Reviewer #1:

Reviewer: The manuscript focuses on the potential of stem cell therapy for treating skeletal muscle atrophy, a condition characterized by the loss of muscle mass due to various physiological and pathological factors, including aging, injury, disuse, or diseases like amyotrophic lateral sclerosis (ALS) and muscular dystrophies. The article emphasizes the current lack of effective treatments for muscle atrophy and explores how stem cell-based therapies could fill this gap. I would like to recommend its publication pending the address of the following comments:

1. The current title is somewhat generic. Consider making it more specific to reflect the core focus of the paper.

Response: Thank you for your kind suggestion. We have changed the title into "Stem Cell Therapy: A Promising Therapeutic Approach for Skeletal Muscle Atrophy".

2. The introduction covers various aspects of muscle atrophy, but the flow is somewhat disjointed. Consider reorganizing the content to ensure a logical sequence: start with the significance of muscle atrophy, introduce existing challenges, and then transition into the potential of stem cell therapies. This will provide a smoother flow of information.

Response: thank you for your valuable suggestion. We have re-organized the sentences of the instruction.

3. The transitions between sections are somewhat abrupt. For instance, after discussin muscle atrophy mechanisms, the manuscript directly moves into treatment strategies. Consider adding transitional sentences or paragraphs to connect topics smoothly, perhaps using rhetorical questions or summary statements to guide the reader.

Response: thank you for your valuable suggestion. We have made corresponding revisions.

4. The discussion section currently lacks depth, particularly regarding the limitations of stem cell therapy (such as immune rejection, ethical concerns,

and clinical challenges). Expanding this section with a critical analysis of these issues and proposing actionable future research directions will strengthen the manuscript's impact.

Response: thank you for your valuable suggestion. We have made corresponding revisions.

5. The formatting of references is inconsistent and adding references to recent studies in the field will also enhance the manuscript's credibility.

Response: thank you for your kind suggestion. We have made corresponding revisions and added recent studies.

6. Ensure that every significant claim is supported by a specific reference. Additionally, updating the references to include more recent studies (2023-2024) will increase the manuscript's relevance.

Response: thank you for your kind suggestion. We have made corresponding revisions and added recent studies.

7. The discussion section currently lacks depth, particularly regarding the limitations of stem cell therapy (such as immune rejection, ethical concerns, and clinical challenges). Expanding this section with a critical analysis of these issues and proposing actionable future research directions will strengthen the manuscript's impact.

Response: thank you for your valuable suggestion. We have made corresponding revisions.

Reviewer #2:

The review of Wang et al. entitled 'The role of stem cells in the treatment of skeletal muscle atrophy' aims to address stem cell-based cell therapy and stem cell derivative therapy to treat muscle atrophy. It would be beneficial to provide a more detailed summary of progress in intricate molecular mechanisms underlying skeletal muscle atrophy and provides an overview of

current therapeutic approaches, with particular emphasis on mesenchymal stem cells, induced pluripotent stem cells, and their derivatives for treating skeletal muscle atrophy. This review is nicely designed and written clearly. However, this is my advice to fix some points in the manuscript:

1 In Section 1, the author provides a comprehensive review of the current molecular mechanisms and associated signaling pathways that lead to muscle atrophy. It is suggested that the author summarize these molecules through a cartoon diagram, which would allow readers to more clearly understand the molecular mechanisms underlying muscle atrophy.

Response: thank you for your kind suggestion. We have drawn a new figure 2.

2 In Section 2, there appears to be a logical issue: the text first discusses pharmacological treatment and then addresses rehabilitation therapy. Is there a relationship between these two approaches in the context of treating muscle atrophy? Since the author mentions that rehabilitation therapy is the major clinical treatment for muscle atrophy, and anti-inflammatory and antioxidant drugs have been mostly used in pre-clinical studies, their efficacy and safety in humans need further investigation. Given this, why not discuss rehabilitation therapy first?

Response: thank you for your valuable suggestion. We have made corresponding revisions.

3 In section3, ". Due to their excellent properties such as unlimited proliferation and directed differentiation," Unlimited proliferation is not the rationale behind the suitability of stem cells for the treatment of muscle atrophy. For instance, the UC-MSCs at lower passages demonstrate superior therapeutic efficacy in vivo on mice with acute graft-versus-host disease, suggesting that excessive proliferation of MSCs may impair their therapeutic outcomes (PMID: 31779707).

Response: thank you for your valuable commend. We agree with the reviewer

that the unlimited proliferative potential of stem cells is not one of the fundamental principles in the treatment of muscular dystrophy. We have made corresponding revisions.

4 In the section 3.1, the author has provided a detailed description of the advancements in the treatment of muscle atrophy using MSCs derived from three different tissue sources. MSCs from these sources can effectively alleviate the symptoms of muscle atrophy and maintain muscle homeostasis by regulating local inflammation, promoting muscle regeneration, and protecting the nerves that innervate muscles. Current literature indicates that MSCs from different origins exhibit heterogeneity, and these differences may impact their therapeutic efficacy in treating muscle atrophy. It is recommended that the author discusses the potential influence of these heterogeneities on the treatment of muscle atrophy with MSCs (PMID: 38124129).

Response: thank you for your valuable commend. We have added the discussion of the potential influence of heterogeneity in "MSCs in the treatment of muscle atrophy" and "Challenges of stem cell therapy".

Reviewer #3:

This paper aims to offer a comprehensive overview of the current understanding of stem cells in treating skeletal muscle atrophy. The authors provide background information on muscle atrophy and its correlation with specific diseases, while briefly addressing the underlying mechanisms of muscle atrophy development, as the focus of the review is primarily on treatment options. The technical aspects of the paper, particularly the figures, are commendable, offering clear visual representations of the molecular mechanisms and therapeutic approaches discussed. The timing of this review is opportune, and I have several recommendations to enhance its impact.

1. The mechanisms of muscle atrophy resulting from denervation, including

reduced neurotrophic factor availability and decreased activity levels, should also be considered.

Response: thank you for your valuable commend. We have added the information and relative reference.

2. The authors have not presented any evidence to support the use of hematopoietic stem cells in the treatment of muscle atrophy. Please include this section.

Response: thank you for your kind suggestion. Hematopoietic stem cells represent a therapeutic cell therapy that is primarily employed in various blood disorders to reconstitute the entire blood and immune system following transplantation. A Study has demonstrated that the transplantation of wild-type mouse hematopoietic stem cells and progenitor cells (HSPCs) into Friedreich's ataxia YG8R mice mitigated muscle atrophy[1]. Additionally, recent research suggests that the gene therapy of HSPCs mediated by lentiviral vectors can serve as a novel approach for treating the hereditary neuromuscular disorder Pompe disease [2]. We have made corresponding revision.

3. The cited references are insufficient. Bodine SC, Sinha I, Sweeney HL. Mechanisms of Skeletal Muscle Atrophy and Molecular Circuitry of Stem Cell Fate in Skeletal Muscle Regeneration and Aging. J Gerontol A Biol Sci Med Sci. 2023;78(Suppl 1):14-18.

Response: thank you for your kind suggestion. We have added some latest references.

4. Has stem cell therapy for skeletal muscle atrophy been applied in preclinical research? Are there any ongoing clinical trials involving the application of stem cell treatments? If so, please provide a list to facilitate assessment of translational progress.

Response: Stem cell therapy for skeletal muscle atrophy has been extensively utilized in preclinical studies. Nevertheless, several limitations, such as potential immune rejection, the risk of tumor formation, and the absence of consensus or standards regarding the route of administration, dosage, and duration of treatment, have impeded the clinical application of stem cells and their derivatives in the treatment of skeletal muscle injury. There are only limited clinical trial reports, and they are mainly regarding the therapy of muscular atrophy in some intractable genetic motor neuron diseases, such as ALS and SMA [3-6]. More clinical trials are necessary in the future to further investigate the efficacy of stem cells and their derivatives for various types of muscular atrophy. We have added the information in the manuscript.

5. Table 1 should provide more comprehensive information, encompassing animal models and types of muscle wasting diseases.

Response: thank you for your kind suggestion. We have added the information in the table.

6. "Although the use of MSCs has some advantages over conventional treatments in clinical trials, most of them fail to achieve the expected therapeutic effects" If the author intends to draw such a conclusion, it is imperative to incorporate relevant literature in support of this perspective.

Response: thank you for your kind suggestion. We have added corresponding revisions.

7. "Cell-free therapies based on stem cell derivatives have better prospects compared to conventional cell transplantation therapy" This conclusion also requires additional evidence for substantiation.

Response: thank you for your kind suggestion. We have added corresponding revisions.

8. There are still some errors in the manuscript writing. Please revise the grammar and other aspects of the manuscript. It is recommended to choose a native speaker to write the manuscript for better quality.

Response: thank you for your kind suggestion. We have polished the language.

1. Rocca CJ, Goodman SM, Dulin JN, Haquang JH, Gertsman I, Blondelle J, Smith JLM, Heyser CJ, Cherqui S: **Transplantation of wild-type mouse hematopoietic stem and progenitor cells ameliorates deficits in a mouse model of Friedreich's ataxia**. *Sci Transl Med* 2017, **9**(413).
2. Yoon JK, Schindler JW, Loperfido M, Baricordi C, DeAndrade MP, Jacobs ME, Treleaven C, Plasschaert RN, Yan A, Barese CN *et al*: **Preclinical lentiviral hematopoietic stem cell gene therapy corrects Pompe disease-related muscle and neurological manifestations**. *Mol Ther* 2024, **32**(11):3847-3864.
3. Reis ALG, Maximino JR, Lage L, Gomes HR, Pereira J, Brofman PRS, Senegaglia AC, Rebelatto CLK, Daga DR, Paiva WS *et al*: **Proteomic analysis of cerebrospinal fluid of amyotrophic lateral sclerosis patients in the presence of autologous bone marrow derived mesenchymal stem cells**. *Stem Cell Res Ther* 2024, **15**(1):301.
4. Berry JD, Cudkowicz ME, Windebank AJ, Staff NP, Owegi M, Nicholson K, McKenna-Yasek D, Levy YS, Abramov N, Kaspi H *et al*: **NurOwn, phase 2, randomized, clinical trial in patients with ALS: Safety, clinical, and biomarker results**. *Neurology* 2019, **93**(24):e2294-e2305.
5. Forotti G, Nizzardo M, Bucchia M, Ramirez A, Trombetta E, Gatti S, Bresolin N, Comi GP, Corti S: **CSF transplantation of a specific iPSC-derived neural stem cell subpopulation ameliorates the disease phenotype in a mouse model of spinal muscular atrophy with respiratory distress type 1**. *Exp Neurol* 2019, **321**:113041.
6. Mohseni R, Hamidieh AA, Shoae-Hassani A, Ghahvechi-Akbari M, Majma A, Mohammadi M, Nikougoftar M, Shervin-Badv R, Ai J,

Montazerlotfelahi H *et al*: **An open-label phase 1 clinical trial of the allogeneic side population adipose-derived mesenchymal stem cells in SMA type 1 patients**. *Neurol Sci* 2022, **43**(1):399-410.

Revision reviewer:

I have no additional comments; the article is deemed suitable for publication. Congratulations.

Response: Thank you for your comments.