I would like to thank the respected Reviewers for their constructive comments on our manuscript "(Manuscript NO.: 85624, Meta-Analysis)". I have considered the comments very carefully and have revised the paper accordingly. I believe that this revised paper has been improved considerably. I hope that the corrections are satisfactory.

Responds to the reviewers' comments:

Reviewer #1

Comment 1: Page 1 at the end. I think it could be very useful to define what a complex PF is.

Response: Thanks for your suggestion. We have defined what a complex PF is in our manuscript. (Complex fistula perianal is defined by those with more muscle involvement, or anterior fistulas in female patients, as well as recurrent fistulas, suprasphincteric fistulas, extrasphincteric fistulas, horseshoes fistulas, fistulas associated with irritable bowel disease, transsphincteric fistulas that involve greater than 30% of the external sphincter and those associated with pre-existing fecal incontinence, inflammatory bowel disease, radiation, malignancy, or chronic diarrhea.

Comment 2: Page 1, last line. "…complex PF, which often causes faecal incontinence…" is not well written. It is not frequent that the PF causes and incontinence, unless the PF soils;

Response: We have re-written this part according to the Reviewer's suggestion. (The most severe form is complex PF, which is difficult to manage with a high rate of recurrence and may cause sphincter damage and fecal incontinence.)

Comment 3: Faecal incontinence could be associated to PF and condition its treatment or could be a devastating consequence of PF surgery. It must be

better explained the importance of FI.

Response: We have elaborated on that in more severe cases of Complex PF, faecal incontinence can occur furthering morbidity. Complex PF is often not permanently cured by surgery, leading to multiple procedures and complications such as faecal incontinence. So, faecal incontinence is associated to complex PF and condition its treatment and also a devastating consequence of PF surgery.

Comment 4: Page 2: "In addition, the risk of the development of neoplasia in patients with complex PF is considered related to perianal disease duration [4]." I think it could be better presented as: "there is a risk of developing a neoplasm in the PF area related with the complexity and perianal disease duration…"

Response: We agree with you and have incorporated this suggestion throughout our paper.

Comment 5: Page 2: Patients with complex PF tend to have poor treatment outcomes or experience frequent relapses, and most interventions are ineffective in providing long-term healing. It could be interesting to provide a range of these mentioned published results. I.e "long term healing under 60% for complex PF…" (not real data).

Response: As suggested by the reviewer, we have added more published results:

(1) Aguilera-Castro L, Ferre-Aracil C, Garcia-Garcia-de-Paredes A, Rodriguez-de-Santiago E, Lopez-Sanroman A. Management of complex perianal Crohn's disease. *Ann Gastroenterol*. 2017;30(1):33-44.

(2) Panes J, Reinisch W, Rupniewska E, et al. Burden and outcomes for complex perianal fistulas in Crohn's disease: Systematic review. *World J Gastroenterol*. 2018;24(42):4821-4834.

(3) Pedersen KE, Lightner AL. Managing Complex Perianal Fistulizing Disease. J Laparoendosc Adv Surg Tech A. 2021;31(8):890-897.)

Comment 6: The following two sentences in that paragraphs start by the same word (additionally), try to use a synonym.

Response: We have rewritten this sentence (~~Additionally~~ Immunomodulators can have serious side effects. Additionally, there is a risk of opportunistic infection associated with the use of biological treatments.)

Comment7: Page 2 last paragraph: [Complex PF is thought to arise from an epithelial defect, which may be caused by ongoing inflammation. As we have mentioned before, Current treatments frequently cannot maintain long-term healing of the disease. Possible alternative treatments include cell therapy, especially MSC therapy. The most performed approach to deliver MSCs is local administration. After being delivered] I suggest consider adding the highlighted sentences.

Response: Thanks for your suggestion. We have added the highlighted sentences in our manuscript. (The most performed approach to deliver MSCs is local administration).

Comment 8: Page 3: [However, the efficacy and safety evaluation time of the study was short and middle term [8].] I think that 2 years of follow-up could be considered more than "short-term".

Response: We agree with you and have incorporated this suggestion in our manuscript. (In 2020, to evaluate whether local MSC therapy for complex PF is effective and safe, we conducted a meta-analysis. That study, with a follow-up of 8 weeks to 2 years, showed that local MSC therapy for complex PF was safe and feasible. However, the efficacy and safety evaluation time of the study was short and middle term).

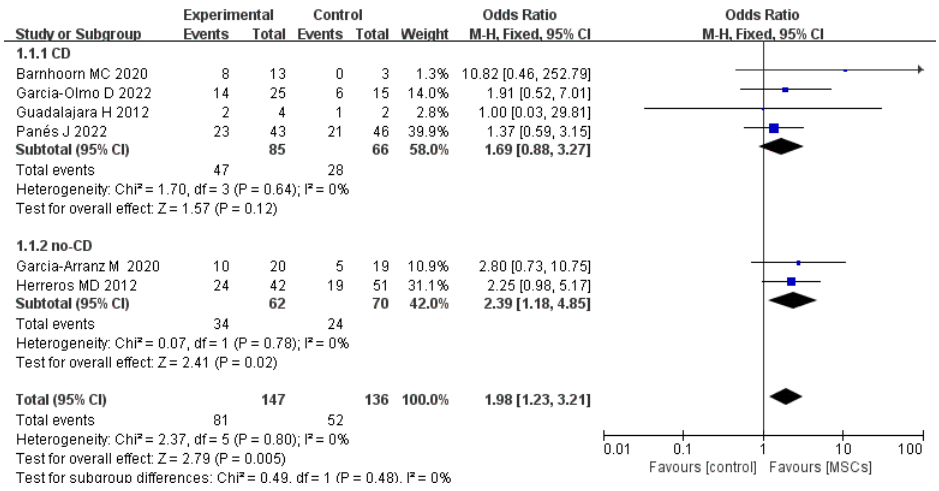
Comment 9: A global commentary… considering the results published by García Olmo D research network, it seems that there are different scenarios to

be considered, or maybe relevant to analyse separately: Autologous MSCs seem to be less effective than allogeneic MSCs. Crohn´s patients maybe have better outcomes with MSCs.

Response: Thank you for this suggestion. It would have been interesting to explore this aspect. There were some limitations of our manuscript (the studies used MSCs of different origins (adipose tissue and bone marrow from autologous as well as allogeneic sources). Due to the limitations of current research, it is difficult to be analyzed. In the future research, we should pay attention to these unresolved questions (such as MSC origin, dosage and modality of intervention) to ensure that PF patients receive optimal treatment. And, in the future, we need more RCTs aim to compare the efficacy of autologous and allogeneic MSCs in the treatment of PF.

Comment 10: It could be possible to perform separated analysis considering these situations (i.e Crohn versus no Crohn?). Maybe it could impact the observed findings…

Response: Thank you for this suggestion. It would have been interesting to explore this aspect. We're trying to subgroup analyze long-term effectiveness of MSCs for complex PF (Crohn versus no Crohn). The pooled analysis showed that no Crohn group (OR=2.39; 95% CI 1.18, 4.85; P= 0.02) has greater long-term efficacy than Crohn group (OR=1.69; 95% CI 0.88, 3.27; P= 0.12). But, due to the limitations of our study (only 2 articles of no-Crohn')), the results may be distorted. On the other hand, there is lack of RCT studies comparing MSCs in the treatment of CD and no CD. Therefore, the result is not worth promoting.



Comment 11: I suggest to include at the beginning a cite to the seminal article in this field from García Olmo D in 2003 (Int J Colorectal Dis. 2003 Sep;18(5):451-4. doi: 10.1007/s00384-003-0490-3).

Response: As suggested by the reviewer, we have included at the beginning of DISCUSSION a cite to the seminal article in this field from García Olmo D in 2003 (Int J Colorectal Dis. 2003 Sep;18(5):451-4. doi: 10.1007/s00384-003-0490-3).

Comment 12: Page 10, when authors speak about MSC significantly improves QoL… It could be useful to compare with long-term data of other available therapeutic options if possible (in terms of effectiveness, QoL, healing rate,…)

Response: Thank you for this suggestion. We have incorporated this suggestion in our manuscript. There were also some studies showed that in patients receiving MSCs transplantation, the closure rate of PF is significantly higher and the time to closure significantly shorter compared to anti TNF drugs and fistulotomy, and to a decrease in the frequency of recurrence of the disease.

(1): Park MY, Yoon YS, Lee JL, et al. Comparative perianal fistula closure rates following autologous adipose tissue-derived stem cell transplantation or treatment with anti-tumor necrosis factor agents after seton placement in patients with Crohn's disease: a retrospective observational study. Stem Cell

Res Ther. 2021;12(1):401. Published 2021 Jul 13.

(2): Knyazev OV, Fadeeva NA, Kagramanova AV, et al. Stem Cell Therapy for Perianal Crohn's Disease. Ter Arkh. 2018;90(3):60-66.

(3): Park MY, Yoon YS, Kim HE, et al. Surgical options for perianal fistula in patients with Crohn's disease: A comparison of seton placement, fistulotomy, and stem cell therapy. Asian J Surg. 2021;44(11):1383-1388.)

Comment 13: Page 11 first paragraph, authors speak about FG: [The use of FG has been found to be uniformly safe, with minimal adverse effects, an early return to normal activity, and no negative impact on continence]. It could be useful to add a commentary about FG alone y PF in the literature, short and long term.

Response: Thank you for your suggestions. I have supplemented the commentary about FG alone was effective for the treatment of PF in our manuscript. (Included short and long term effective).

Comment 14: Page 11, last paragraph: [Notably, this is a minimally invasive surgery (with curetted fistula, locally injected MSCs, and closed internal opening) and does not produce faecal incontinence]. Some technical aspects appear to be essential to obtain the better results as it has been published by Georgiev-Hristov et al. J Gastrointest Surg. 2018 Nov;22(11):2003-2012. doi: 10.1007/s11605-018-3895-6.

Response: Thank you for this suggestion. We have detailed the technical aspects of MSCs injection, and cited this published literature by Georgiev-Hristov et al. J Gastrointest Surg. 2018 Nov;22(11):2003-2012. doi: 10.1007/s11605-018-3895-6.

Comment 15: Page 13: [In addition, all eligible patients suffering from complex PF may have branches with multiple tracks involving an extensive area that

cannot always be adequately treated with a fixed dose of cells. Maybe the cell dosage must be related to the length of fistula tracts and cavities. In future research,] I suggest adding highlighted sentence.

Response: Thank you for this suggestion. We have added the highlighted sentences in our manuscript. (Maybe the cell dosage must be related to the length of fistula tracts and cavities.)

Comment 16: Page 13, last paragraph (about limitations). [(3) All patients underwent surgical procedures. This may be beneficial to the clinical remission of the fistula and cause our results to be overestimated]. Which surgeries? Explain better… For example Garcia-Olmo et al proposed and performs minimally aggressive surgeries (curettage + internal opening closure) not comparable to the standard surgical procedures (fistulectomy, flaps, LIFT,….). It is a very interesting issue to remark, with a very minimal aggressive procedure, with minimal risk for continence, MSCs obtain similar or better results to surgery…

Response: In our study, the surgical protocol for the treatment of PF with MSCs include such as deep curettage. Deep curettage may have a positive effect on fistula closure. However, some studies observed an increased number of long-term recurrences among control participants. Therefore, we speculate that the inflammatory focus persists, deep curettage may not provide lasting resolution. So, we have re-written this part according to the Reviewer's suggestion. (All patients underwent surgical procedures such as deep curettage. This may be beneficial to the short-term clinical remission of the fistula. But, whether deep curettage will benefit long-term healing is currently uncertain.)

Comment 17: Number 4: the first author is Panes, not Anes.

Response: We are very sorry for our incorrect writing, and we have corrected the "Anes" into "Panes".

Reviewer #2:

Comment 1: Language Quality: Grade B (Minor language polishing)

Response: We have re polished our article, and we have provided the English Language Certificate issued by a professional English language editing company. We hope the revised manuscript could be acceptable for you.

Specific comments from EIC:

First: Figure titles must carry self-explanatory information. An ideal figure title should give complete information to the reader even without reading the text. The figure should be provided with a governing title followed by the descriptive interpretation of panel contents. All the figure legend descriptions were not written in keeping this point in mind in the current manuscript version. e.g., "Figure 4 Long-term effectiveness of mesenchymal stem cells plus fibrin glue for treating complex perianal fistula. MSCs: Mesenchymal stem cells; CI: Confidence interval." In the text: "Long-term efficacy of MSCs for complex PF (MSCs + FG vs FG alone) Cell therapy strategies using MSCs carried in FG have shown promising results in regenerative medicine. The biological properties of FG as a growth environment for MSCs have been reported in several studies[20]. However, the use of local FG plus MSC therapy in complex PF cases is not supported by sufficient evidence. In our study, three studies were identified[14,15,17], with low heterogeneity between the studies (I2 = 0%). In a fixed-effects model, MSCs plus FG had more long-term efficacy for fistula healing than FG alone (OR = 2.30; 95%CI: 1.21, 4.36; P = 0.01) (Figure 4)." Neither the text nor the figure legend gave sufficient details of why and what, and how these studies should be interesting and of importance. The above text of conflicting: "However, the use of local FG plus MSC therapy in complex PF cases is not supported by sufficient evidence. In our study, three studies were identified[14,15,17]," – what did they mean by "our study?" In fact, none of the citations of 14, 16, or 17 was "THEIR study."

Response: We have re—written some figure titles of our manuscript. And, we also have re—written this part according to the editor-in-cheif's suggestion.(Cell therapy strategies using MSCs carried in FG have shown promising results in regenerative medicine. FG is a natural polymer involved in the coagulation process. In regenerative medicine, FG can be used as a delivery system for drugs, biomolecules, growth factors and cells. FG also provides a temporary structure that favors angiogenesis, extracellular matrix deposition and cell-matrix interactions and it also FG maintains the local and paracrine functions of MSCs, providing tissue regeneration through less invasive clinical procedures. The biological properties of FG as a growth environment for MSCs have been reported in several studies[20]. Now, local FG combined with MSCs therapy is still a relatively new treatment and has not yet gained popularity. So,the need for the local FG combined with MSCs therapy remains unknown. In our meta-analysis, three studies were included that reported local FG combined with MSCs therapy for PF [14,15,17], with low heterogeneity between the studies (I2 = 0%). In a fixed-effects model, MSCs plus FG had more long-term efficacy for fistula healing than FG alone (OR = 2.30; 95%CI: 1.21, 4.36; P = 0.01) (Figure 4). So, we think local FG combined with MSCs therapy have synergistic effect on PF.) Second, their definitions of the terms crawl around the pages without strict boundaries. E.g., what was the QC of MSC used in each study by what standard and from what sources?

Response: Our meta-analysis is the first to evaluate the long-term efficacy and safety of MSCs for PF treatment. Inevitably, this article has some limitations: (1) The studies

used MSCs of different origins (adipose tissue and bone marrow from autologous as well as allogeneic sources).(2) diferent dosages and modalities of administration. So, our study was limited by its multiple centers and heterogeneity in the study inclusion criteria, mesenchymal stem cell origin, dose and frequency of delivery, and definition and time point of fistula healing. In the future, more patients must be evaluated in long-term follow-ups to optimize the efficacy and safety of MSCs for PF treatment. Third, "The key long-term therapeutic goals for the treatment of complex PF are to: (1) Resolve fistula discharge; (2) achieve fistula healing; (3) prevent fistula recurrence; (4) maintain fecal continence; (5) avoid long-term diversion (protectomy with stoma), and hence; and (6) improve and maintain QoL for patients." They should make a table of these elements and compare these criteria with their citations that specifically met them.

Response: Thank you for this suggestion. It would have been interesting to explore this aspect. We have proposed the key long-term therapeutic goals for the treatment of complex PF in our manuscript. Our study aimed to explore the long-term effectiveness and safety of MSC therapy for complex PFs. The healing rate (HR) of perianal fistula was regarded as the main endpoint in our meta-analysis. Due to the limitations of current research, it is difficult to be analyzed the all key long-term therapeutic goals. Fourth, clarity is missing in certain spots. E.g., "Therefore, fecal incontinence is associated with complex PF and not only affects its treatment but also is a devastating consequence of PF surgery" [out of context]. "In recent years, local injection of mesenchymal stem cells (MSCs) has shown notable promising results in the treatment of PFs[11]. MSCs are a heterogeneous subset of stromal stem cells. They can be isolated from a wide variety of tissues and expanded in vitro to obtain large quantities. MSCs are characterized by multilineage differentiation and powerful immunomodulatory effects and are able to mitigate inflammatory states." what was the QC of MSCs of what organs were used in each study by what standard and from what managements?

Response: (1) We haved removed "Therefore, fecal incontinence is associated with complex PF and not only affects its treatment but also is a devastating consequence of PF surgery" in our study.(2) In our article, of the final 6 studies selected for inclusion in the review. Guadalajara et al[14], Herreros et al[15] and Garcia-Arranz et al[16], patients received at least one dose of autologous adipose-derived mesenchymal stromal cells. Barnhoorn et al[17], patients received a single administration of allogeneic bone marrow-derived mesenchymal stromal cell. Panés et al[18] and Garcia-Olmo et al[19], patients received a single administration of allogeneic adipose-derived mesenchymal stem cells. Fifth, "To our knowledge, this is the first meta-analysis to evaluate the long-term safety and efficacy of local MSC therapy for complex PFs" – what was the definition of the meta-analysis" if they got only "After screening, 6 studies met the inclusion criteria?" such as the sources of stem cells, the routes of administration, the effect criteria or the outcome measures should be consistent. E.g., Table 1 did not specify the nature of the sources: ASCs, BMSCs, are different in stem

cell capacity. Neither could the authors draw comparisons nor specify the differences but stated MSCs as a generic agent. Again, this statement is misleading, as you can compare apples and oranges.

Response: Our study aimed to explore the long-term effectiveness and safety of MSCs therapy for complex PFs. After all criteria were applied, 6 articles were included in our meta-analysis. MSCs can be isolated from various tissues including the bone marrow, adipose tissue, and human umbilical cord. Due to the easy gain property of adipose tissue-derived stem cells (ASCs) and the low immunogenicity property of bone marrow-derived stem cells (BMSCs) compared with other stem cells, both ASCs and BMSCs are the main stem cells to treat fistulas. Our study showed that both ASCs and BMSCs can improve the HR. But only one BMSCs study was included in our meta-analysis. This is the limitation of our study. It also should be noted that human adipose tissue is plentiful and easily accessible, and contains a large number of stem cells compared to other tissues in the body. Obtaining adipose tissue minimizes side efects on donors (regardless whether patient or healthy donor). To obtain bone-marrow MSCs in some special donors such as patients with a history of myocardial infarction is dangerous. There were differences between bone-marrow-and adipose tissue-derived MSCs.There were some studies suggests that adipose tissue-derived MSCs might be superior to bonemarrow MSCs in suppressing immune responses in vitro. Therefore, adipose tissue-derived MSCs may can be considered a priority in the future. There were only one study used bone-marrow-derived MSCs, which was a limitation of our study. Due to the limitations of current research, it is difficult to directly compare the efficacy of BMSCs and ASCs.So, limitations of current studies and the optimal dosage and source of MSCs for the treatment of fistula remained unclear. But, we should know different sources, different differentiation properties and regeneration capacity of MSCs, and different cell isolation and culture and the patient's age, severity of the disease, and other factors may also affect the efficacy of MSCs. Standardization is crucial to assess the efficacy of current and future local MSC treatment strategies.In the future,more RCTs should compare their effcacy and safety. We have explained the limitations of our study in the discussion section of our manuscript (such as: (1) the studies used MSCs of different origins (adipose tissue and bone marrow from autologous as well as allogeneic sources),(2) different criteria for defining fistula healing were applied in the studies, (3) the number of included studies and the sample size were limited, and extrapolation of the meta-analysis results was limited to some extent. So, our study was limited by its multiple centers and heterogeneity in the study inclusion criteria, mesenchymal stem cell origin, dose and frequency of delivery, and definition and time point of fistula healing. In the future, more patients must be evaluated in long-term follow-ups to optimize the efficacy and safety of MSCs for PF treatment. Sixth, Discussion: "We speculate that for localized digestive tract diseases, local application and delivery seems more logical because side effects can be minimized and the cells are kept in direct contact with the at-risk tissue. Therefore, local MSC therapy seems to be a more promising treatment approach for further research. In our study, all eligible patients received a fixed dose of MSCs (one-time local injection or a second

dose). Although most patients in our study received two doses of MSCs, some studies have indicated a relationship between cell dose—or even the number of doses and efficacy [35,36].” Neither citations #35 nor #36 belong to the authors’ study, which misled the readers. Thus, their discussion did not tie in with their results.

Response: We have re—written this part in our manuscript according to the editor-in-cheif’s suggestion. Seventh, A meta-analysis is a statistical method used in research that systematically analyzes and combines data from multiple independent studies on a specific topic or research question. It is a quantitative approach that aims to provide a more comprehensive and precise estimate of the effect or relationship being investigated by pooling data across studies. A reader comes to read such a study, expecting to gain vision in the field, which is beyond the regurgitalith of the original articles; however, this manuscript did not show such vision. Neither did they identify any problems, nor did they provide solutions.

Response: Local mesenchymal stem cell (MSC) therapy for complex perianal fistulas (PFs) has shown considerable promise. But, the long-term safety and efficacy of MSC therapy in complex PFs remain unknown. Our study aimed to explore the long-term effectiveness and safety of MSC therapy for complex PFs (48 wk to 4 years of follow-up after MSC therapy). Although there have been only 6 trials conducted with control arms, existing data demonstrate MSC treatment is a safe and effective method that can significantly improve the long-term healing of complex PFs, and this method confers no risk of MSC-related AEs. The limitations of our study were that the study was limited by its multiple centers and heterogeneity in the study inclusion criteria, mesenchymal stem cell origin, dose and frequency of delivery, and definition and time point of fistula healing. Considering the limitations mentioned above, and extrapolation of the meta-analysis results was limited to some extent. However, it provides a certain guiding direction for future clinical practice. In the future, more patients must be evaluated in long-term follow-ups to optimize the efficacy and safety of MSCs for PF treatment.