Reviewer #1:
Scientific Quality: Grade C (Good)
Language Quality: Grade A (Priority publishing)
Conclusion: Minor revision
Specific Comments to Authors: This review is based on 38 articles in the clinical trials of stem cell treatment of spinal cord injury. The evaluation and review of "Operative Considerations in Spinal Cord Stem Cell Therapy" in terms of treatment time, administration method, and number of treatments are carried out. And it suggests that longer treatment paradigms soon after injury may be most beneficial. The conclusions of the review can provide scientific data support for further clinical research, but in some aspects, there are still some problems. This article is short in length. Although it can clearly show the key analysis content, many details have not been specifically explained and discussed. The description is not complete. It is recommended that the description of the number of cells injected, the effects of the acute, subacute and chronic phases of SCI, and the time when cell therapy begins to intervene is to be complete, or some possible analysis reasons can be added.

We thank the reviewer for their comments. The article was meant to be a concise description of operative techniques. We do agree that more in depth explanation for the effects of chronicity (acute/subacute/chronic) and the number of cells injected could be undertaken. To address this we have done a few things.

- First we have broadened table 1 from 14 representative articles to 29 articles making it a complete description of all clinical trials in SCI.
- Second we have substantially added to the section “time from injury to treatment”. This section now reads:

“Time from injury to treatment

Generally SCI is characterized based on chronicity into acute, subacute and chronic phases. While the time course for each period is highly variable, the acute phase generally spans days post-injury, the subacute phase weeks post-injury and the chronic phase months post-injury. Although some studies in chronic SCI show improvement following stem cell injection, [8,11] studies in the acute or subacute SCI population seem to show more dramatic return of function.[10,21,22] A study by Yoon et al. stratified patients into acute (<13 days), subacute (14 days to 8 weeks) and chronic (> 8 weeks) groups with all patients receiving intramedullary injection of BM-MSCs with systemic GM-CSF treatment. Notably 30.4% of the acute and subacute treated patients improved AIS grade (AIS A to B/C) while none of the chronic patients showed any improvement.[23] This was not limited to intramedullary cell transplantation. Bansal et al. demonstrated that lumbar puncture for delivery of BM-MSCs led to ASIA grade improvement in 6/10 patients who were less than 6 months from injury with no patients over 6 months from injury achieving functional improvement.[22]

A study by Cheol Shin et al, used human fetal tissue derived neural stem cell progenitor cells and had both a control group and an intramedullary cell transplantation group. Notably while 26% of patients in the transplantation group had AIS grade improvement, only 6.6% had improvement in the control group.[20] This disparity between transplantation and control group patients was also seen by Karamouzian et al. These authors injected purified BM-MSCs via lumbar puncture and noted that 45 patients in the cell transplantation group had AIS improvement compared to 15% in the control group ($p=0.09$).[24] This helps answer the major critique that some degree of functional improvement happens in the acute/subacute period naturally and may explain the functional improvements seen in the acute to subacute cell transplantation studies. Also the fact that both studies with control groups used different stem cell types and different methods of injection (lumbar puncture vs. intramedullary injection) also highlights the importance of the treatment window versus mechanism of treatment. This is further supported by the studies by Bansal et al. and Yoon et al. These studies had patients in multiple treatment windows, with intramedullary or lumbar puncture delivered BM-MSCs and noted that patients in the acute to subacute period from injury achieved greater functional improvement.[22,23] In fact, of the studies that focused on the

acute/subacute SCI population, only one out of seven studies showed no motor or functional improvement with the rest having a subset of patients that had AIS grade improvement. The single study with no functional improvement notably used a novel form of stem cells derived from peripheral schwann cells and also included subjects from 4-7 weeks following injury - generally on the upper limit as compared to other acute/subacute studies. [25]"

- We have also added the following to answer the question regarding the number of cells injected.

"Furthermore, understanding the number of cells transplanted and its effect on outcomes is more difficult. Various trials have transplanted cell numbers ranging from $1x10^5$ to $40x10^7$. There was no consistent relationship between cell number and outcome over the trials reported."

Reviewer #2:
Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)
Conclusion: Accept (General priority)
Specific Comments to Authors: Good systematic review on spinal injuries and therapy

We thank the reviewer for their time and comment.

Reviewer #3:
Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)
Conclusion: Accept (General priority)
Specific Comments to Authors: This is a well written summary of the available literature on stem cell transplantation in human clinical trials. My one concern lies in your recommendation that debridement of the scar tissue may be beneficial to the ultimate outcome. Although one clinical trial suggested this as a possibility, most animal data shows worsening of neurologic condition. This should at least be discussed.

We agree this is a controversial statement. To further support this finding we have added two other clinical trials to support this statement and we have tempered the overall language to be more reflective of the actual surgical techniques described. The current paragraph now reads:

"Whether the preservation of existing spinal cord tissue is technically necessary is up for debate. While the previously described studies focused on chronic SCI, a study by Xiao et al. in acute SCI patients completely excised the necrotic spinal cord around the SCI lesion. The authors placed a collagen scaffold impregnated with human umbilical cord MSCs in this area and showed nerve conduction across the SCI lesion plus functional improvement from ASIA A to ASIA C in two patients.[12] This study built off previous work by Lima et al. where necrotic scar tissue was excised and an olfactory mucosal autograft was placed. Importantly, this radical technique of laminectomy, scar excision and mucosal autograft replacement in chronic cervical and thoracic SCI led to marked functional improvement. In the earlier study by Lima et al. 28% of patients had recovery of bladder sensation or voluntary anal contraction,[13] while in the more recent study 55% of patients had ASIA improvement of at least 1 grade.[14] Importantly this second study involved intensive rehabilitation following autograft transplantation. It is important to note that olfactory mucosa grafts have been associated with spinal masses pointing to their increased regenerative potential but also to their increased side effect profile.[15,16] As a technical consideration, excision of scar may allow for increased regeneration of neural white matter tracts and may be an

important technical consideration even with other injection techniques that do not use a graft substrate or scaffold."

Reviewer #4:
Scientific Quality: Grade D (Fair)
Language Quality: Grade B (Minor language polishing)
Conclusion: Major revision
Specific Comments to Authors: This review analyze efficacy of operative techniques and technical considerations of stem cells delivery for treatment of spinal cord injury (SCI) performed in phase I/II of clinical trials. The Authors state that 38 papers from PubMed meet the criteria for clinical trials selected for "spinal cord injury" and "stem cell", and that these articles included all published results from the active and completed clinical trials. However, the Table 1 summarize results from only 14 papers.
We thank the reviewer for their time and comments. We agree this was a shortcoming and have rectified table 1 to now include all clinical trials that resulted from the search (29).

Functional improvement was observed when intervention was performed in the acute to subacute treatment phase and when multiple stem cells injections were utilized. There is a lack of information on biological properties of MSCs injected. Applied MSCs were isolated from different tissue sources, this also have an impact on the desired regenerative response. For effective SCI treatment, important role plays not only operative technique and routes of MSCs delivery but also tissue source and biological properties of MSCs. This issue need to be discuss.
We think this is a very important point. It is, however, beyond the scope of this paper as multiple other manuscripts have reported on the biological properties of stem cells used (example: 32985458). To address this we have added the following portion to our "Future directions" section.
"The aim of this manuscript is to help optimize clinical trial parameters, patient selection and cell transplantation techniques. Comparative clinical trials using different types of stem cells are necessary to determine what type of cells are most efficacious in improving patient functional outcomes."

To also further ensure we have addressed our aims - to accurately answer the time from injury and types of surgical techniques that are associated with improved outcomes, we have added the following sections as described in our response to Reviewers 1 and 3.

How functional assessment was performed? In analyzed clinical trials reported improvements in ASIA scores and motor function, was this performed at the similar time-point after MSCs injection? How long the patients were observed?
In most cases we have only reported on reproducible and consistent elements of functional improvement including ASIA score, voluntary anal contraction and motor or sensation scores. The motor improvements are consistently reported as motor function across key muscle groups. We have added this information to the manuscript.

Also we have examined all papers to see the follow-up period. All studies that reported functional outcomes (versus simply safety) had a follow-up period of greater than 10 months. We have added the following to the manuscript as well to help address this:

"Where motor improvement is reported, this references changes in key muscle group function based on a five point grading scale as standard in spinal cord injury literature. Follow-up across all clinical trials reporting functional outcomes, versus safety profile, was at a minimum of 10 months."

This manuscript is superficial and do not fully reflect the aim of this review. The Core Tip do not summarize presented analysis, need to be improved.

We have added substantially to the manuscript and hope it is able to answer the key questions related to operative techniques across stem cell transplantation clinical trials in spinal cord injury. We thank the reviewer for their time in revision.