04	(0.94-4.46)	0.073
Microsatellite instability (MSI-H versus MSS)	0.65	(0.37-1.14)	0.129	0.72	(0.40-1.52)	0.388
TP53 (mutated versus wild type)	**2.16**	**(1.34-3.47)**	**0.002**	**2.32**	**(1.14-4.71)**	**0.020**
PIK3CA (mutated versus wild type)	0.66	(0.42-1.05)	0.077	1.02	(0.52-2.03)	0.944
ARID1A (mutated versus wild type)	0.72	(0.45-1.15)	0.175	1.13	(0.60-2.16)	0.692
PTEN (mutated versus wild type)	**0.61**	**(0.38-0.97)**	**0.038**	0.69	(0.35-1.33)	0.264

CI, confidence interval; HR, hazard ratio; MSI-H, microsatellite instability-high; MSS, microsatellite stable; OS, overall survival; PFS, progression-free survival; TCGA, The Cancer Genome Atlas.
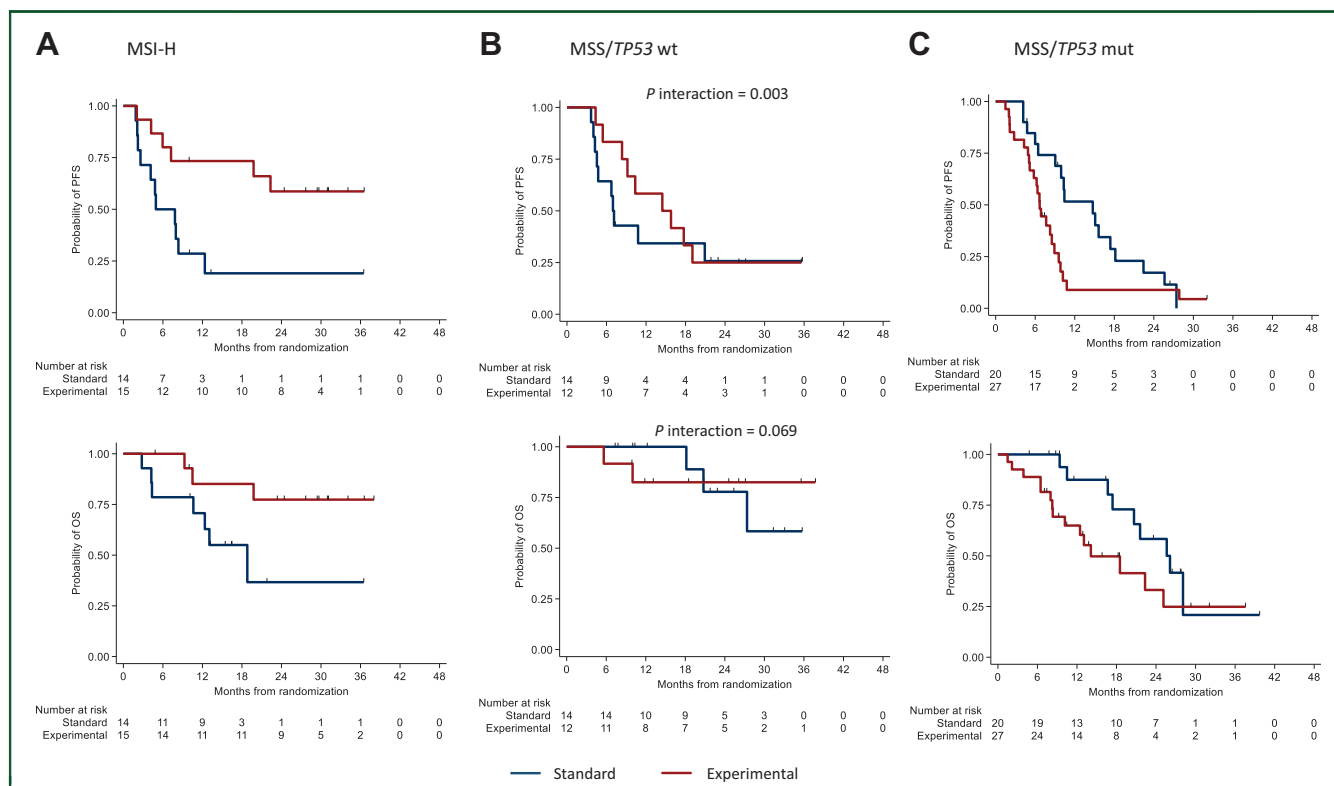Significant statistically P values are reported in bold.

Figure 3. Kaplan—Meier curves of the effect of treatment on progression-free survival (upper panel) and overall survival (lower panel) according to TCGA categories. (A) MSI-H; (B) MSS/TP53 wt; (C) MSS/TP53 mut. Experimental arm: avelumab plus CBDCA and PTX; standard arm: CBDCA plus PTX. Vertical black lines represent censoring.

CBDCA, carboplatin; MSI-H, microsatellite instability-high; MSS/TP53 mut, microsatellite stable and TP53 mutated; MSS/TP53 wt, microsatellite stable and TP53 wild type; PTX, paclitaxel; TCGA, The Cancer Genome Atlas.

regulating M2 macrophage polarization.[27] An analysis of TCGA has recently shown that ovarian high-grade serous carcinoma patients with mutant TP53 had significantly higher macrophage infiltration than those with TP53 wt ($P < 0.05$) and a worse prognosis associated.[28]

Despite the not fully clear mechanisms of the poor efficacy of immunotherapy in p53-mutant EC patients enrolled in our study, these patients show a complex molecular profile that includes several potentially targetable mutations. We have observed PIK3CA mutations in 44% of the MSS patients and 44 of the TP53-mutated cases. In addition, PTEN and ARID1A mutations have been observed in 21% and 14% of the TP53-mutated cases, respectively. These data confirm that other targetable mutations are present in MSS EC patients, thus suggesting that new target agents or combinations of these drugs with immunotherapy could be explored in this disease. Target-based therapy is under investigation in recurrent EC with drugs affecting the PIK3CA and PTEN pathways (EndoMAP trial NCT04486352).

In conclusion, we found that avelumab was ineffective in patients carrying a mutation of TP53. MSS patients, including those TP53 mutated, show several different targetable mutations, suggesting that new target agents can be investigated in first-line therapy of advanced EC.
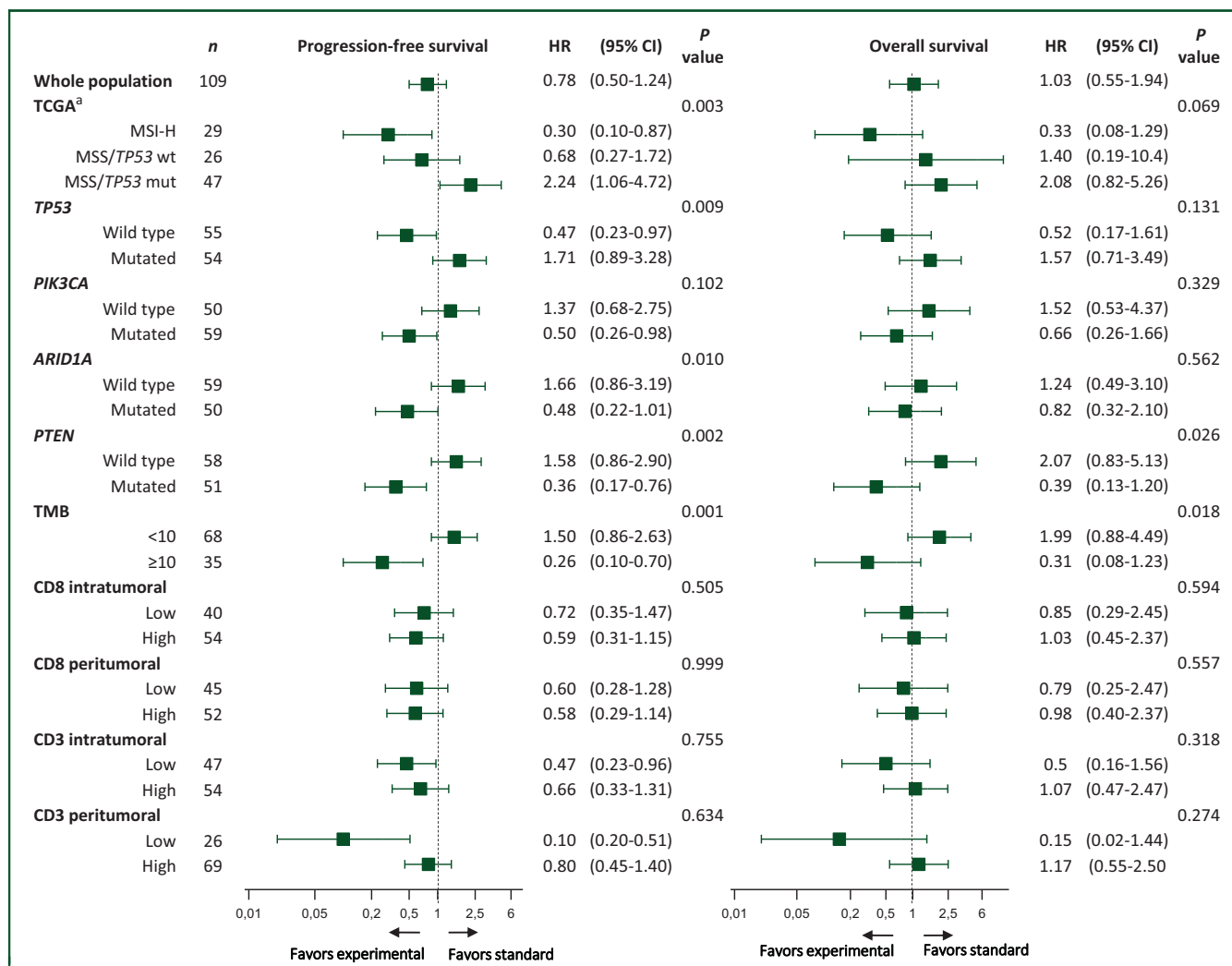
FUNDING

DISCLOSURE

Figure 4. Forest plots of the treatment effect on PFS (left) and OS (right) according to TCGA, most frequently mutates genes, TMB, TILs, and PTLs. Experimental arm: avelumab plus CBDCA and PTX; standard arm: CBDCA plus PTX. The dashed vertical line shows the HR in the overall population.

CBDCA, carboplatin; CI, confidence interval; HR, hazard ratio; MSI-H, microsatellite instability-high; MSS/*TP53* mut, microsatellite stable/*TP53* mutated; MSS/*TP53* wt, microsatellite stable and *TP53* wild type; OS, overall survival; PFS, progression- free survival; PTLs, peritumoral lymphocytes; PTX, paclitaxel; TCGA, The Cancer Genome Atlas; TILs, tumor-infiltrating lymphocytes; TMB, tumor mutational burden.

[a]Seven patients had missing information on TCGA and MSI status.

ROLE OF THE FUNDER

Pfizer had no role in study design, protocol writing, data collection, data analysis, data interpretation, and writing of the report. There was no professional medical writer. The corresponding author had final responsibility for the decision to submit.

DATA SHARING

The core dataset from this study is available online (https://doi.org/10.5281/zenodo.10605957). Full data will be shared upon publication after a reasonable request to the corresponding author. Some deidentified individual participant data (i.e. baseline patient characteristics, treatment data, safety data, and follow-up data) will be available for sharing, with no time limit.

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