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ORIGINAL RESEARCH

The impact of single agent PD-1 or PD-L1 inhibition on advanced endometrial cancers: meta-analysis

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Background: Immune checkpoint inhibitor (ICI) therapy is an emerging option for advanced endometrial cancer (EC). Mismatch repair (MMR) status is widely regarded as a biomarker predictive of response to ICIs. The predictive value of MMR based on small, single-arm trials, however, is conflicting. In this meta-analysis, we aimed to assess the activity of single-agent ICI in advanced EC, and compared the magnitude of treatment benefit in MMR deficient (dMMR) and MMR proficient (pMMR) EC.

Methods: We carried out an electronic search to identify prospective trials of single-agent ICI in advanced EC. Data on objective response rate (ORR) and progression-free survival (PFS) were extracted and pooled. ORR was estimated using the inverse variance method and subgroup difference by MMR status was examined. PFS difference according to MMR status was summarized using the Kaplan—Meier approach.

Results: From eight trials with 492 women, the pooled ORR was 19% [95% confidence interval (CI) 16% to 22%]. ORR was significantly greater in dMMR ($n = 281$) than pMMR EC ($n = 211$) (dMMR: 46%, pMMR: 8%; risk ratio 5.74, 95% CI 3.58-9.21; interaction $P < 0.001$). Complete response was 11% and 0.05% and median PFS was 8.3 and 2.1 months in dMMR and pMMR EC, respectively (hazard ratio PFS 0.58, 95% CI 0.38-0.89; $P = 0.01$). The 12-month PFS rates were 42.0% and 20.7%, respectively.

Conclusion: Single-agent ICI is associated with a 5.74 times greater objective response and 42% reduction in risk of disease progression or death in dMMR compared with pMMR EC. MMR status should be determined prospectively and be used as a stratification factor in future trials of advanced EC. Further translational analysis is urgently required to identify the cause of dMMR and allow subclassification of EC into different dMMR molecular subtypes.

Key words: mismatch repair, MMR, endometrial cancer, immunotherapy

INTRODUCTION

Women with advanced endometrial cancer (EC) have a poor prognosis with an estimated 5-year survival of 17%.[1] Following progression from first-line platinum-taxane chemotherapy, subsequent lines of chemotherapy have a response rate of only 20% or less.[2] Until recently, estrogen receptor expression has been used as a predictive biomarker to guide decision making about endocrine therapy, but systemic treatment approaches have otherwise been generally unselective.[3]

As with many other tumor types, there has been an evolutionary discovery of the use of immune checkpoint inhibitors (ICIs) in EC. The activity of pembrolizumab, an anti-programmed cell death protein 1 (PD-1) inhibitor, was demonstrated in DNA mismatch repair deficient (dMMR) tumor types in a study which included a small number of EC cases.[4] Up to 30% of ECs exhibit a microsatellite instability-high (MSI-H) phenotype, but they have different molecular driver pathways which can include somatic (sporadic) or germline (hereditary) causes.[5] MMR assessment using immunohistochemistry (IHC) has been thought to be analogous to microsatellite assessment. The majority of MSI-H ECs have acquired hypermethylation of the *MLH1* promoter region, resulting in downstream silencing of the *MLH1* gene. Other important but less common oncogenesis pathways include germline pathogenic mutations, or double

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somatic mutations, of one of the MMR genes (Lynch syndrome genes *MLH1*, *MSH2*, *MSH6* and *PMS2*), or of EpCAM (with downstream methylation of *MSH2*).[6] MSI-H is commonly associated with accumulated uncorrected errors within the repetitive DNA sequence during replication leads, resulting in high tumor mutational burden (TMB). As a consequence, there are increased levels of neopeptide antigen production, which potentially explains the observed peritumoral CD3+ and CD8+ T-cell responses.[7] ECs with high TMB and MSI-H have been reported to respond better to ICI therapy.[8]

In advanced EC, both single-agent ICI, and a combination of ICI with other therapies, have been tested in second-line or beyond settings. Many of these EC trials included both microsatellite stable (MSS)/MMR proficient (pMMR) and MSI-H/dMMR tumors. To date, KEYNOTE-775 is the only randomized, controlled trial (RCT) in advanced EC comparing pembrolizumab plus lenvatinib (an oral multikinase inhibitor) with chemotherapy following progression of at least one platinum-based chemotherapy regimen.[9] The trial showed that pembrolizumab plus lenvatinib led to significantly longer progression-free survival (PFS) and overall survival (OS) than did chemotherapy. Subgroup analysis showed that greater benefit was seen with pembrolizumab plus lenvatinib over chemotherapy in dMMR compared with pMMR EC. This trial, however, was neither designed nor powered to test for differences in this subgroup. Further, not all women could tolerate combination therapy with ICI and multikinase inhibitor, as there is significantly greater toxicity with the combination therapy compared with single-agent ICI.[9] Importantly, no RCT comparing single-agent ICI against chemotherapy has been reported, and the majority of the available data have been generated from small, single-arm studies. The predictive value of MMR status as a biomarker for ICI benefit remains conflicting and poorly defined in the clinical setting.

We carried out this meta-analysis with the aim of assessing the activity of single-agent ICI in advanced EC. In particular, we sought to better quantify and compare the magnitude of treatment benefit based on objective response rate (ORR) and PFS in dMMR and pMMR EC, respectively.

MATERIAL AND METHODS

Eligible studies were prospective trials that investigated the activity of single-agent ICI in advanced EC. Two authors (PSK and YCA) independently reviewed the eligibility of abstracts and published papers. We carried out electronic searches of MEDLINE, Embase and the Cochrane Central Register of Controlled Trials for studies registered between 1 January 2016 and 31 January 2022. The following terms were used: (advanced or metastatic) AND (endometrial/uterine) AND (cancer/neoplas*/tumo?r/carcinoma) AND (mismatch repair/MSI/microsatellite) AND (atezolizumab/avelumab/nivolumab/pembrolizumab/ipilimumab/tremelimumab/cytotoxic T lymphocyte antigen 4/CTLA4/programmed death-1/PD-1/programmed death-ligand 1/PD-L1). We also hand-searched conference abstracts, posters and presentations from websites of the American Society of Clinical Oncology, Society of Gynecologic Oncology and European Society of Medical Oncology. We excluded trials of combination therapies of ICI with other agents.

The primary endpoint was pooled ORR with secondary endpoints which included depth of response and PFS. We further evaluated differences between tumor subgroups based on MMR status. Pooled ORR treatment estimates and 95% confidence intervals (CIs) were calculated using the inverse variance method. A test for subgroup differences in ORR was carried out according to MMR status. We used the χ^2 Cochrane Q test and I^2 statistics to detect any heterogeneity across the different trials.

We also provided graphical displays to demonstrate differences in depth of response by MMR status. For each trial, we extracted the change in sum of target lesions from the waterfall plot(s) of each study. In trials where PFS Kaplan–Meier curves were available, we also extracted data coordinates from these published curves using the DigitizeIt program. Individual patient data were subsequently reconstructed using a method that was previously described.[10]

Risk of bias was assessed utilizing the Quality in Prognosis Studies tool in Review Manager version 5.3 (RevMan 5.3) software, by Cochrane Training.[11] All analyses were carried out with Stata statistical software (version 15; StataCorp).

RESULTS

We found 56 articles which included activity of ICI in dMMR EC. Of these, 48 were excluded (13 were mixed dMMR of endometrial and non-endometrial cancers, where ORR in EC could not be extracted; and 35 were duplicates). Where multiple presentations or publications occurred, only the latest data were included. Eight eligible trials tested single-agent ICI in the second-line and beyond setting, including only women with advanced EC (Figure 1).[8,12-20] Five trials tested treatment with pembrolizumab[4,8,14,18,19] and a single trial each tested treatment with durvalumab [anti-programmed death-ligand 1 (PD-L1) inhibitor],[13] avelumab (anti-PD-L1)[15] and dostarlimab (anti-PD-1).[17] The sample sizes of the trials included ranged from 9 to 103 women with dMMR EC, and 16 to 142 women with pMMR EC. Median follow-up of these trials ranged from 16.3 to 42.6 months. Methods used to classify MMR status include IHC ($n = 5$),[4,13,15,17,18] polymerase chain reaction ($n = 5$)[12,15,17-19] and next generation sequencing ($n = 3$).[8,15,17] For one trial, the method was not reported.[14] The characters of each trial are included in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2022.100635.

In eight trials with a total of 492 women, the pooled ORR for single-agent ICI therapy was 19% (95% CI 16% to 22%). With data available from seven trials involving 281 women with dMMR EC, the pooled ORR was 46% (95% CI 40% to 52%). There was no significant heterogeneity in the results across these trials ($I^2 = 0\%$, $P = 0.50$). From four trials involving 211 women with pMMR EC, the pooled ORR was

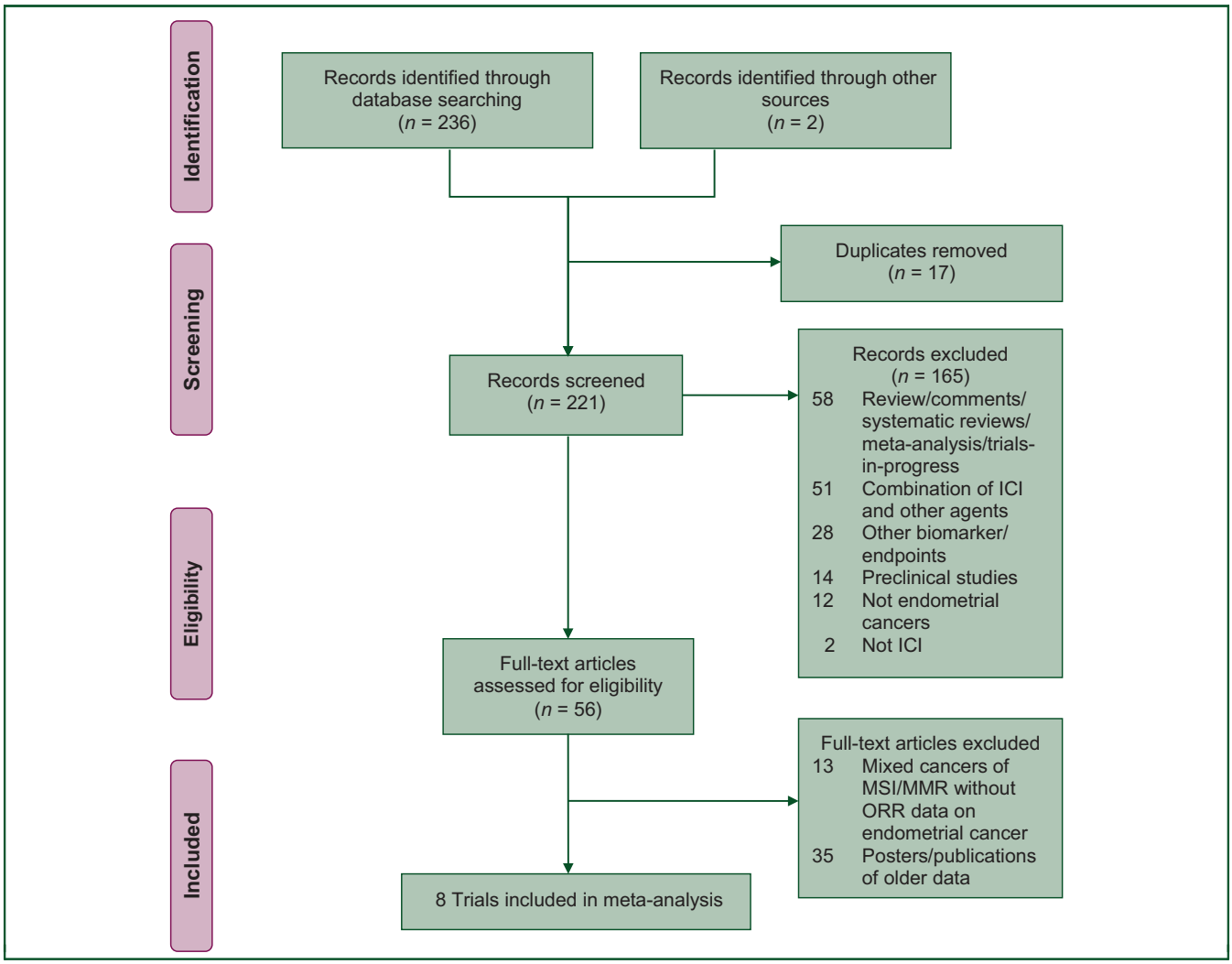

Figure 1. Flow diagram of study inclusion and exclusion.
ICI, immune checkpoint inhibitor; MMR, mismatch repair; MSI, microsatellite instability; ORR, objective response rate.

8% (95% CI 5% to 12%) ($I^2 = 60\%$, $P = 0.06$) (Figure 2). The difference in ORR between dMMR and pMMR was statistically significant ($P < 0.001$). dMMR EC had a 5.74 increase in chance of achieving an objective response than pMMR EC when treated with ICI (relative risk 5.74, 95% CI 3.58-9.21).

The waterfall plots showed deep responses in women with dMMR EC (Figure 3A). Significantly more complete responses were seen in dMMR than pMMR tumors (Figure 3B) (11% versus 0.05%).

PFS data were available from four trials with 149 women (dMMR 66%, pMMR 34%) with a total of 75% PFS events (dMMR 59%, pMMR 84%). The median PFS for all of these women was 2.9 months. The median PFS was significantly different for dMMR and pMMR subgroups, respectively: 8.3 versus 2.1 months, hazard ratio 0.58, 95% CI 0.38-0.89, $P = 0.01$ (Figure 4). The 12-month PFS rates were 42.0% and 20.7%, respectively.

All trials were single-arm, non-randomized trials and therefore had high risks of selection bias and performance bias. Overall, there were low to moderate detection, attribution and reporting bias (Figure 5).

DISCUSSION

In women with advanced EC, our meta-analysis has shown that single-agent PD-(L)1 inhibitor in second and subsequent lines was associated with only a modest ORR of 19% and a median PFS of 2.9 months. Single-agent PD-(L)1 inhibitor, however, is associated with a 5.74 times greater objective response and 42% reduction in risk of disease progression or death in dMMR EC compared with pMMR EC. Our data indicate that dMMR and pMMR ECs are distinct disease entities, with different prognoses when treated with single-agent ICI.

The significant differences in ORR and PFS between dMMR and pMMR EC when treated with single-agent ICI suggest that MMR status could predict treatment benefit from ICI therapy. We acknowledge that the predictive value of MMR status, however, could only be conclusively determined via (i) a prospectively conducted RCT comparing single-agent ICI versus non-ICI treatment and (ii) an adequately powered RCT to demonstrate difference in survival outcomes according to MMR status. Combination of pembrolizumab plus lenvatinib met the former criterion,

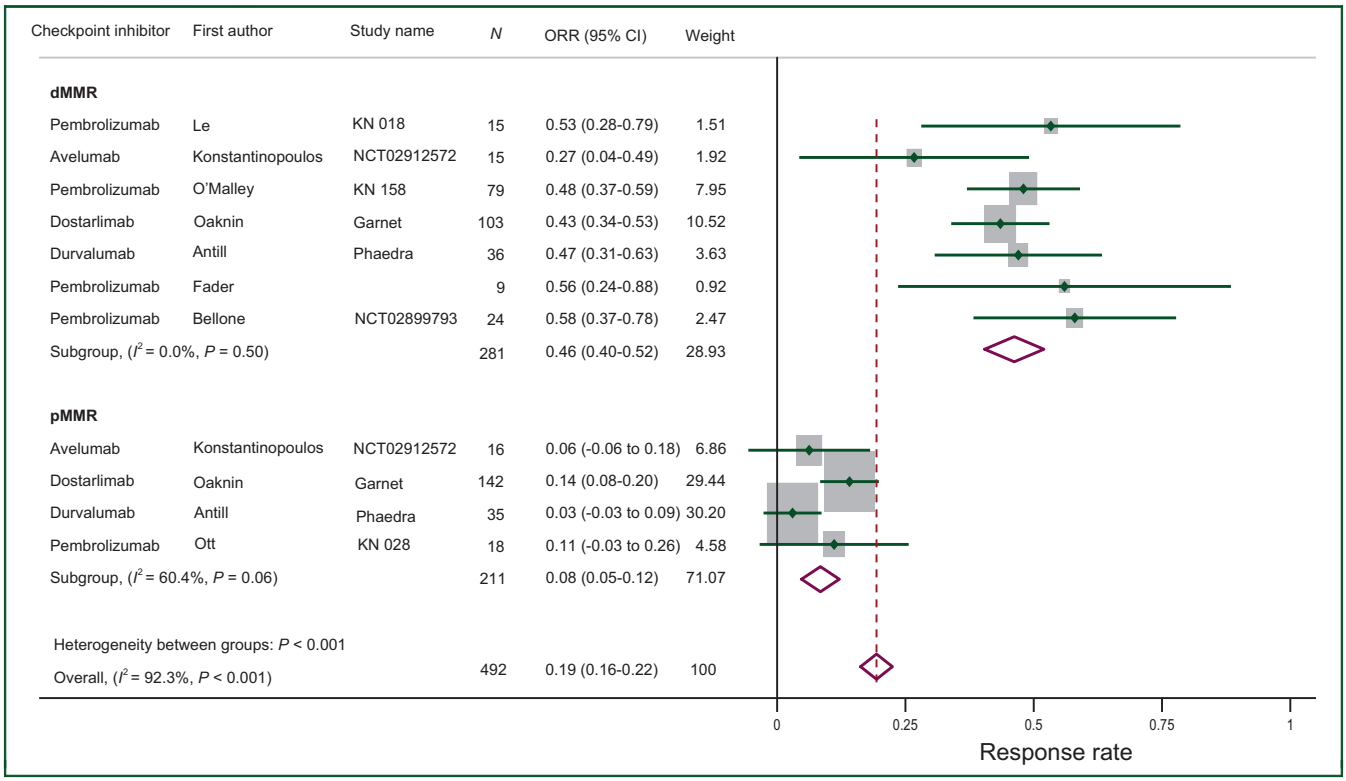

Figure 2. Forest plot of ORR, based on MMR status. ORR for each trial is represented by the squares and the horizontal line crossing the square represents the 95% confidence interval (CI). The diamonds represent the overall effect, based on the meta-analysis fixed-effect method. All statistical tests were two-sided.
dMMR, deficient mismatch repair; MMR, mismatch repair; ORR, objective response rate; pMMR, proficient mismatch repair.

with superior ORR, PFS and OS over chemotherapy.[9] It did not, however, meet the latter criterion as it was not powered to assess the efficacy of treatments in the smaller dMMR cohort ($n = 130$). In its dMMR population, ORR was 40% with a median PFS of 3.7 months. These results are not dissimilar to our meta-analysis findings [pooled ORR was 46% (95% CI 40% to 52%) and the median PFS was 8.3 months for dMMR EC], where there were a comparable number of women ($n = 281$) who received single-agent ICI therapy. The unanswered question remains as to whether lenvatinib adds any additional benefit to pembrolizumab for dMMR EC and if so, to which subsets of dMMR EC? Importantly, this treatment combination was associated significant adverse events, with dose reduction of lenvatinib

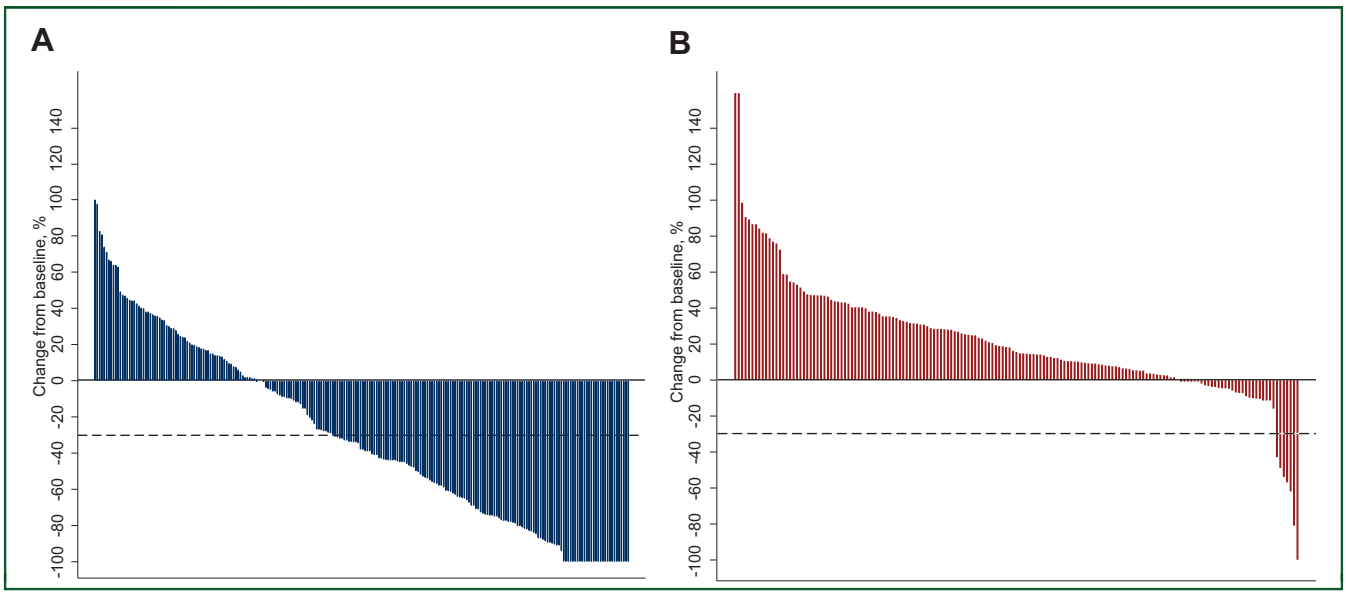

Figure 3. Sum of target lesions change. (A) dMMR. (B) pMMR. In this waterfall plot, the bars indicate the largest percentage change in sum of target lesions from baseline.
dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

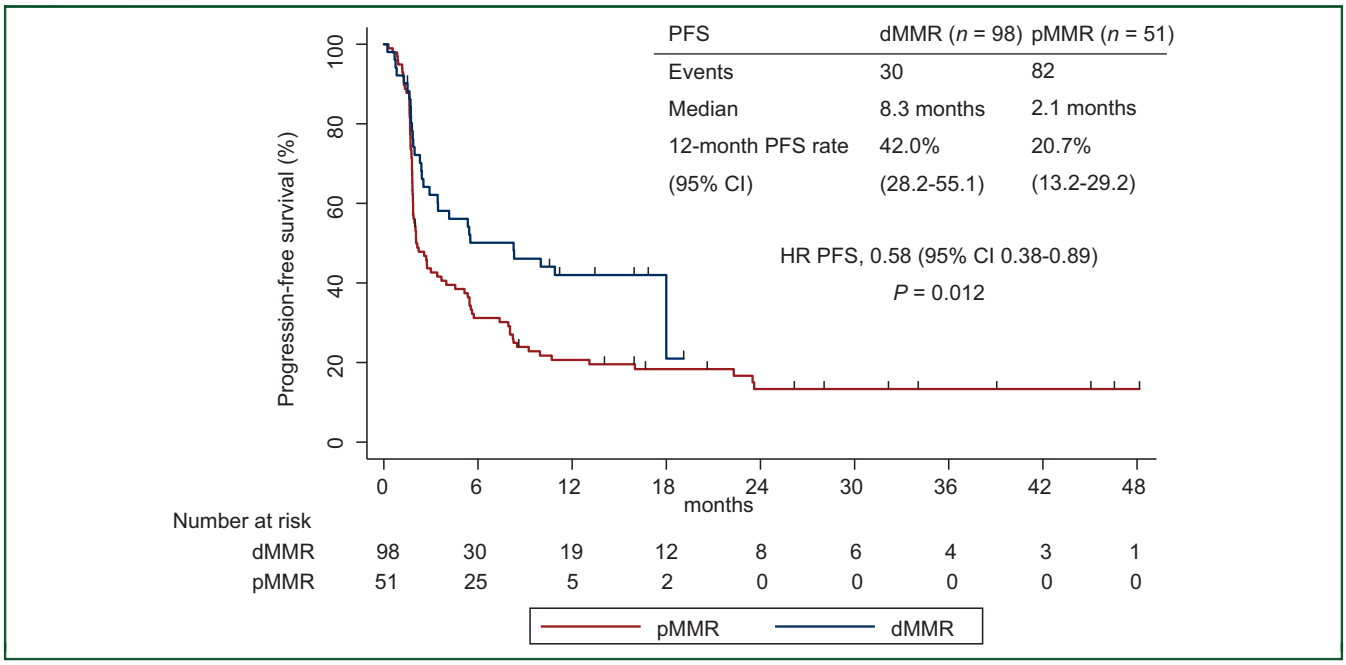

Figure 4. Kaplan–Meier curves for PFS, based on MMR status.
CI, confidence interval; dMMR, deficient mismatch repair; HR, hazard ratio; PFS, progression-free survival; pMMR, proficient mismatch repair.

occurring in 67%, interruption of trial drugs in 69% and trial drug discontinuation in 33% of women.

Of relevance to the question of which women with EC should receive combination IO therapy, emerging data suggest that amongst women with dMMR EC, different molecular mechanisms of carcinogenesis will result in different responses to ICI therapy. In a small study of 25 women, Bellone et al.[8] reported an ORR of 100% ($n = 6$) in sporadic Lynch-like MMR gene-mutated EC, but only 44% ORR ($n = 18$) in sporadic *MLH1* promoter-methylated EC ($P = 0.02$). The 3-year PFS rates were 100% and 30% ($P = 0.02$) for MMR gene-mutated EC versus *MLH1* promoter-methylated EC, respectively. The 3-year OS were 100% and 40% ($P = 0.04$), respectively. Whilst this study was small, these striking findings necessitate confirmation in both future prospective studies and in robust translational correlative analyses of EC collected from previously completed single-agent ICI trials.

If the Bellone data are confirmed, women with MMR gene-mutated EC could potentially be treated with single-agent ICI and be spared from toxicities associated with multikinase inhibitor combination therapy. dMMR gene-mutated EC would be expected to include both inherited and somatic cases, which at present has not been formally shown, as due to small numbers no inherited MMR-mutated cases were present in the Bellone study.[8] On the other hand, women with dMMR *MLH1* promoter-methylated EC were less likely to have sustained benefit from single-agent ICI in the Bellone study and might perhaps be more appropriately treated with combination ICI and multikinase inhibitor.

Our meta-analysis has shown a low ORR of 8% and a median PFS of 2.1 months for women with pMMR EC treated with single-agent ICI. When treated with pembrolizumab plus lenvatinib,[9] however, women with pMMR EC had significant improvement of outcomes with an ORR of 30%, and a median PFS and OS of 6.6 and 17.4 months, respectively. To date, this combination remains a superior choice for women with pMMR EC. Inducing an immune response in pMMR EC with single-agent ICI is very uncommon (as we show here, for pMMR EC: ORR 8% and complete response rate 0.05%) and hence the potential additional toxicity of combination therapy is justified. Many trials are also assessing other combination strategies for pMMR EC, including ICI with polyadenosine diphosphate-ribose polymerase (PARP) inhibitors, and ICI with chemotherapy.

TMB expression is commonly investigated as a predictor for ICI benefit and indeed was the hallmark for the evolution of use of ICIs. The relationship of TMB with dMMR is, however, poorly understood. To investigate the hypothesis that ICI response in dMMR tumors may be related to higher TMB and therefore greater neoantigen peptide presentation, it is important to acknowledge that not all dMMR ECs are associated with MSI-H.[15,17] ORR was almost double in high TMB tumors compared with the low TMB subgroup (44% versus 21%) in the dostarlimab study.[17] Interestingly, 46% of women with pMMR EC but high TMB were also more likely to respond to dostarlimab. A proportion of ECs with isolated loss of *MSH6* expression has been reported to be associated with an MSS state rather than MSI-H. This was supported by results from the dostarlimab study where those with loss of *MSH6* function had lower ORR (23%) than those with *MLH1* loss (47%) or *MSH2* functional loss (64%).[17] Further, median TMB was lower for the *MLH1* hypermethylated subgroup than other dMMR ECs. MMR

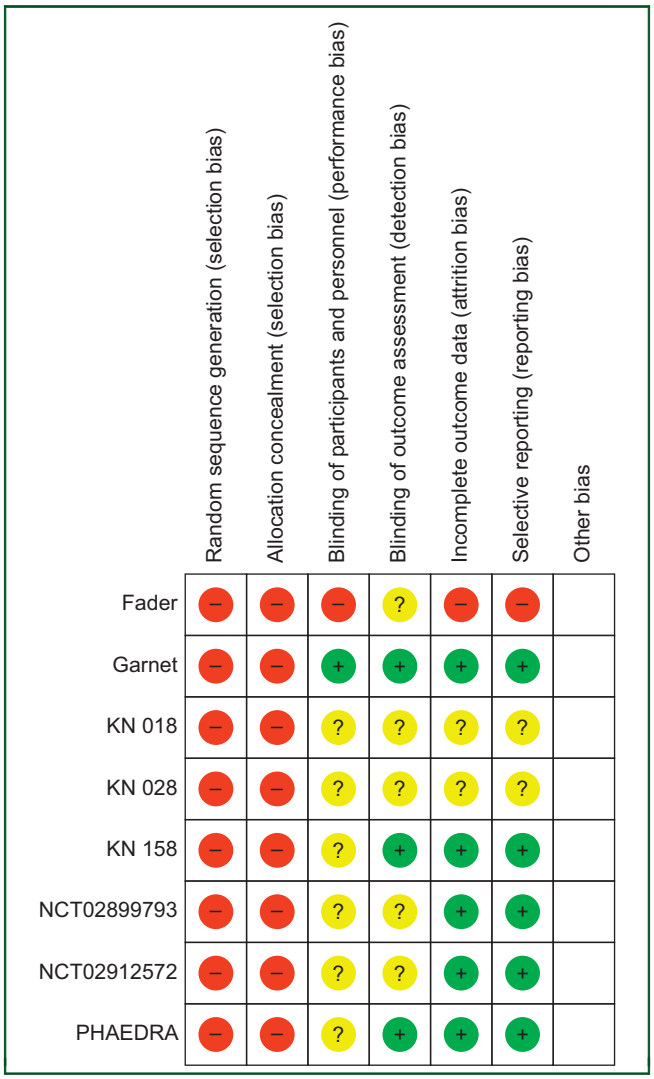

Figure 5. **Summary of risk of bias.**

assessment by IHC is readily available and is a more reliable choice compared with MSI assessment in classifying and selecting women with advanced EC for ICI therapy.

This meta-analysis has several strengths. We carried out a comprehensive review using the most up-to-date trial data. We also overcame the problem of inadequate power of individual trials where the majority had small sample sizes to allow us to examine subgroup outcomes by MMR status. There are also limitations of our meta-analysis. Given that all included studies were of non-randomized designs, single-arm and unblinded trials, they were associated with some risks of bias. We assumed that all ICI agents have equivalent therapeutic efficacy. The use of MSI and IHC expression of MMR proteins varied between studies, and with small patient numbers we were unable to determine differences in response to ICIs based on different modes of assessment of MMR status. Our method of grouping all women with dMMR might also be limited, as the response to ICIs might differ depending on the cause of the MMR deficiency. We have no access to individual patient data with comprehensive baseline characteristics to allow us to carry out multivariable analyses to adjust for potential confounders that could affect the outcomes. Despite these limitations, to our knowledge this meta-analysis remains the only study so far that incorporates results from eight trials with almost 500 women, where the findings could impact on practice and design of future clinical trials. In future, publication of EC ICI clinical trial outcomes or updates (for previously published trials) without fairly simple biomarker analysis [dMMR gene mutations (somatic or germline) versus somatic *MLH1* methylation], even as exploratory endpoints, is as unhelpful to women as publishing ovarian cancer PARP inhibitor studies without *BRCA1/2* and DNA repair homologous recombination deficiency status.

Conclusion

This meta-analysis shows that women with dMMR advanced EC derive greater response from single-agent ICI compared with the more limited benefit seen for those women with pMMR EC. MMR status should be determined prospectively and be used as a stratification factor in future trials of advanced EC. In order to further subclassify EC into different molecular subtypes, especially dMMR subtypes, and to better define those who may be more likely to benefit from single-agent versus combination therapies, further prospective studies are required. Collaboration between clinical trial groups and industry to ensure appropriate translational analyses of current and future EC samples collected from clinical trials will be crucial. These translational analyses should be funded appropriately so that they can be carried out during the lifetime of the trial, compared across trials and reported at the time of first trial reporting, to maximize the effort expended by women who have taken part in these trials.

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DISCLOSURE

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