

PEER-REVIEW REPORT

Name of journal: World Journal of Clinical Oncology

Manuscript NO: 55936

Title: Forkhead box and indoleamine 2,3-dioxygenase co-expression in Pakistani triple negative breast cancer patients

Reviewer's code: 02445433

Position: Editorial Board

Academic degree: PhD

Professional title: Professor, Research Assistant Professor, Senior Scientist

Reviewer's Country/Territory: Italy

Author's Country/Territory: Pakistan

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Reviewer chosen by: Ya-Juan Ma

Reviewer accepted review: 2020-07-16 06:19

Reviewer performed review: 2020-07-22 11:02

Review time: 6 Days and 4 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input checked="" type="checkbox"/> Grade A: Priority publishing <input type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input checked="" type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Peer-reviewer statements	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

The authors described the evaluation of the co-expression of FOXP3 and IDO biomarkers in TNBC. The paper is potentially interesting but it requires major and minor revisions. In particular, many data presentations need to be revised and the discussion section is very poor of clarity and comparison with literature. The detailed queries are below indicated:

1. **AIM:** Our aim was to evaluate the co-expression of FOXP3 and IDO in triple-negative breast cancer (TNBC) patients from Pakistan. The aim should be more specific, e.g. co-expression of FOXP3 and IDO in TNBC with respect to hormone-positive BC or with respect to the aggressiveness of the tumor.
2. **RESULTS:** Out of 100 breast tumors, 25% expressed FOXP3 positive T-regs. A significant association of FOXP3 and IDO co-expression was observed among patients with TNBC ($p = 0.01$). The authors must add a comparison, e.g. with respect to hormone-positive BC.
3. FOXP3 has prognostic significance in triple-negative breast cancer (TNBC)[8]. The authors must specify what kind of prognostic significance, e.g. good or worse.
4. The authors analyzed in a retrospective manner the expression of the biomarkers in relation to patients' survival. I point out that the correlation with the PFS could be also very interesting and should be added as an investigation in the paper.
5. A blind histopathologic assessment was conducted by pathologists. How many pathologists?? The number should be indicated, as well as the choice in case of discordance.
6. The scores were further categorized as low, medium, and high. This description must be specified, the authors are invited to define what they mean for low, medium, and high with respect to the assigned scores. For example, 0-1 low, 2 medium and 3 high
7. The baseline characteristics of female breast cancer patients ($n=100$) are summarized in Table I. The mean age at the time of diagnosis was 48.28 ± 11.83 . The table I must be completely revised. The authors must describe the two groups of the study, i.e. TNBC

and hormone-positive BC, with the clinicopathological characteristics. It is important to add the number of patients analyzed for each group (the sum of the two groups should be 100). Moreover, the mean age must be indicated for the two groups (TNBC versus hormone-positive BC) to point out the homogeneity of the population of the study. 8.

Based on IHC analysis, the FOXP3 expression had nuclear localization. I think that FOXP3 protein expression has been already evaluated in the literature, the authors should underline if the localization is as expected or not. 9. Out of 100 patients, 25 expressed FOXP3-positive T-regs and 75 expressed FOXP3-negative T-regs (Figure 1).

The authors must provide a table, also as supplementary materials, where they show positive and negative FOXP3 cases with the main clinicopathological characteristics, or, alternatively, describe this in the text. 10. There is contradictory data regarding the involvement of FOXP3+ T-regs in breast cancer patients. The fact that in literature there are contradictory results about the role of FOXP3 should be discussed extensively, giving more detail and in comparison, with the results obtained by the authors. Moreover, in my opinion, this could be cited as an aim. 11. FOXP3 expression was identified in 25 breast cancer patients and the majority of these patients displayed TNBC phenotype. The authors must discuss the FOXP3 positive cases on the basis of: the % of positive vs negative in TNBC and % of positive vs negative in hormone-positive BC. The authors are invited once more, to compare their results with those of literature. 12.

FOXP3+ T-regs have prognostic implications in TNBC[8]. IDO expression is also associated with TNBC[26]. The authors must specify what kind of prognostic implications FOXP3 positive T-regs have, and to discuss their results in comparison with those of literature. Moreover, the kind of implications of IDO expression in TNBC must be discussed, as the request above indicated for FOXP3. 13. Finally, our study did not compare results of the IDO enzymatic activity with IDO protein expression due to retrospective nature of study. However, this is the subject of our ongoing prospective

study. The sentence is confusing. The authors must explain in a more detailed and clear form why IDO enzymatic activity measurement should be interesting and its relation with protein expression on the basis of literature data. 14. FOXP3

monitoring and IDO manipulation in TNBC patients may be an effective therapeutic strategy. The term manipulation is not very clear; what the authors do mean? 15.

Figure1. The authors are invited to show examples of low, medium, and high scores for both markers. The staining of IDO seems to be very diffuse and the co-expression with FOXP3 showed in the figure is not completely sound. For example, I noted that the orientation of the slice seems to be not right. Moreover, the authors must provide for comparison the pictures of IHC of normal breast tissue (e.g. tissue derived from reductive mammoplasty) analyzed for the two biomarkers. 16. Figure: 2

Kaplan-Meier curve of overall survival for total patients diagnosed with breast cancer. TNBC is a subgroup of BC that generally accounts for 10-15% of BC, therefore a table with about 50% of TNBC can lead to misinterpretation. I suggest analyzing separately the survival curve for TNBC versus hormone-positive BC. Finally: An immunosuppressive enzyme, indoleamine 2, 3 dioxygenase (IDO) catabolizes the tryptophan in to kynurenines. Maybe, there is a typo "in to"

RE-REVIEW REPORT OF REVISED MANUSCRIPT

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Reviewer chosen by: Jia-Ping Yan

Reviewer accepted review: 2020-08-25 12:54

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Review time: 1 Day and 7 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
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Peer-reviewer statements	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

The authors did not completely satisfy the queries. A further revision is required, as specified below

1) In the Abstract (Result section): A significant association of FOXP3 and IDO co-expression was observed among patients with TNBC ($P=0.01$) as compared to the hormone-positive breast cancer. New query: The authors did not explain what kind of association... or the English language is not clear for me (may be the authors means: a positive association between FOXP3 and IDO in TNBC or a significant co-expression of FOXP3 and IDO ?

2) Query 3. FOXP3 has prognostic significance in triple-negative breast cancer (TNBC)[8]. The authors must specify what kind of prognostic significance, e.g. good or worse. Ans: Lee et al. observed the prognostic significance of FOXP3- positive T-regs in TNBC. They suggested that assessment of FOXP3- positive T-regs in combination with other risk factors could improve the prognostic stratification of TNBC. Therefore, further studies were required to categorize FOXP3- positive T-regs for good or worse prognosis. New query: The above considerations cannot be a reply to the reviewer: They must be inserted in the context.

3) Query 5. A blind histopathologic assessment was conducted by pathologists. How many pathologists?? The number should be indicated, as well as the choice in case of discordance. Ans: The two pathologists were involved in the study. The mean score of both pathologists was considered as final score. New query: Firstly, the above answer has not been introduced in the materials and methods. Furthermore, I do not completely agree with the method used to assign the score. This method could be acceptable only if the two pathologists have assigned the same score or a different score in the same category (low, medium or high). On the contrary, there should be a third pathologist for the assignment of the score and the final score should be a consensus of all pathologists. Thus, the authors must follow this recommendation and, if necessary, revise the database. The description of the assigned score is crucial and must be carefully explained in the materials and methods.

4) Query 8. Based on IHC analysis, the

FOXP3 expression had nuclear localization. I think that FOXP3 protein expression has been already evaluated in the literature, the authors should underline if the localization is as expected or not. Ans: According to the literature, the FOXP3 expression had nuclear localization and we found the same results. New query: The authors must introduce this concept in the text and add the appropriate reference. 5) Query 9. Out of 100 patients, 25 expressed FOXP3-positive T-regs and 75 expressed FOXP3-negative T-regs (Figure 1). The authors must provide a table, also as supplementary materials, where they show positive and negative FOXP3 cases with the main clinicopathological characteristics, or, alternatively, describe this in the text. Ans: The data of 75 out of 100 FOXP3 negative cases is provided in supplementary data (Table 1). New query: In the table 1, I would like to know, at least, which molecular subtype resulted positive or negative to FOXP3. 6) Query 12. FOXP3+ T-regs have prognostic implications in TNBC[8]. IDO expression is also associated with TNBC[26]. The authors must specify what kind of prognostic implications FOXP3 positive T-regs have, and to discuss their results in comparison with those of literature. Moreover, the kind of implications of IDO expression in TNBC must be discussed, as the request above indicated for FOXP3. Ans: As described previously, Lee et al. observed the prognostic significance of FOXP3-positive T-regs in TNBC. They suggested that assessment of FOXP3- positive T-regs in combination with other risk factors could improve the prognostic stratification of TNBC. Therefore, further studies were required to categorize FOXP3- positive T-regs for good or worse prognosis. Furthermore, high IDO expression was detected in the TNBC patients. Previously FOXP3 and IDO co-expression has been detected in breast cancer patients but the co-expression of IDO and FOXP3 in TNBC has recently been identified.

New query: The authors must insert in the text a summary of the above answer. 7)

Query16. Figure: 2 Kaplan-Meier curve of overall survival for total patients diagnosed with breast cancer. TNBC is a subgroup of BC that generally accounts for

10-15% of BC, therefore a table with about 50% of TNBC can lead to misinterpretation. I suggest analyzing separately the survival curve for TNBC versus hormone-positive BC.

Ans: Our hypothesis was to quantify the FOXP3 expression in relation with IDO expression and to study the overall survival in patients diagnosed with breast cancer from Pakistan. We observed that there was no statistically significant difference in the overall survival between patients with FOXP3-positive or -negative tumors (p-value = 0.73) as shown in (Figure 2). TNBC versus hormone-positive BC was not the scope of our current study. New query: I don't agree with the answer of the authors because the aim of the study is the expression of FOXP3 in TNBC, also the Kaplan-Meier curve of all BCs can be confusing. 8)New query: Finally, in the abstract and discussion sections the sentence "FOXP3 and IDO expression monitoring in TNBC patients may provide an effective therapeutic strategy." is not acceptable in this form. The authors must carefully explain how, in their opinion, the monitoring of FOXP3 and IDO expression could be useful to provide effective therapeutic strategy.