

The present manuscript discusses the use of medical ozone to treat SOD1^{G93A} ALS disease. According to the presented data, model mice, treated with medical ozone, exhibit deceleration of trigeminal motoneuron degeneration, attenuation of microglial proliferation, and preservation of monocyte levels in mesenteric lymph nodes.

The work was carried out competently, yet there exist concerns over several points that are remarked below:

1. In the introduction, the statement “The discovery of a glycine to alanine mutation in the SOD1 gene in 1993 was the earliest description of a genetic cause associated with fALS [4]” should be corrected to read “The discovery of a glycine to alanine mutation in the SOD1 gene in 1993 was the earliest description of a genetic cause associated with fALS [4]”.
2. Throughout the manuscript, the term “motoneurons” should be replaced by “motor neurons”.
3. In the introduction, the statement “Contrarily, advanced stages of ALS are characterized by M1 microglia, which release pro-inflammatory cytokines ...” should be corrected to read “On the contrary, advanced stages of ALS are characterized by M1 microglia, which release pro-inflammatory cytokines ...”.
4. In lines 232-236 of the same section, the description of the statement “Human SOD1-related vacuolization was attenuated after medical ozone treatment in the motor trigeminal nucleus at end-stage (15.30 ± 2.81) when compared to sham treatment ($p = 0.03$), but not in the facial motor” was obscured by the Figure. The statement should be visible for evaluation.
5. In the legend of Figure 3, the various subfigures should have capital letters, designating the experiments, in line with the graphs themselves.
6. In section 2.4, “2.4. Therapeutic treatment of SOD1G93A mice with medical ozone attenuates 252 microglial reactions”, the discussion of the results projects figures 3A-3F. Such figures do not exist. Do the authors mean figures 4A-4F? Attention to detail should be given for a journal of this caliber.
7. In the legend of Figure 4, the various subfigures should have capital letters, designating the experiments, in line with the graphs themselves.
8. In section 2.5, the statement “Again, the number of lymphocytes as well as granulocytes in the mesenteric lymph nodes did not changed in an age- or treatment-dependent manner.” should be corrected to read “Again, the number of lymphocytes as well as granulocytes in the mesenteric lymph nodes did not change in an age- or treatment-dependent manner.”.
9. In the legend of Figure 5, the various subfigures should have capital letters, designating the experiments, in line with the graphs themselves.
10. In the discussion section, the statement “SOD1G93A mice that received bone marrow transplantation from mice deficient in myeloid differentiation primary response protein 88, MyD88, exhibited an earlier disease onset and a shorter survival compared to mice that received a normal bone marrow transplant [51].” should be re-written to read “SOD1G93A mice that have undergone bone marrow transplantation from mice deficient in myeloid differentiation primary response protein 88, MyD88, exhibited an earlier disease onset and a shorter survival compared to mice that received a normal bone marrow transplant [51].”.
11. The title of section 4.5 should be corrected to read “Magnetic Resonance Imaging”.

12. Toward the end of the discussion, the authors attempt to provide an explanation for the observed effects seen upon use of medical ozone (the composition of which is provided in the Materials and methods section) in the ALS model systems-animals employed. What one sees, however, is a very shallow explanatory description on how the mixture works, and to that end, how ozon works in the presence of oxygen. One would expect more than just a mere articulation of the effects observed and used in the face of the results presented. Based on the fact that molecular mechanisms of action are not clear at this point, an insightful molecular description (proposed model) would be useful for subsequent molecular studies.

On the basis of the aforementioned, the manuscript should be revised and re-evaluated prior to any consideration.