nature portfolio

Peer Review File

Neoadjuvant immune checkpoint blockade in women with mismatch repair deficient endometrial cancer: a phase I study

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author): with expertise in endometrial cancer, therapy

Manuscript # NCOMMS-24-11421-T

The authors present a phase I study exploring the neoadjuvant use of pembrolizumab in patients with MMRd EC, aiming to assess its safety and efficacy. With an exclusive focus on MMRd EC patients planned for primary surgery, the study intriguingly positions itself at the intersection of immunotherapy application in a pre-surgical setting—a context not extensively explored in current literature for this cancer subtype. The completion of treatment without severe toxicities in all patients underscores the potential feasibility and safety of introducing neoadjuvant immunotherapy in MMRd EC.

However, despite these promising aspects, several concerns that should be addressed. The discussion on immunologic predictors and correlates of response provides valuable insights, yet it lacks depth in interpreting the discrepancies between observed increases in T cells, B cells, clonal expansion, and the lack of association with radiologic or pathologic responses. While the manuscript mentions some discrepancies with previous studies, it falls short in discussing potential reasons behind these differences.

For example,

・The observation that most responders were characterized by a baseline immune "desert" phenotype is interesting. This phenotype is often associated with poor responses to immunotherapy. A deeper discussion could explore why, in this context, pembrolizumab treatment was effective.

・The significant monoclonal expansion observed in radiologic responders and both MPR cases, compared to no expansion in partial pathologic responders or non-responders, offers a insight into the T cell response dynamics following pembrolizumab treatment. Exploring T cell receptor diversity and its impact on immunotherapy outcomes may enrich this discussion.

・（line 85）One patient with pleural effusion and ascites is mentioned, but no detailed explanation is shown. This is a Phase 1 trial which evaluate the safety and feasibility, and the data of them may be crucial. Further explanation should be added about this patient.

・（line117, 253-267）It has been reported that there may be differences in the efficacy of ICB depending on whether MLH1 is hypermethylated or mutated (ie. Gynecol Oncol. 2023 Oct;177:132-141. PMID: 37683549), and the effect of ICB may be decreased in hypermethylated populations. The discussion on this point, however, seems to be insufficient as to why there is a difference.

Minor:

・One patient had a pleural effusion and ascites, which required drainage. Please provide the causes of the pleural effusion and the underlying detail information.

・The percentage of endometrioid adenocarcinoma in Table 1 is 90%, not 80%.

（Table 1）

・Is the FIGO classification the 2008 edition?

・Are the stages described clinical diagnoses? If so, both pathological and clinical stages should be described.

・The percentage of endometrioid adenocarcinoma may is wrong, maybe 90% is correct.

Reviewer #2 (Remarks to the Author): with expertise in endometrial cancer, therapy

Thank you for the opportunity to review the manuscript by Eerkens and colleagues reporting the clinical and translational results of a phase I trial assessing the role of two cycles of neoadjuvant pembrolizumab in MMRd endometrial cancer. The pilot sydt is interesting for the gynecologic oncology community and ongoing larger studies are assessing it. The enriched pre- and post-treatment tissue samples allow performance of complementary translational research.

The concept of “neoadjuvant” is not as relevant in endometrial cancer as in other oncology disease sites, given that endometrial cancer hysterectomy is not as morbid as other surgeries, and changes in the surgical technique or organ sparing surgery may impact patients’ quality of life (i.e. requirements of an ostomy in rectal cancer). This point has not been discussed in the discussion section of the manuscript. Nevertheless, I believe the study

is innovative, and provides the first data of a new concept in the management of MMR deficient endometrial carcinoma.

Please find below some comments for the authors to consider addressing:
-Abstract (45-46): Please rephrase the way radiological and pathological response is reported in a more clear and transparent way (for example: 2 non-evaluable, X PR). They way it is written it is not clear what happens with the 2 non-evaluable patients.
-Abstract (50-51): You report that it is a ¨favorable response¨. How is favorable defined as per protocol? This should also be discussed in methods.
-57: MMRd is not synonymous with TMBh. Consider saying: that there is “higher TMB” in MMRd. But not necessarily all MMRd are TMBh.
-Results 73-74: The 2 patients that were not treated were screen fails? It is not clear to me on the way its written if they started therapy but did not receive less than 2 cycles of pembrolizumab.
-83: Safety is not synonymous of tolerability. No PROs are collected as part of the trial, consider rephrasing.
-Results 99: Can you specify if this is by RECIST 1.1 criteria? How many of the included patients had “measurable disease” as per RECIST at baseline?
-Results 101: How is the pathological response defined. Can you reference the choice of cut-off of 15-50% for PPR?
-Results 110: Given that you are providing the DFS on the trial, please start by the median duration of follow-up to give a general overview.
-Results 110: Consider providing an overview of the about adjuvant treatment post-surgery according to the stage (there is summary in Figure 2, but I think it is not too comprehensive and not highlighted in the written text), as this may have an impact in DFS.
-Results 116: Specify if it is both pathologic and radiologic non-responders.
-118: Can you state in the text number of patients that are hypermethylated? It is stated in the figure, but I think that it would help the reader to have this in the text, as comparisons are performed.
-Figure 2: It is difficult to read the way its displayed (upside down radiological response)
-Discussion 253-254: Following the publication of front-line study assessing Pembrolizumab with chemotherapy by Eskander et al. in NEJM, the group reported the role of MLH1

hypermethylation and outcomes in ESMO 2023 showing no significant differences in outcomes. Consider including this in the discussion (larger trial, earlier line did not show a correlation with response).

-Discussion 266-267: Grade 3 vs 1-2: “Whether these differences reflect underlying immune biology such as a higher TMB and/or influx of lymphocytes or kinetic differences in response remains to be determined”. Although limited by your small sample size, did you see any changes on this in your patients?

-Discussion 268: “non-responding tumours”: pathologically, radiologically or both?

-Discussion: Discuss the relevance of “neoadjuvant”. The organ preservation is not as important in EC, EC histerectomy is not usually very morbid.

-Methods 324: Which is the rationale to perform only MMR testing in <70 yrs old? Median age at endometrial cancer diagnosis is 60 yrs, and many patients in clinic will be >70. I think that this adds a bias to the study, because you are selecting that testing is only performed in younger patients and potentially older patients (who could be more frail, differences in immune system) were not assessed for the neoadjuvant treatment. I think that this should be highlighted as a bias in the limitations of the study.

-Methods 326: Typo: “active other cancer” should read “other active cancer”.

-Methods 331: Typo “met our in- and exclusion criteria“: Met the inclusion and none of the exclusion.

-Methods 334: What about adjuvant chemotherapy? Given that some of the included patients were stage III chemotherapy also may have a role as per guidelines.

-Methods 340, 342-346: Please include definitions of PRR. Are there any guidelines or scoring to ensure objectively when assessing the necrosis, inflammation, or others in the tumour?

-Methods 347: Typo “Hereto, tumour volume”

-Methods 349: Disease free survival is reported in the results. Was this an objective of the study (not reported here)?

-Methods: Was other staging such as CT scan mandatory for patients with more advanced disease?

-Methods: Was there any sample size calculation for the pilot study, or assumption of what is considered favorable?

-Table 2. Are these AEs related or emergent? I am wondering this because ascites and

pleural effusion are included as “related” AEs.

-Figure 1: The order of patients is different in each graph (A: higher response first, B: by number), follow the same order to present the results for clarity.

-Figure 1: Can you include the staging in the image 1A (potentially below the histology)?

-Figure 1B: No chemotherapy was administered in patients with stage III disease?

Reviewer #3 (Remarks to the Author): with expertise in biostatistics, clinical trial study design

COMMENTS FOR THE AUTHORS

Nature Communication: manuscript ID NCOMMS-24-11421-T

Neoadjuvant immune checkpoint blockade in mismatch repair deficient endometrial cancer

My review will focus on the statistical aspects because of my background.

Overall summary:

This is a single institution investigator-initiated Phase 1 clinical trial to evaluate the efficacy and safety and tolerability of Neoadjuvant immune checkpoint blockade (ICB) on patients with MMRd endometrial cancer (EC). The study enrolled ten patients after screening 12 patients. The primary objectives of this study is to assess efficacy (responses), tolerability (adverse events according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0) and molecular correlates of response.

The analyses of the study data are primarily descriptive and are written accordingly.

They reported that 6 (out of 8 evaluable) patients had radiologic responses, with 3 (out of 8) exhibited partial radiologic response, leading to ORR of 37.5%. In addition, 5 out of 10 had pathological responses, and 0 out of 10 patients experienced grade 3 AEs. Median follow-up time was 22.5 months, and no death was observed till the end of this study. They observed two disease recurrence in two non responders (NR) at 47 and 93 weeks after surgery, respectively.

They concluded: "Neoadjuvant ICB with pembrolizumab proved safe and induced favourable pathologic, radiologic, and immunologic responses in MMRd EC, warranting further exploration of extended neoadjuvant treatment.”.

Trial registration: NCT04262089.

Overall Comments:
Overall, the paper is very well written: the design part is clear, the analyses (mostly descriptive) are appropriate and clearly described. I did not read the correlative studies part very carefully because it is not my area of expertise. However, I found this part interesting and quite informative. I have no other comments/concerns about the manuscript.

Reviewer #4 (Remarks to the Author): with expertise in endometrial cancer, therapy
The authors report preliminary data of a phase 1 study with neoadjuvant pembroizumab in patients with dMMR endometrial cancer. There are clear signals of activity and having paired samples some hypothesis on the T cell population activated in responders are presented

Major comments
The number of pathological response is limited due the short course of therapy with only 2 administrations. In the new trial planned the authors are exploring the activity of more cycles as already done in rectal cancer. The results of this trial will clarify better the potentiality of pembrolizumab in this dMMR setting. The number of complete responses is important and, thus, the clinical conclusions of this trial are limited and this need to be discussed

Results: I cannot find initial tumor staging according to the FIGO classification that is also useful to interpret the clinical responses

Lines 245-247: authors cite the study of anti-PD1 monotherapy in dMMR colorectal cancer. In that trial the clinical response was 100% in contrast with the current report where

response rate is far lower. This should be further discusses

Line 253-255: authors are reporting improved outcomes and efficacy of immunotherapy in LS- endometrial cancer. Author should also cite the larger subgroup analysis presented at ESMO 2023 of the NRG-GY018 showing no difference in efficacy of pembrolizumab plus chemotherapy in dMMR endometrial cancer caused by MLH1 methylation or MMR mutations
(ESMO 2023 LBA Updated response data and analysis of progression free survival by mechanism of mismatch repair loss in endometrial cancer (EC) patients (pts) treated with pembrolizumab plus carboplatin/paclitaxel (CP) as compared to CP plus placebo (PBO) in the NRG GY018 trial; Annals of Oncology (2023) 34 (suppl_2): S1281-S1282. 10.1016/S0923-7534(23)X0011-8)

Lines 306-309: conclusions are too strong according to results presented. I suggest delating the last sentence since the potentiality of immunotherapy to become SoC in this setting are out of the scope of the current study and far from be tested in properly designed trial.

Methods: more information on how dMMR was tested should be added

Minor comments
Line 303: not pembrolizumab, dostarlimab

Reviewer #5 (Remarks to the Author): with expertise in endometrial cancer, therapy
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

RESPONSE TO REFEREES

Reviewer #1 (Remarks to the Author): with expertise in endometrial cancer, therapy

Manuscript # NCOMMS-24-11421-T

The authors present a phase I study exploring the neoadjuvant use of pembrolizumab in patients with MMRd EC, aiming to assess its safety and efficacy. With an exclusive focus on MMRd EC patients planned for primary surgery, the study intriguingly positions itself at the intersection of immunotherapy application in a pre-surgical setting—a context not extensively explored in current literature for this cancer subtype. The completion of treatment without severe toxicities in all patients underscores the potential feasibility and safety of introducing neoadjuvant immunotherapy in MMRd EC.

We thank the reviewer for the kind words and thorough review of the manuscript, and we wholeheartedly agree on the promise of neoadjuvant immunotherapy in this patient group.

However, despite these promising aspects, several concerns that should be addressed. The discussion on immunologic predictors and correlates of response provides valuable insights, yet it lacks depth in interpreting the discrepancies between observed increases in T cells, B cells, clonal expansion, and the lack of association with radiologic or pathologic responses. While the manuscript mentions some discrepancies with previous studies, it falls short in discussing potential reasons behind these differences.
For example,

・The observation that most responders were characterized by a baseline immune "desert" phenotype is interesting. This phenotype is often associated with poor responses to immunotherapy. A deeper discussion could explore why, in this context, pembrolizumab treatment was effective.

We agree with the reviewer and have expanded the discussion (line 281-288) on why pembrolizumab may have been effective in these immune “desert” patients.

・The significant monoclonal expansion observed in radiologic responders and both MPR cases, compared to no expansion in partial pathologic responders or non-responders, offers a insight into the T cell response dynamics following pembrolizumab treatment. Exploring T cell receptor diversity and its impact on immunotherapy outcomes may enrich this discussion.

We agree with the reviewer and have expanded the discussion on clonal expansion of T cells in our cohort and the broader literature (lines 289-309).

・（line 85）One patient with pleural effusion and ascites is mentioned, but no detailed explanation is shown. This is a Phase 1 trial which evaluate the safety and feasibility, and the data of them may be crucial. Further explanation should be added about this patient.

We have provided more details on the cause and composition of the pleural effusion between lines 85 and 89.

・（line117, 253-267）It has been reported that there may be differences in the efficacy of ICB depending on whether MLH1 is hypermethylated or mutated (ie. Gynecol Oncol. 2023 Oct;177:132-141. PMID: 37683549), and the effect of ICB may be decreased in hypermethylated populations. The discussion on this point, however, seems to be insufficient as to why there is a difference.

This point on the differences in response between hypermethylated or mutated MMR genes was also raised by reviewer #2. Specifically, reviewer #2 added:

“Following the publication of front-line study assessing Pembrolizumab with chemotherapy by Eskander et al. in NEJM, the group reported the role of MLH1 hypermethylation and outcomes in ESMO 2023 showing no significant differences in outcomes. Consider including this in the discussion (larger trial, earlier line did not show a correlation with response).”

We have integrated both comments into the broader discussion on hypermethylated or mutated MMR genes in the discussion section on line 259-280.

Minor:

・One patient had a pleural effusion and ascites, which required drainage. Please provide the causes of the pleural effusion and the underlying detail information.

We have provided more details on the cause and composition of the pleural effusion between lines 85 and 89 of the manuscript .

・The percentage of endometrioid adenocarcinoma in Table 1 is 90%, not 80%.（Table 1）
We have adjusted the percentages in the text on line 80 of the manuscript and table 1 accordingly.

・Is the FIGO classification the 2008 edition?

The revised 2009 FIGO staging for endometrial cancer was used according to standard-of-care in the Netherlands. This information has been added as a footnote to Table 1.

・Are the stages described clinical diagnoses? If so, both pathological and clinical stages should be described.
Only the pathological FIGO stages were described in the original manuscript. We have added information on the pre-treatment clinical stage to table 1. Post-treatment both pathological and clinical staging where the same since the clinical diagnosis was in the end based on the pathological staging after standard-of-care hysterectomy, we’ve added a clarification to table 1 and added the post-treatment FIGO to fig. 1a.

・The percentage of endometrioid adenocarcinoma may is wrong, maybe 90% is correct.
We have adjusted the percentages in the text of line 80 and table 1 accordingly.

Reviewer #2 (Remarks to the Author): with expertise in endometrial cancer, therapy

Thank you for the opportunity to review the manuscript by Eerkens and colleagues reporting the clinical and translational results of a phase I trial assessing the role of two cycles of neoadjuvant pembrolizumab in MMRd endometrial cancer. The pilot sydt is interesting for the gynecologic oncology community and ongoing larger studies are assessing it. The enriched pre- and post-treatment tissue samples allow performance of complementary translational research. The concept of "neoadjuvant" is not as relevant in endometrial cancer as in other oncology disease sites, given that endometrial cancer hysterectomy is not as morbid as other surgeries, and changes in the surgical technique or organ sparing surgery may impact patients' quality of life (i.e. requirements of an ostomy in rectal cancer). This point has not been discussed in the discussion section of the manuscript. Nevertheless, I believe the study is innovative, and provides the first data of a new concept in the management of MMR deficient endometrial carcinoma.

We thank the reviewer for the kind words and thorough review of the manuscript. We have adjusted the specific comments raised by the reviewer in a point-by-point manner below.

Please find below some comments for the authors to consider addressing:
-Abstract (45-46): Please rephrase the way radiological and pathological response is reported in a more clear and transparent way (for example: 2 non-evaluable, X PR). They way it is written it is not clear what happens with the 2 non-evaluable patients.
We have rephrased the way radiological and pathological response is reported in line 45-48; page 2 in a more clear and transparent way.

-Abstract (50-51): You report that it is a ¨favorable response¨. How is favorable defined as per protocol? This should also be discussed in methods.
We thank the reviewer for pointing this out. Favourable was not meant as a characterization of a per protocol analysis and we have removed this qualification from the abstract in line 51 accordingly.

-57: MMRd is not synonymous with TMBh. Consider saying: that there is "higher TMB" in MMRd. But not necessarily all MMRd are TMBh.
We have adjusted the sentence on line 56 to 59 accordingly as:
"20-30% of cases demonstrate DNA mismatch repair deficiency (MMRd), due to germline defects in MMR genes MLH1, MSH2, MSH6 or PMS2 (Lynch syndrome) or somatic MLH1 loss, resulting in a higher tumour mutational burden (TMB) in the majority of MMRd EC cases."

-Results 73-74: The 2 patients that were not treated were screen fails? It is not clear to me on the way its written if they started therapy but did not receive less than 2 cycles of pembrolizumab.
To clarify, we have added the sentence:
"Two patients were excluded during screening prior to initiation of treatment"
to line 73 of the manuscript.

-83: Safety is not synonymous of tolerability. No PROs are collected as part of the trial, consider rephrasing.
We have adjusted the sentence:
"Neoadjuvant pembrolizumab was well tolerated and feasible"
On line 83 to:
"Neoadjuvant pembrolizumab was feasible and could be administered safely"

-Results 99: Can you specify if this is by RECIST 1.1 criteria? How many of the included patients had "measurable disease" as per RECIST at baseline?

We have added the use of RECIST v1.1 and more information on the amount of patients that had measurable disease as per RECIST baseline to line 96 of the manuscript.

-Results 101: How is the pathological response defined. Can you reference the choice of cut-off of 15-50% for PPR?
We have clarified the definition of pathological response to the methods section on line 375-381 as follows:
"Aligning with the tumour regression grading system of Mandard et al., the following percentages are used (our adaptation): >90% tumour cells, 50-90% tumour cells, 10-50% tumour cells and MPR was characterized as ≤10% residual tumour cells, and complete responses as no residual tumour cells at all. It is a bit arbitrary to combine the middle categories. The Mandard paper does not suggest that and there is no consensus regarding PR definition post-immunotherapy. Therefore, it may be totally fine to combine the categories in uncharted waters, such as neo-adjuvant ICB for MMRd EC.

-Results 110: Given that you are providing the DFS on the trial, please start by the median duration of follow-up to give a general overview.
We have adjusted line 114 of the revised manuscript accordingly.

-Results 110: Consider providing an overview of the about adjuvant treatment post-surgery according to the stage (there is summary in Figure 2, but I think it is not too comprehensive and not highlighted in the written text), as this may have an impact in DFS.
We have added a sentence to line 117 to clarify the adjuvant treatment post-surgery. We have also provided a table (table 3) with an overview of the FIGO stage and adjuvant treatment per patient.

-Results 116: Specify if it is both pathologic and radiologic non-responders.
We have adjusted line 116 of the revised manuscript to specify pathologic and radiologic non-responders.

-118: Can you state in the text number of patients that are hypermethylated? It is stated in the figure, but I think that it would help the reader to have this in the text, as comparisons are performed.
We have added the number of patients in the text on line 123.

-Figure 2: It is difficult to read the way its displayed (upside down radiological response)
We apologize for the difficult to read figure 2. We agree with the reviewer that the y axis labelling gives the upside down view of response. We have now adjusted the naming throughout all figures to: "Histopathological Tumour Regression" and/or "Radiologic Tumour Regression" for consistency and clarity. Fig 1a and Fig. 2 are adjusted accordingly.

-Discussion 253-254: Following the publication of front-line study assessing Pembrolizumab with chemotherapy by Eskander et al. in NEJM, the group reported the role of MLH1 hypermethylation and outcomes in ESMO 2023 showing no significant differences in outcomes. Consider including this in the discussion (larger trial, earlier line did not show a correlation with response).
This point on the differences in response between hypermethylated or mutated MMR genes was also raised by reviewer #1. Specifically, the reviewer added:

"It has been reported that there may be differences in the efficacy of ICB depending on whether MLH1 is hypermethylated or mutated (ie. Gynecol Oncol. 2023 Oct;177:132-141. PMID: 37683549), and the effect of ICB may be decreased in hypermethylated populations. The discussion on this point, however, seems to be insufficient as to why there is a difference."

We have integrated both comments into the broader discussion on hypermethylated or mutated MMR genes in the discussion section on page 259-280.

-Discussion 266-267: Grade 3 vs 1-2: "Whether these differences reflect underlying immune biology such as a higher TMB and/or influx of lymphocytes or kinetic differences in response remains to be determined". Although limited by your small sample size, did you see any changes on this in your patients?
2 of the 4 grade 1/2 tumours were characterized by an elevated tumour mutational burden (TMB). However, both of these patients were diagnosed with Lynch syndrome, a confounder for the heightened TMB compared to sporadic MMR-mutation cases. Analysis of lymphocytic infiltration and potential kinetic variations revealed no significant differences between tumour grades within our cohort, but as the reviewer mentions this may easily be due to the small sample size of the study.

-Discussion 268: "non-responding tumours": pathologically, radiologically or both?
We have clarified this point in the revised discussion section.

-Discussion: Discuss the relevance of "neoadjuvant". The organ preservation is not as important in EC, EC histerectomy is not usually very morbid.
We have added a paragraph to the discussion section (line 259 to 263) regarding the relevance of neo-adjuvant use of pembrolizumab in EC.

-Methods 324: Which is the rationale to perform only MMR testing in <70 yrs old? Median age at endometrial cancer diagnosis is 60 yrs, and many patients in clinic will be >70. I think that this adds a bias to the study, because you are selecting that testing is only performed in younger patients and potentially older patients (who could be more frail, differences in immune system) were not assessed for the neoadjuvant treatment. I think that this should be highlighted as a bias in the limitations of the study.
We apologize for the confusion caused by this section. To clarify: in our regional oncology network, MMRd testing is standard-of-care for patients <70 years old, in line with Dutch guidelines. For the current trial, we extended the testing to include patients older than 70 for all recruiting hospitals (MMRd expression analysis performed according to standard-of-care, but reimbursed via study funding). We have clarified this point in the corresponding method section in line 351-353.

-Methods 326: Typo: "active other cancer" should read "other active cancer".
We have adjusted line 356 accordingly.

-Methods 331: Typo "met our in- and exclusion criteria": Met the inclusion and none of the exclusion.
We have adjusted line 360 accordingly.

-Methods 334: What about adjuvant chemotherapy? Given that some of the included patients were stage III chemotherapy also may have a role as per guidelines.
The Dutch guideline for endometrial cancer mentions that chemotherapy may be considered alongside radiotherapy for stage III disease. As a result, chemotherapy does not hold a fixed position in the treatment of patients included in this trial. Based on physician assessment, none of the trial patients with stage III disease (2/10) were judged to receive adjuvant chemotherapy. The use of adjuvant chemotherapy was therefore not documented in the methodology.

-Methods 340, 342-346: Please include definitions of PRR. Are there any guidelines or scoring to ensure objectively when assessing the necrosis, inflammation, or others in the tumour?
We have added information on the definitions of the pathologic response rate on lines 375-381.

-Methods 347: Typo "Hereto, tumour volume"
We have changed this sentence (line 382) to:

"The longest diameter of the target lesion (tumour) was assessed by Magnetic Resonance Imaging (MRI) at baseline and after administration of two cycles of pembrolizumab, but prior to surgery."

-Methods 349: Disease free survival is reported in the results. Was this an objective of the study (not reported here)?
DFS was not a primary objective of the study, but have we aimed to provide insight into the patients' post-treatment outcomes in an exploratory manner. Consequently, we included this information in the results section. However, as it was not a predefined endpoint, it was not explicitly detailed in the methods.

-Methods: Was other staging such as CT scan mandatory for patients with more advanced disease?
We have added a sentence on line 353-354 to clarify this matter. "9/10 patients underwent abdominal and thoracic CT scans and/or thoracic X-ray as part of the routine diagnostic evaluation for EC prior to study enrolment."

-Methods: Was there any sample size calculation for the pilot study, or assumption of what is considered favorable?
No official sample size calculation was performed for this pilot study. Based on the available literature at time of submission of the protocol, we hypothesized that a sample size of 10 patients would be sufficient for an estimated objective response rate of 50% with a 95% confidence interval of +/- 31%, sufficient to determine whether follow-up studies might be warranted. As also discussed by reviewer #3 with expertise in biostatistics and study design, we believe this design and analysis was appropriate for the current pilot.

-Table 2. Are these AEs related or emergent? I am wondering this because ascites and pleural effusion are included as "related" AEs.
We apologize for the oversight. All AEs mentioned in Table 2 are related to pembrolizumab. We have removed ascites and pleural effusion as these were deemed treatment unrelated.

-Figure 1: The order of patients is different in each graph (A: higher response first, B: by number), follow the same order to present the results for clarity.
We have re-ordered the patients in Fig. 1B according to the order in Fig. 1A.

-Figure 1: Can you include the staging in the image 1A (potentially below the histology)?
We have added the FIGO stage to Fig. 1A.

-Figure 1B: No chemotherapy was administered in patients with stage III disease?
The Dutch guideline for endometrial cancer mentions that chemotherapy may be considered alongside radiotherapy for stage III disease. As a result, chemotherapy does not hold a fixed position in the treatment of patients included in this trial. Based on physician assessment, none of the trial patients with stage III disease (2/10) were judged to receive adjuvant chemotherapy.

Reviewer #3 (Remarks to the Author): with expertise in biostatistics, clinical trial study design

COMMENTS FOR THE AUTHORS

Nature Communication: manuscript ID NCOMMS-24-11421-T

Neoadjuvant immune checkpoint blockade in mismatch repair deficient endometrial cancer
My review will focus on the statistical aspects because of my background.

Overall summary:
This is a single institution investigator-initiated Phase 1 clinical trial to evaluate the efficacy and safety and tolerability of Neoadjuvant immune checkpoint blockade (ICB) on patients with MMRd endometrial cancer (EC). The study enrolled ten patients after screening 12 patients. The primary objectives of this study is to assess efficacy (responses), tolerability (adverse events according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0) and molecular correlates of response.

The analyses of the study data are primarily descriptive and are written accordingly.

They reported that 6 (out of 8 evaluable) patients had radiologic responses, with 3 (out of 8) exhibited partial radiologic response, leading to ORR of 37.5%. In addition, 5 out of 10 had pathological responses, and 0 out of 10 patients experienced grade 3 AEs. Median follow-up time was 22.5 months, and no death was observed till the end of this study. They observed two disease recurrence in two non responders (NR) at 47 and 93 weeks after surgery, respectively.

They concluded: "Neoadjuvant ICB with pembrolizumab proved safe and induced favourable pathologic, radiologic, and immunologic responses in MMRd EC, warranting further exploration of extended neoadjuvant treatment.".

Trial registration: NCT04262089.

Overall Comments:
Overall, the paper is very well written: the design part is clear, the analyses (mostly descriptive) are appropriate and clearly described. I did not read the correlative studies part very carefully because it is not my area of expertise. However, I found this part interesting and quite informative. I have no other comments/concerns about the manuscript.

We thank the reviewer for the kind words and thorough review of the manuscript.

Reviewer #4 (Remarks to the Author): with expertise in endometrial cancer, therapy

The authors report preliminary data of a phase 1 study with neoadjuvant pembroizumab in patients with dMMR endometrial cancer. There are clear signals of activity and having paired samples some hypothesis on the T cell population activated in responders are presented

Major comments

The number of pathological response is limited due the short course of therapy with only 2 administrations. In the new trial planned the authors are exploring the activity of more cycles as already done in rectal cancer. The results of this trial will clarify better the potentiality of pembrolizumab in this dMMR setting. The number of complete responses is important and, thus, the clinical conclusions of this trial are limited and this need to be discussed.

We thank the reviewer for the kind words and thorough review of the manuscript. We have expanded the discussion on limitations of our study to address in more detail the (complete) response rates of the trial and limitations thereof. See line 325-326.

Results: I cannot find initial tumor staging according to the FIGO classification that is also useful to interpret the clinical responses.

We have added the clinical/radiological FIGO stage before treatment to the patient characteristics in table 1.

Lines 245-247: authors cite the study of anti-PD1 monotherapy in dMMR colorectal cancer. In that trial the clinical response was 100% in contrast with the current report where response rate is far lower. This should be further discusses

We have revised the discussion section of the manuscript according to the reviewer's suggestion.

Line 253-255: authors are reporting improved outcomes and efficacy of immunotherapy in LS-endometrial cancer. Author should also cite the larger subgroup analysis presented at ESMO 2023 of the NRG-GY018 showing no difference in efficacy of pembrolizumab plus chemotherapy in dMMR endometrial cancer caused by MLH1 methylation or MMR mutations (ESMO 2023 LBA Updated response data and analysis of progression free survival by mechanism of mismatch repair loss in endometrial cancer (EC) patients (pts) treated with pembrolizumab plus carboplatin/paclitaxel (CP) as compared to CP plus placebo (PBO) in the NRG GY018 trial; Annals of Oncology (2023) 34 (suppl_2): S1281-S1282. 10.1016/S0923-7534(23)X0011-8)

We have included the citation specified by the reviewer into the broader discussion on LS versus sporadic MMRd. However, it should be noted that our study does not report on improved efficacy in LS-endometrial cancer.

Lines 306-309: conclusions are too strong according to results presented. I suggest delating the last sentence since the potentiality of immunotherapy to become SoC in this setting are out of the scope of the current study and far from be tested in properly designed trial.

We adjusted the last sentence of the discussion section as follows:

"Extended treatment is warranted to determine if complete pathologic responses can be achieved."

Methods: more information on how dMMR was tested should be added

Expression of MMR proteins was tested according to standard-of-care immunohistochemical analysis. We have added this information to our methods in subheading “Immunohistochemical (IHC) analysis on lines 411-412 + 417-418.

Minor comments

Line 303: not pembrolizumab, dostarlimab

We have changed pembrolizumab to dostarlimab accordingly (line 329 in the revised manuscript).

Reviewer #5 (Remarks to the Author): with expertise in endometrial cancer, therapy

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

We thank the reviewer for the thorough co-review of the manuscript, and would like to extend our support to the initiative by Nature Communications to facilitate training in, and recognition for, peer review by Early Career Researchers.

REVIEWERS' COMMENTS

Reviewer #2 (Remarks to the Author):

Thank you for providing a response to my comments. Most of my comments have been addressed in the revised version of the manuscript.

-Discussion (259-263, tracked version): I think that the implications of "neoadjuvant" in endometrial cancer compared to other disease sites is not fully addressed in the discussion. Authors report: "A notable portion of MMRd EC patients currently undergo adjuvant radio(chemo)therapy, which affects quality of life (QoL). Although adjuvant therapy improves locoregional control, it does not improve disease-specific survival." Could you provide a reference for this sentence? PORTEC-3 trial data shows benefit in stage III disease, and clinical practice in many centres and guidelines (ESGO/ESTRO/ESP - stage III/IVa MMRd considered high risk. Recomendation: EBRT with concurrent and adjuvant chemotherapy (I, A) or alternatively sequential chemotherapy and radiotherapy is recommended (I, B). Chemotherapy alone is an alternative option (I, B)) includes treating these patients with chemo-radiotherapy.

I think it is still relevant to comment on the potential role of organ sparing procedures (as performed in rectal cancer), although it does not seem that sparing a simple hysterectomy is that relevant.

Reviewer #4 (Remarks to the Author):

Thanks for doing the changes proposed

RESPONSE TO REFEREES

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I think it is still relevant to comment on the potential role of organ sparing procedures (as performed in rectal cancer), although it does not seem that sparing a simple hysterectomy is that relevant.

Thank you for this comment. To further elucidate and clarify our statement, we have added references in line 267-268 on the effect of radiotherapy in MMRd EC patients on disease-specific survival and (loco-regional) disease control, and a reference on the difference in effect of EBRT and EBRT combined with chemotherapy in this patient population. Furthermore, we added a passage about the possible use of pembrolizumab as organ-sparing treatment in subgroups of the patient population.

Reviewer #4 (Remarks to the Author):

Thanks for doing the changes proposed

You're welcome. We want to thank the reviewer again for their time to review the manuscript and providing feedback on its contents.