Supplemental Online Content

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This supplemental material has been provided by the authors to give readers additional information about their work.

eMETHODS

Study Design and Procedures

The primary objective of this investigator-initiated phase 2 study was to evaluate the activity of the PARPi talazoparib plus the anti-PD-L1 antibody avelumab as determined by the frequency of patients who survived progression-free for at least 6 months (PFS6) after initiating protocol therapy or had objective response (OR) by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. Secondary endpoints included duration of progression-free survival (PFS), overall survival (OS), and nature and degree of toxicity of avelumab and talazoparib. Avelumab was administered on an outpatient basis at 10 mg/kg intravenously every 2 weeks in combination with talazoparib 1 mg PO daily continuously until progression or unacceptable toxicity. Patients with moderate renal impairment (defined as an estimated creatinine clearance of 30-59 mL/min) received a reduced starting dose of talazoparib at 0.75 mg PO daily. The clinical trial was approved by the institutional review boards of all participating institutions (NCT02912572). This investigator-initiated study (IND holder PK) was funded by Pfizer, as part of an alliance between Pfizer and the healthcare business of Merck KGaA, Darmstadt, Germany, which also provided avelumab and talazoparib. Full protocol is included in the supplement (our protocol was recently amended to also include evaluation of an additional immunotherapy combination (avelumab/axitinib) in a separate cohort of patients with MMRP EC (this cohort is currently enrolling)).

Eligibility

Eligible participants had recurrent or persistent EC of any histology that was MMRP as determined by normal immunohistochemical nuclear expression of mismatch repair (MMR) genes *MSH2, MSH6, MLH1* and *PMS2*. Other eligibility criteria included measurable disease by RECIST 1.1, no upper limit of prior therapies but ≥1 prior chemotherapeutic regimen, ECOG performance status of ≤1, availability of a formalin fixed paraffin embedded (FFPE) block of cancer tissue and normal organ and marrow function. Key exclusion criteria included prior treatment with any ICI or PARPi, known brain metastases, systemic corticosteroids at physiologic doses exceeding 10 mg/day of prednisone or equivalent and active autoimmune disease.

Biomarker evaluation

Archival formalin-fixed paraffin embedded specimens were obtained from participating patients where available. Targeted panel next-generation sequencing (NGS) was performed using Dana-Farber Cancer Institute's OncoPanel platform as previously described[1-3]. For one patient, targeted NGS from Foundation Medicine was previously performed. Immunohistochemistry (IHC) was performed for CD4, CD8 and PD-L1 on FFPE samples.

Statistical Analysis

Statistical considerations were developed for co-primary objectives of objective response rate (ORR) and rate of PFS6, with a two-stage design that allowed for early stopping for

futility. A two-stage test was constructed using the method of Sill, Rubinstein, Litwin and Yothers[4] with the goal of stopping early for futility to limit patient exposure to an inactive agent while restricting the probabilities of type I and type II errors to approximately 10% and 15%, respectively. For the co-primary endpoints, a true ORR of 5% or less and a rate of PFS6 of 10% or less would not be of clinical interest (H0: $\pi OR \leq 5\%$ AND $\pi PFS6 \leq 10\%$), whereas an improvement to a 20% ORR or 30% PFS6 rate would warrant further investigation of avelumab. In the first stage, 16 patients would be enrolled. If ≥2 objective responses or ≥2 patients who were progression-free at 6 months were observed, accrual would continue to the second stage where an additional 19 patients would be enrolled. Overall, if ≥4 treated patients with an objective response or ≥8 patients who are progression-free at 6 months were observed, the null hypothesis would be rejected and avelumab/talazoparib would be considered worthy of further study.

ORR was defined to be the rate of achieving either complete or partial response by RECIST 1.1 among patients evaluable for response. The coprimary end point of PFS6 was defined to be a binary outcome indicating whether or not a patient was progression-free for at least 6 months. For both binary end points, the exact 95% CIs were estimated. In addition, Kaplan-Meier estimates of the progression-free survival function were estimated, which incorporated censored outcomes in the analysis. The pointwise 95% CI was constructed using Greenwood's formula for the variance estimate. Prespecified exploratory objectives included association of immunogenomic features with clinical activity. The statistical significance of biomarker associations with objective response and

clinical benefit were evaluated using Fisher's exact test or Mann Whitney U Test. Log rank test was used to test biomarker association with progression-free survival.

eRESULTS

Biomarker Analyses

Tumor immunogenomic profiling was performed to identify subsets of patients who may benefit from avelumab/talazoparib. Prespecified exploratory objectives included association of immune markers (PD-L1 status and presence of tumor infiltrating lymphocytes) and of specific genomic alterations (including HRR alterations) with response as measured by objective response or clinical benefit (i.e., meeting either objective response or PFS6).

PD-L1 status and presence of CD3+ and CD8+ tumor infiltrating lymphocytes (TILs) were assessed by immunohistochemistry in 34 patients. Based on a PD-L1 combined positive score (CPS) cutoff of 1, 15 (44.1%) of 34 tumors were PD-L1 positive; 11 (32.4%) had CPS ≥ 5 and 6 (17.6%) had CPS≥10. Regardless of the CPS cutoff, PD-L1 status was not associated with objective response or clinical benefit; similarly, there was no association when CPS was analyzed as a continuous variable (Supplementary Table 5). Furthermore, there was no statistically significant association observed between CD3+ or CD8+ TILs and clinical outcomes, measured either by objective response or clinical benefit (Supplementary Table 5).

Archival specimens from 29 patients were available for molecular characterization by targeted NGS using the OncoPanel platform (for one patient, targeted NGS from Foundation Medicine was available). Tumors from all 29 patients (which were MMRP by

IHC as required by eligibility) were all subsequently confirmed to be MMRP genomically by targeted NGS. No tumor was *POLE*-mutated. Tumor mutational burden (TMB) was not associated with response to avelumab/talazoparib measured either by objective response (8.6 mutations/Mb in responders vs 6.9 mutations/Mb in non-responders, p=0.489) or clinical benefit (8.4 mutations/Mb in those with clinical benefit vs 6.6 mutations/Mb in those without, p=0.241).

Twenty-one (72.4%) of 29 tumors were *TP53* mutated, 14 (48.3%) harbored PI3K pathway alterations, 9 (31%) had *CCNE1* amplification, 7 (24.1%) had SWI/SNF complex alterations (5 involving *ARID1A*, 1 SMARCAL1 and 1 SMARCA4) and 6 (20.7%) had HRR alterations involving *BRCA1* (n=1), *BRCA2* (n=1), *BRIP1* (n=1), *CDK12* (n=2) and *FANCA* (n=1). No statistically significant association was observed between *TP53* mutations, PI3K pathway alterations and *CCNE1* amplification with objective response or clinical benefit from avelumab/talazoparib (Supplementary Table 4). However, HRR pathway alterations were associated with clinical benefit from avelumab/talazoparib (83.3% of HRR altered tumors derived clinical benefit vs 17.4% of non HRR altered tumors, p=0.005) while SWI/SNF complex alterations were associated with a trend towards absence of clinical benefit (none of the tumors with SWI/SNF alterations derived clinical benefit vs 40.9% of those without SWI/SNF alterations, p=0.066), Supplementary Table 4. The median PFS of HRR altered tumors was 9.1 months vs 3.3 months in non HRR altered tumors, p=0.03.

Given that response to platinum is a well-established clinical surrogate of response to PARPi, we explored whether patients with a platinum-free interval (PFI, defined as the time between the last cycle of platinum and evidence of disease progression) of at least 6 months exhibited better outcome. As shown in Supplementary Table 4, PFI≥6 months was associated with clinical benefit from avelumab/talazoparib (55.6% of patients with PFI≥6 derived clinical benefit vs 15.4% of those with PFI<6 months, p=0.03); the median PFS of patients with PFI≥6 months was 9.1 months vs 3.3 months patients with PFI<6 months, p=0.009.

Overall, 7 of the 9 patients who derived benefit from avelumab/talazoparib exhibited PFI≥6 months and/or HRR alterations, Supplementary Table 4. Analysis of the 2 remaining patients (with PFI<6 months and no HRR alterations) who derived clinical benefit revealed that one patient harbored a hotspot mutation in the U2AF1 spliceosome gene; this tumor had an immune inflamed phenotype with high TILs and positive PD-L1 (CPS=30), Supplement Figure 1. The second patient who derived benefit had a low tumor mutational burden (3.8 mutations/MB), no HRR alterations but had an increased number (>20/HPF) of CD3+ and CD8+ TILs and a CPS of 6.

eTABLE 1. Demographic and Baseline Characteristics of Patients

	Number	Percent
Overall	35	100.0
Ethnicity[a]	23	65.7
Non-Hispanic		
Ethnicity Not Known	12	34.3
Race[b]		
White	18	51.4
Black or African American	5	14.3
Other	11	31.4
More than one race	1	2.9
Stage at diagnosis		
I	9	25.7
II	3	8.6
III	6	17.1
IV	17	48.6
Histology		
Endometrioid	11	31.4
Grade I	2	
Grade II	6	
Grade III	3	
Serous	12	34.3
Clear Cell	3	8.6
Carcinosarcoma	4	11.4
Mixed[c]	5	14.3
ECOG Performance Status		
0	14	40.0
1	21	60.0

[a] There were no Hispanic and no Latino patients.

[b] There were no Asian, Native Hawaiian or Other Pacific Islander, American Indian or Alaskan Native patients. The “Other” category reflects that the race was unknown for 11 patients.

[c] 1 Mixed endometrioid/clear cell, 3 Mixed endometrioid/serous and 1 Mixed serous/clear cell

eTABLE 2. Objective Response (confirmed) and PFS6

RESPONSE	Patients, No
	Overall (N=35)
Best Overall Response	
CR	0
PR	4 (11.4%) (2 endometrioid and 2 serous)
SD	20 (57.1%)
PD	9 (25.7%)
Not evaluable	2 (5.7%)
ORR, %	11.4 (3.2-26.7)
PFS6	
Yes	8 (22.9) (1 carcinosarcoma, 1 clear cell, 3 endometrioid, 3 serous)
No	27 (77.1)
PFS6, %	22.9 (10.4 - 40.1)

eTABLE 3. Treatment-Related Adverse Events (TEAEs) of any grade in ≥10% of patients and G3+ TEAEs in any patient

Adverse Events	Grade 1-2[a]	Grade 3[a]	Grade 4[a]
Anemia	9(26%)[b]	16(46%)	0(0%)
Platelet count decreased	15(43%)	7(20%)	3(9%)
Fatigue	12(34%)	4(11%)	0(0%)
Neutrophil count decreased	8(23%)	4(11%)	0(0%)
Hypothyroidism	4(12%)	0(0%)	0(0%)
Diarrhea	7(20%)	0(0%)	0(0%)
Nausea	12(35%)	0(0%)	0(0%)
Edema limbs	4(12%)	0(0%)	0(0%)
White blood cell decreased	4(12%)	0(0%)	0(0%)
Anorexia	5(14%)	0(0%)	0(0%)

[a] Maximum grade

[b] The denominator to all calculated percentages is N=35, the number of patients who received at least one dose of study drug

eTABLE 4. Associations Between Molecular Biomarkers and Clinical Activity, as Measured by Objective Response or Clinical Benefit

	Objective Response			Clinical Benefit		
Alteration	**No**	**Yes**	**p value**	**No**	**Yes**	**p value**
HRR pathway[a]			0.18			0.005
Absent (n=23)	21 (91.3%)	2 (8.7%)		19 (82.6%)	4 (17.4%)	
Present (n=6)	4 (66.7%)	2 (33.3%)		1 (16.7%)	5 (83.3%)	
SWI/SNF complex[a]			0.546			0.066
Absent (n=22)	18 (81.8%)	4 (18.2%)		13 (59.1%)	9 (40.9%)	
Present (n=7)	7 (100.0%)	0 (0.0%)		7 (100.0%)	0 (0.0%)	
CCNE1 amplification[a]			0.568			0.999
Absent (n=20)	18 (90.0%)	2 (10.0%)		14 (70.0%)	6 (30.0%)	
Present (n=9)	7 (77.8%)	2 (22.2%)		6 (66.7%)	3 (33.3%)	
PI3K pathway[a]			0.598			0.427
Absent (n=15)	12 (80.0%)	3 (20.0%)		9 (60.0%)	6 (40.0%)	
Present (n=14)	13 (92.9%)	1 (7.1%)		11 (78.6%)	3 (21.4%)	
TP53 mutations[a]			0.999			0.675
Absent (n=8)	7 (87.5%)	1 (12.5%)		5 (62.5%)	3 (37.5%)	
Present (n=21)	18 (85.7%)	3 (14.3%)		15 (71.4%)	6 (28.6%)	
Platinum Status[b]			0.268			0.03
PFI<6 months (n=26)	24 (92.3%)	2 (7.7%)		22 (84.6%)	4 (15.4%)	
PFI≥6 months (n=9)	7 (77.8%)	2 (22.2%)		4 (44.4%)	5 (55.6%)	
PFI≥6 months and/or HRR			0.279			0.014
HRR altered and/or PFI≥6 months (n=12)	9 (75.0%)	3 (25.0%)		5 (41.7%)	7 (58.3%)	
PFI<6 months and no HRR alterations (n=17)	16 (94.1%)	1 (5.9%)		15 (88.2%)	2 (11.8%)	

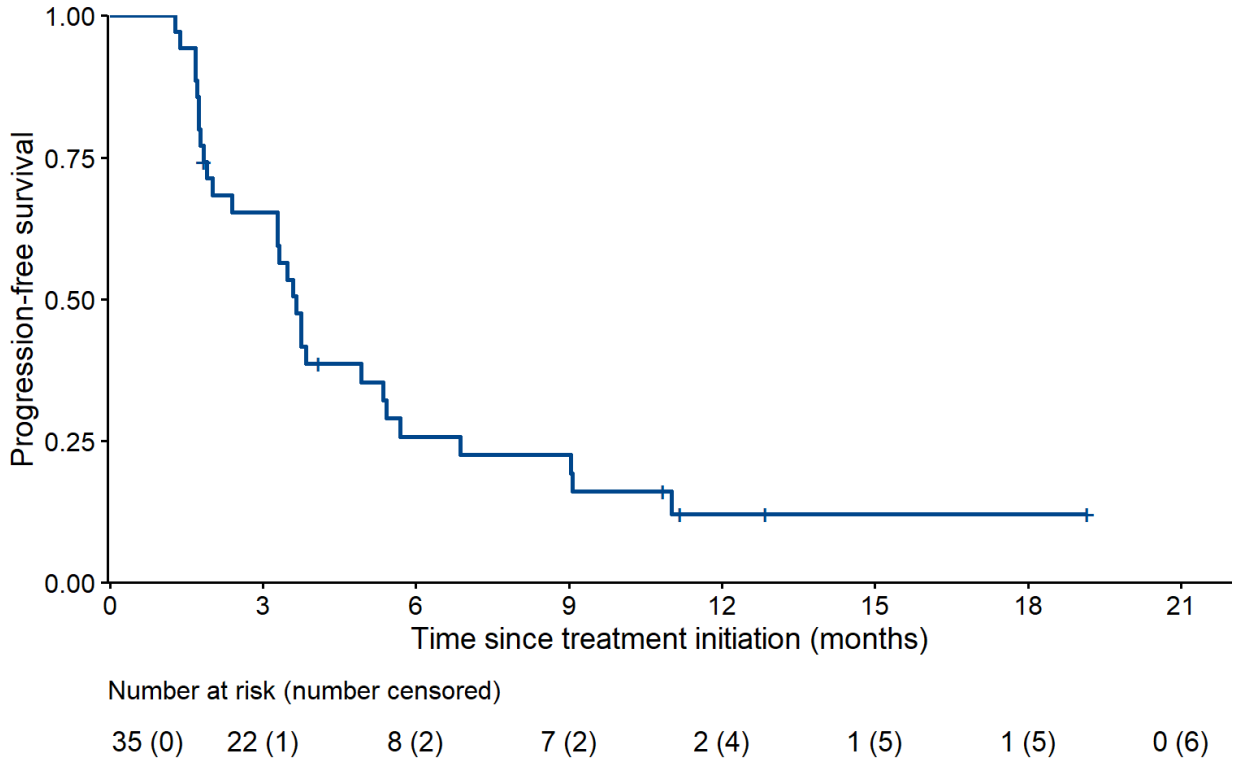
[a] Assessed in 29 patients whose tumors were profiled by targeted NGS

[b] Platinum status was available for all 35 patients

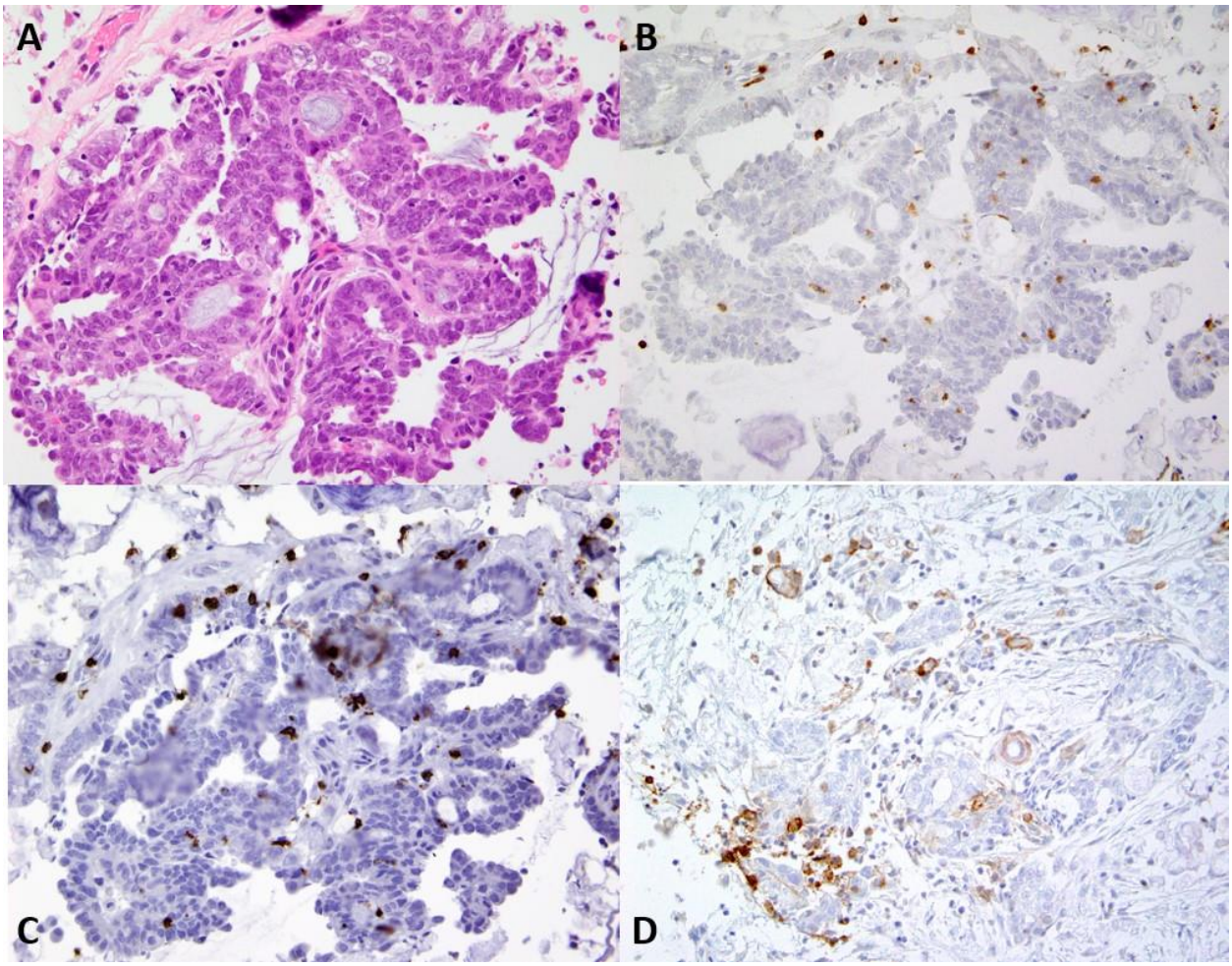
eTABLE 5. Associations Between Immune Biomarkers and Clinical Activity, as Measured by Objective Response or Clinical Benefit

	Objective Response			Clinical Benefit		
	No	**Yes**	**p value**	**No**	**Yes**	**p value**
CD8 Semiquantitative			0.999			0.644
+2/+3	6 (85.7%)	1 (14.3%)		6 (85.7%)	1 (14.3%)	
0/+1	24 (88.9%)	3 (11.1%)		19 (70.4%)	8 (29.6%)	
CD8 Count per HPF			0.437			0.969
Mean (SD)	8 (6.2)	19 (22.2)		8 (5.8)	12.7 (16.1)	
Median (Q1, Q3)	6 (3, 12)	12 (6.3, 24.8)		6 (3, 12)	8 (2, 16)	
CD3 Semiquantitative			0.283			0.704
+2/+3	11 (78.6%)	3 (21.4%)		11 (78.6%)	3 (21.4%)	
0/+1	19 (95.0%)	1 (5.0%)		14 (70.0%)	6 (30.0%)	
CD3 Count per HPF			0.592			0.531
Mean (SD)	16.1 (16.7)	20.8 (17.6)		17 (18.1)	15.9 (12.4)	
Median (Q1, Q3)	8.0 (6.3, 18.5)	17.5 (13.5, 24.8)		8 (6, 19)	17 (8, 18)	
CPS ≥ 1			0.999			0.999
Absent	17 (89.5%)	2 (10.5%)		14 (73.7%)	5 (26.3%)	
Present	13 (86.7%)	2 (13.3%)		11 (73.3%)	4 (26.7%)	
CPS ≥ 5			0.58			0.425
Absent	21 (91.3%)	2 (8.7%)		18 (78.3%)	5 (21.7%)	
Present	9 (81.8%)	2 (18.2%)		7 (63.6%)	4 (36.4%)	
CPS ≥ 10			0.559			0.306
Absent	25 (89.3%)	3 (10.7%)		22 (78.6%)	6 (21.4%)	
Present	5 (83.3%)	1 (16.7%)		3 (50.0%)	3 (50.0%)	
CPS continuous			0.659			0.699
Mean (SD)	9.3 (19.8)	16.5 (29.1)		9.2 (21.0)	12.7 (20.7)	
Median (Q1, Q3)	0 (0, 6)	3 (0, 19.5)		0 (0, 6)	0 (0, 18)	

eFIGURE 1. Progression Free Survival in all patients: Estimated median PFS was 3.6 months (95% CI: 2.4 to 5.4) and estimated PFS at 6 months was 25.8% (95% CI: 12.4% to 41.4%).



eFIGURE 2. IHC evaluation of the tumor with a hotspot mutation in the U2AF1 spliceosome gene: A) H&E stain, B) CD3+ TILs, C) CD8+ TILs and D) PD-L1.



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