Author’s response to reviews

Title: The expression of MDM2 in gastrointestinal stromal tumors: immunohistochemical analysis of 35 cases.

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Author’s response to reviews:

Dear Editor

We gratefully thank the reviewers for their valuable assessment of our manuscript. Here are below our responses/comments about their suggestions. In the manuscript, comments or modifications suggested by the reviewers are highlighted in red, blue and green (reviewer 1, 2 and 3 respectively).
Reviewer 1:

1. The major problem with the manuscript is that it is unclear what hypothesis is being tested. The authors conduct 16 different chi-square tests to investigate many different possible associations with MDM2. This creates a risk of spurious findings. Many of the tested associations are not connected to MDM2. For example, in table 2, only one row is a test related to MDM2. The authors should either test one or two well-formulated hypotheses, reduce the number of incidental tests, or use a statistical adjustment procedure to control for Type I error. I recommend cutting down on the number of statistical tests to focus on associations with MDM2. Further, I would limit the tests for associations with MDM2 to a few clinically important associations (e.g. risk score, metastasis).

Response:

✓ In the current study, we have studied the immunohistochemical expression of MDM2 in gastrointestinal stromal tumors (GIST) as well as its correlation with certain clinicopathologic features of these tumors.

✓ We completely agree with the reviewer for his pertinent comments on the large number of statistical tests we have initially carried out. As suggested, we have now limited these tests for associations with MDM2 and some important clinical features of GIST (risk score, tumor site, size, mitotic count, necrosis, and metastasis). Thus, we have withdrawn Tables 2 and 3. The manuscript has now only 2 tables: Table 1 and Table 2 (initially Table 4).

2. The title does not represent the content of the paper. This is not a prognostic study because there is no outcome data. You can infer that MDM2 is associated with a poor prognosis but there is no data showing this directly. To do this, you would need to perform survival analysis and show that MDM2 is an independent prognostic factor in addition to metastases, mitoses, etc.

Response:

✓ We agree with this comment. In fact in the manuscript, we were investigating MDM2 immunohistochemical expression with some histoprognostic features (rather than clinical prognostic features). As our study was not a prognostic study, we have now changed the title to: The expression of MDM2 in gastrointestinal stromal tumors: immunohistochemical analysis of 35 cases.
3. In table 2, I believe the Cochrane-Armitage test for trend would be a stronger test than the chi-square test. I suspect you may be able to show a statistically significant association between MDM2 and AFIP risk if you use this stronger test.

Response:

✓ In accordance with the previous comments of the reviewer (Comments No. 1), we have withdrawn tables No.2 and 3. In fact, we have maintained only the table 1 and the table 4 (now table 2). Due to a small sample in our study, we have conducted a chi-square test or a Fisher exact-test when appropriate (when the sample size <5 cases), to investigate association of MDM2 immunoeexpression with some clinicopathologic characteristics of GIST.

Reviewer 2:

o There is no method and clinical validation study for the MDM2 expression studied for the primary question of the study.

Response:

✓ In fact, MDM2 expression is not yet a validated or a routine practice in GIST, as stated by the reviewer. GIST as mesenchymal neoplasm, previous studies have tried to assess MDM2 expression in these tumors (the MDM2 pathway has been found to be deregulated in various human sarcomas). In our current study, we have tried to investigate MDM2 immunohistochemical expression in GIST and its correlation with some clinicopathologic features.

o The clinical significance of the test in the discussion is that the molecule has been implicated in relation to 53 pathways in the literature. There are no markers of p53 pathway in the cases. For this reason, clinical significance should not be discussed in this context.

Response:

In fact, we have studied the immunoeexpression of MDM2 in GIST, not p53 as noticed by the reviewer. The previous studies on this subject have in fact, focused mainly on MDM2 amplification by using FISH techniques (Wallander ML et al. Gastrointestinal stromal tumors: clinical significance of p53 expression, MDM2 amplification, and KIT mutation status. Appl Immunohistochem Mol Morphol. 2013;21(4):308-12). However, the MDM2 overexpression cannot be only the consequence of MDM2 gene amplification, it is why we think that immunohistochemical evaluation of MDM2 in GIST offers the simplest way to investigate the deragulation of this molecule. We agree with the reviewer that it would be more interesting to associate p53 markers with MDM2 overexpression in our study. However, it is now widely
accepted in the literature that the main role of the MDM2 oncoprotein (as well as its analogs) is to inhibit p53 (Wade M et al. MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer. 2013;13(2):83-96). It is why we have discussed this issue in our study. Also, the clinical significance of the MDM2 deregulation in tumors is to target it, mainly its interaction with p53. Some studies show promising or conflicting results in this subject (Pishas KI et al. Nutlin-3a efficacy in sarcoma predicted by transcriptomic and epigenetic profiling. Cancer Res. 2014;74(3):921-31.).

No statistics were provided for the sample size. For subgroup analysis, sample size is small.

Response:

The statistic issue in this manuscript has been widely discussed by the reviewer 1. As noticed by the reviewer, the sample size is small, it is why we have carried out the appropriate statistic tests to show association of MDM2 immunoexpression with some clinicopathologic features. For this, we have conducted a chi-square test or a Fisher exact-test when appropriate (when the sample size is too small, less than 5 cases).

Reviewer 3:

The manuscript is well written and well organized. The result showed a significant prognostic value of MDM2 in these patients despite the limited number of cases. I think this research is valuable for publication and it will encourage more scientists to research a larger number of patients regarding MDM2. The figures are of fair quality. I just noticed few spelling and grammar errors need to be addressed.....

Response:

✓ We gratefully thank the reviewer for his pertinent comments. As suggested, we have corrected the notified grammatical errors as well as all other language mistakes.

In conclusion, we thank again all reviewers and the editor for their valuable assessment of our manuscript, and hope that we have carried out all their suggestions in order to improve its quality.

Best regards.

The authors.