

## Reviewer: Yadira Palacios

### 1. Basic reporting

Comment on areas where the article fails to meet our standards, with suggested improvements. This text is sent to the author. Enter 'no comment' if you have nothing to add.

1. The presentation of the text, figures, and figure legends must be improved in quality and presentation. For example, the figures must homologate the lines of significance between groups. Figures 2, 3, and 4 are shown with superimposed panels and texts, which are not legible and displayed entirely out of order (unlike Figure 1). This should not happen, even in the manuscript revision stage.
2. The figure legends must also be approved in the content of the information. In particular, Figure Legend 4 presents a small description of the observed correlations, but it needs to be included for panel E. Additionally, it is suggested that the figure legend describes the statistical analysis carried out, the level of significance for all cases, the 'n' of each represented group, and also Rho for correlations.
3. The English must be improved. Likewise, space tabulations need to be included throughout the text.
4. Although an English version file from the Ethic Committee Approval is included, there is no marking that this is an official translation. In any case, it is a requirement that the Journal's editorial team must decide if it is necessary.

### 2. Experimental design

The work was carried out with a clear and straightforward experimental analysis that provides an interesting description of the identified cell populations. However, some aspects must be clarified.

1. Did the authors pre-tested for normal distribution of the data? Please describe to clarify the selection of the statistical analysis you performed.
2. Did the authors analyze the prevalence of comorbidities in COPD patients? As epidemiological studies have shown that COPD is frequently associated with comorbidities, it is essential to mention the prevalence in the studied population and include it in Table 1.
3. Additionally, the study considered the analysis of smoking association with the findings; however, other risk factors, such as diet and physical activity, could be relevant. Please comment if these factors were considered. If so, they should be included in Table 1. If not, please justify.

### 3. Validity of the findings

In the research from Mingqiang Zhang et al., the authors focus on the clinical significance of PD-1/PD-L1 axis expression in the accumulation of circulating MDSCs subsets, especially M-MDSCs, induced Tregs expansion and activation in COPD patients.

1. Although the study focuses on Treg cells, did the authors identify any other CD4+ T cell population that may be relevant? This could be interesting because, as the authors present in Figure 3I, PD1 is increased in the overall CD4+ population. So, this molecule is not exclusively overexpressed in Tregs. Please describe to clarify the discrimination of other CD4+ populations in the study.

2. As has been broadly described in several reports, Tregs are cells with dual roles in the modulation of immune responses, so this population does not present only a negative regulatory activity, as has been affirmed in line 219 of the manuscript.

3. The authors conclude that myeloid-derived suppressor cell recruitment, Treg accumulation, and up-regulation of CTLA-4 on Treg in COPD, accompanied by an increased level of PD-1/PD-L1, suggest MDSCs may induce the expansion of highly suppressive Treg cells through the PD-1/PD-L1 pathway.

The previous is in agreement with the observations done. However, do the authors have the possibility to evaluate any other immune inhibitory factor that may lead to a more specific mechanism in MDSCs? For example, when activated in a pathogenic situation, MDSC cells overexpress immune inhibitory factors such as nitric oxide synthase, arginase 1, and peroxynitrite. Please justify.

4. At the end of the introduction, the authors promise to associate the experimental results with the clinical significance. However, it is a promise that research does not fulfill. Beyond analysis in patient groups, a correlation of the speculated mechanism with clinical data is needed. This could be enriched with a figure that integrates the findings, in which cell populations, regulatory molecules, possible cellular mechanisms triggered, and their impact at a clinical level should be interrelated.

#### 4. Additional comments

Please make any general comments not covered by the 3 areas above. This text is sent to the author.

The work requires improving the quality of its presentation in the text, figures, table, and figure legends. Although the editorial team must supervise it, the authors should have previously supervised it.

An integrative idea is also necessary to describe possible molecular mechanisms that may be involved in both cell populations according to the molecules identified.