ANSWER TO THE REVIEWERS COMMENTS AND SUGGESTIONS:

R1:
Comments and Suggestions for Authors
The manuscript titled "ADVANCES IN THE DIAGNOSIS OF ATYPICAL POLYPOID ADENOMYOMA THROUGH NEXT GENERATION SEQUENCING (NGS)" by Javier azua romeo et al.

- The NGS employed isn't whole genome sequencing. As a matter of fact, only 59 genes were checked, it is a rather low throughput analysis:
There are many references that support that the key to this technology does not have to be in the size of the panel, and that depending on the use we give it, it is perfectly defensible that we use targeted panels with dozens of genes, as long as it serves our purposes. I have modified the text of materials and methods a little and I indicate several references that support that we can use the term NGS even if it is a relatively small panel. Please find the references regarding the use of a limited panel, we didint include them in the paer, nevertheless, if you consider them of interest we will do it ASAP:

- Jennings LJ, Arcila ME, Corless C, Kamel-Reid S, Lubin IM, Pfeifer J, Temple-Smolkin RL, Voelkerding KV, Nikiforova MN. Guidelines for Validation of Next-Generation Sequencing-Based Oncology Panels: A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists. J Mol Diagn. 2017 May;19(3):341-365. doi: 10.1016/j.jmoldx.2017.01.011. Epub 2017 Mar 21. PMID: 28341590; PMCID: PMC6941185.
- Han SM, Park J, Lee JH, Lee SS, Kim H, Han H, Kim Y, Yi S, Cho JY, Jang IJ, Lee MG. Targeted Next-Generation Sequencing for Comprehensive Genetic Profiling of Pharmacogenes. Clin Pharmacol Ther. 2017 Mar;101(3):396-405. doi: 10.1002/cpt.532. Epub 2016 Nov 24. PMID: 27727443.
- Mosele F, Remon J, Mateo J, Westphalen CB, Barlesi F, Lolkema MP, Normanno N, Scarpa A, Robson M, Meric-Bernstam F, Wagle N, Stenzinger A, Bonastre J, Bayle A, Michiels S, Bièche I, Rouleau E, Jezdic S, Douillard JY, Reis-Filho JS, Dienstmann R, André F. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. Ann Oncol. 2020 Nov;31(11):1491-1505. doi: 10.1016/j.annonc.2020.07.014. Epub 2020 Aug 24. PMID: 32853681.
- Kerr KM, Bibeau F, Thunnissen E, Botling J, Ryška A, Wolf J, Öhrling K, Burdon P, Malapelle U, Büttner R. The evolving landscape of biomarker testing for non-small cell lung cancer in Europe. Lung Cancer. 2021 Apr;154:161-175. doi: 10.1016/j.lungcan.2021.02.026. Epub 2021 Feb 22. PMID: 33690091.
- Mosele MF, Westphalen CB, Stenzinger A, Barlesi F, Bayle A, Bièche I, Bonastre J, Castro E, Dienstmann R, Krämer A, Czarnecka AM, Meric-Bernstam F, Michiels S, Miller R, Normanno N, Reis-Filho J, Remon J, Robson M, Rouleau E, Scarpa A, Serrano C, Mateo J, André F. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol. 2024 Jul;35(7):588-606. doi: 10.1016/j.annonc.2024.04.005. Epub 2024 May 27. PMID: 38834388.

- Tumor heterogeneity is an important issue, even tumor tissues in different region of the tumor mass of the same patient differ in the genetic makeup. How to ensure the NGS results can represent the overall genetics of these tumor cells.

Ensuring that next-generation sequencing (NGS) results accurately represent the overall genetic diversity of a tumor, particularly in cases of tumor heterogeneity, involves several key strategies:

1. **Multi-Region Sampling**: Collect samples from multiple areas of the tumor, as well as from adjacent normal tissue. This approach helps to capture genetic variations that may exist within different regions of the tumor.
2. **Using Core Biopsies**: Instead of relying solely on fine-needle aspirations, consider larger core biopsies that allow for greater tissue volume and potential for capturing heterogeneous cells.
3. **Random Sampling**: For large tumors, random sampling techniques can be employed to ensure various tumor regions are included in the analysis.
4. **Tumor Microenvironment Consideration**: Take into account the microenvironment surrounding the tumor, as interactions within different tumor regions might influence genetic expression and characteristics.
5. **Single-Cell Sequencing**: This advanced technique allows for the analysis of individual cells, providing insights into the genetic diversity present in different cell populations within a tumor.
6. **Integrating Data**: Use bioinformatics tools to aggregate and analyze data from multiple samples. This can help identify common mutations as well as unique alterations in different regions.
7. **Time Points**: If feasible, sampling at multiple time points during treatment can provide insights into how tumor genetics evolve over time.
8. **Collaborative Analysis**: Collaborate with pathologists and oncologists to interpret the genetic data in the context of histological findings, aiding in understanding tumor heterogeneity.

By implementing these strategies, you can enhance the chances that NGS results provide a more comprehensive view of the genetic characteristics of a tumor, accommodating its inherent heterogeneity.
In this sense we did not explain this issue in the paper as we believe it is not really neccesary, please feel free to suggest what you consider in order to improve the comprehension of the text.

- The content is a research paper but the template indicates it is a review article, and the online system indicates it is a case report? Which one is correct by the way? We have changed the title:
Advances in the Diagnosis of Atypical Polypoid Adenomyoma combining immunohistochemical and molecular-based approaches: case report and review of literature

- The tables are poorly prepared as it is unclear what the meaning of + and numbers are. For the evaluation of immunostaining, a simplification strategy was employed to optimize thecomprehension of results obtained from different articles as follows:

- No staining was scored as 0 or negative
- Staining from 1% to 33% of cells was scored as + (1) (low expression)
- Staining from 34% to 66% of cells was scored as ++(2) (moderate expression)
- Staining from 67% to 100% was scored as +++(3) (high expression)

Also, it is also weird that the words in first column of tables are in capital letters. Ok Changed

It is also unclear what IHQ meant in the table? It is a misspelling, as it should say IHC

- Scale should be provide in the IHC pictures.
Scales are provided in the figures and also in the text:

> **Figure 1. A)** Morular, stromal, and glandular area observed. **(**H-E augmentation 22.50x). **B)** Actin positive immunostaining of the stroma highlighted (Augmentation 7.90x). C) Keratin AE1-AE3 positivity for glandular lining cells and complete staining of the morula (Augmentation 11.85x) **D)** p16 positive immunoexpression exclusively in morules (Augmentation 11.85x) **E)** ER positive in glandular epithelium and intense, weak, or absent stroma in morules (Augmentation 11.85x) **F)** PR positive immunostaining in glandular epithelium, sparse in stroma and absent in morules (Augmentation 7.90x).

The IHC pictures are blur and unclear too. We have changed the resolution and the distribution to fit in one page

- All figure panels of a figure should be arranged in one page, not spanning two pages.
We have changed the resolution and the distribution to fit in one page

- At the end, it is unclear how the results in this case report could be translated to benefit the patients with this disease.
We believe it is already state in the “conclusions” as we say: The study has provided a solid foundation for the use of these biomarkers in clinical diagnosis and suggests areas for future research that could improve our understanding and treatment of these pathological conditions. Additional studies with larger samples are recommended to validate these preliminary findings and potentially discover new diagnostic and therapeutic markers.
Which really menas that the performed IHC combined with the genetic mutations, especially in genes such as *CTNNB1*, underscore the distinctions in the biology of these lesions, favouring the diagnosis of APA instead of the malignant endometrioid carcinoma.

R2:
Comments and Suggestions for Authors
Overview

This study investigated APA, a rare uterine pathologic condition. The methodology and text writing are overall safe and sound.

Critical comments

1 Title. “Advances in the Diagnosis of Atypical Polypoid Adenomyoma Through Next Generation Sequencing; Case Report and Review of Literature” may better represent the contents of the study. Please reconsider.
We have changed the title:
Advances in the Diagnosis of Atypical Polypoid Adenomyoma combining immunohistochemical and molecular-based approaches: case report and review of literature

2 Abstract. “Atypical Polypoid Adenomyoma (APA) is a benign uterine lesion with a premalignant potential and occurs in women of reproductive age” may be a better description. Please reconsider.
Changed: **Abstract:** Atypical Polypoid Adenomyoma (APA) is a benign uterine lesion with a premalignant potential and occurs in women of reproductive age. The histological pattern is characterized by irregular epithelial proliferation and muscular stroma

3 Table 2&3. What did authors mean by “2+”, “3+”, “N”? Were they staining intensity or negativity? Please specify in the table legend.

Done: For the evaluation of immunostaining, a simplification strategy was employed to optimize thecomprehension of results obtained from different articles as follows:

- No staining was scored as 0 or negative
- Staining from 1% to 33% of cells was scored as + (1) (low expression)
- Staining from 34% to 66% of cells was scored as ++(2) (moderate expression)
- Staining from 67% to 100% was scored as +++(3) (high expression)

4 Tables & Discussion. Can the authors explain the reasons that the results of immunohistochemistry on caldesmon are inconsistent among the studies?
We have explained this issue in the discussion section: “…, in the reported series we can find a high grade of variability which may be due differences in the IHC protocols, such as the choice of antibodies, antigen retrieval techniques, and staining conditions, can lead to variable results. Variations in sensitivity and specificity of different antibody clones used may also affect outcomes.; as may happen with any other IHC marker, tumors can exhibit heterogeneity, meaning that within a single lesion, different areas may express markers differently. This biological variability can influence results over different samples or case series.”

5 Discussion and References. Recent publication by Chau Minh Bui et al., Stromal p16 and SATB2 Expression Does Not Distinguish Atypical Polypoid Adenomyoma (APA) From its Benign Mimics Int J Gynecol Pathol. 2024 Nov 1;43(6):586-594. doi: 10.1097/PGP.0000000000001023. Epub 2024 May 29, may be introduced and quoted in the manuscript.
We have elimitaed the old refernce for this one