Author’s response to reviews

Title: Regression of solid breast tumours in mice by Newcastle disease virus is associated with production of apoptosis related-cytokines

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Author’s response to reviews:

Dear Linda Gummlich,

We are hereby to submit a revised version of our manuscript entitled “Regression of solid breast tumours in mice by Newcastle disease virus is associated with production of apoptosis related-cytokines” with manuscript number: BCAN-D-17-02230. In this version, we have addressed the concerns of the editor and the reviewers. We thank you for the helpful comments and suggestions.

We have revised the manuscript based on the suggestions and advice of the reviewers. A point-by-point response to their comments is updated in the revised version of the manuscript. We hope that these revisions successfully address their concerns and requirements, and that this manuscript will be consider for publication in your prestigious journal.

Thanks, hearing your response soon.
Responses to Reviewer 1:

We thank you Reviewer 1 (John D. Haley) for the critical comments and helpful suggestions. We have taken all these comments and suggestions into account, and they have improved our manuscript considerably. We fixed the paper based on your valuable comments as I have briefly summarized, and explained in the table below.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Comments/Questions/Suggestions</th>
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<tbody>
<tr>
<td>1</td>
<td>The Results section precedes the Materials and Methods section which adds to the confusion. The section has been moved to its appropriate place in the manuscript accordingly.</td>
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<tr>
<td>2</td>
<td>The extensive use of Tables is off-putting. Much of this data should be plotted and the Tables moved to Supplementary. This has been addressed and could be found in the updated version of the manuscript. Other tables are moved to supplementary as suggested.</td>
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<tr>
<td>3</td>
<td>Page 3: The minor impact of NDV on human health is noted, but no description of toxicity in the murine host is mentioned. What immune responses and toxicopathology are observed in mouse? This may contribute to the activity observed. This has been updated and can be found page 4 of the new manuscript version.</td>
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</table>

While in mouse it is reported to stimulate production of type I interferon response upon infection and resistance of both normal and neoplastic cells to virus replication [19].
4 Page 14: Table is not clear. The 100ul addition of differing virus titers is made to differing amounts of PBS in a way not described. Are the inoculation volumes constant between virus titers? Yes, the inoculation volume is constant between 8 HA, 16 HA, 32 HA and 64 HA virus titres.

5 The cytokine Discussion section, reads as a review with little integration of the data and little interpretation of its significance to the activity of NDV in the cancer setting. This should be largely rewritten to focus on the biology at hand. This has been corrected.

6 Minor:

Page 13: 3.5: Tris-HCl Comment has been fixed and can be found in page 6 of the new version of the manuscript.

Responses to Reviewer 2:

Dear Reviewer 2 (Evgenii Leonidovich Zavjalov, Ph.D), we appreciated and thank you for the critical observations and the valuable questions and suggestions raised. Your great comments and suggestions were considered, and followed accordingly. We corrected the paper following your good observations and that improved our manuscript, making it need. Below is the summarized version of the response together with the raised questions.

S/N Comments/Questions/Suggestions Responses

1 -line 3 page 4: Table 1. VVNDV - unknown abbreviation? Suggestion has been corrected in page 13 of the new version of the manuscript.

Velogenic Viscerotropic(VVNDV)

2 -line 4 page 4: Table 1 have the label with "Mean ± SD" included also in section 3.16. Statistical Analysis. However, that of is not given in the signatures to Tables 3-9. It is necessary to indicate uniformly Mean ± S.D. or Mean ± SD without dots. This has been corrected accordingly (choosing Mean ± S.D.) in the new manuscript version in table 2 and section 2.16 respectively.
3 - line 7 page 4: Table 1. NG - the abbreviation that are absence in the table. This was written clearly in the table caption of the old version of the manuscript. The NG basically means (negative) as written on top of the table.

4 - line 37 page 5: unknown group NNDV? Was actually a mistake. It was referring to NDV alone not as written NNDV. We corrected that in the new version of the manuscript currently located in table 3 caption page 14.

5 - line 38 page 5: what is the purpose of n = 6? To which group does this apply? Similar to Table 1, it is indicated that the data are expressed as Mean ± SD, whereas this information is given in Section 3.16. The n = 6 means the number of experimental replicates that was performed. Correction were made in both section 2.16 (currently) and table 1 regarding expression in Mean ± SD.

6 - line 45 page 5: it remains unclear how the blood was taken from animals and how much blood was taken from animals for so many studies. Corrections were made in the revised version of the manuscript in page 14.

Approximately one milliliter of plasma/serum from mice was collected following CA Argmann and J Auwerx protocol [32]

7 - line 35 page 12: need specify the source of the virus and the storage condition. This has been written in section 2.2 page 5 of the new version of the manuscript. Storage condition has also been mentioned in section 2.5 page 7.

The velogenic viscerotropic NDV strain AF2240 was obtained from Institute of Bioscience of the Universiti Putra Malaysia. This virus was isolated in the late 1960s from a local outbreak [29].

8 - line 56 page 13: incomprehensible scheme for obtaining 8HA, 16NA, 32H and 64HA. Why 8NA obtained from 100 µl of 108 NDV + 900 µl of PBS? Why is the doubling of the titer was obtained by diluting 1/9, 1/8, 1/7? Basically, this is the explanation of the table 9. We had a virus titre of 28 (in ten-fold means108) which is 256, from the original stock. So, to create a dilution of 8 HA virus titre from the stock for treatment, we simply take 256/8 = 32. Which means, take 1 part of NDV stock into 32 part of 1 x PBS.
9 - line 37 page 14: what is the "...hygienic conditions and were provided with standard animal feed". Need to show a specific data on temperature and humidity in the animals housing room, photoperiod, type or name of feed, method for study of animal health. This area has been modified and corrected accordingly in section 2.10, page 8

The animals were reared in the animal house of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, where they were kept in a sterile plastic cage under hygienic conditions and were provided with food and water ad libium. Studies were conducted in Cancer Research Laboratory of Institute of Bioscience.

10 - line 3 page 15: how was determined the mass of the tumor on the first day of the study? This is to observed whether the tumour volume is progressively decreasing as compared with the mass in the last day after NDV inoculation.

11 - line 4 page 16: it is more correct to write that "some viruses have oncolytic properties ..." This has been corrected accordingly which will currently be found in page 21 of the new version of the manuscript.

Some viruses have oncolytic properties [32-35].

12 - line 32 page 16: it is not true that the increase in body weight is caused by the growth of the tumor. Such an assumption can be applied only to animals from the group CNDV + T 32 and 64 (Table 1) in which the body weight + tumor mass at the end of the experiment is comparable to the total mass of animals at the beginning of the experiment. Corrections is made

13 - line 50 page 16: 2 times the word "that ... that" is written Correction has been made currently in page 23

14 - line 50 page 16: the authors write that the IL-10 decreases in CNDV + T mice, but from the data in Table 6 it can be seen that the decrease is observed in the 4th week for all groups except NC. A similar picture observed for all cytokines, but the conclusion about the effect of NDV + T made only for this cytokine. Revision were made accordingly.
15 - line 54 page 16: probably instead of the word "biliary stricture" you should write "biliary structure". The spelling errors there has been corrected accordingly in page 23 of the revised version of the manuscript.

16 From the text remains unclear scheme of virus treatment: day of treatments, virus was injected together with tumor cells or separately, the point of injection, how many times. This is now clearly stated in section 2.10 page 8 of the revised version of the manuscript.

Tumour-bearing mice received an intratumoural injections of NDV for 4 days period. Injection of virus and the tumour cells were made separately.

A concentration of 1 x 10^4 cells/ml (0.1 cc injection per mouse) was used to induced tumour in the mice. The animals were not subjected to any form of suffering during the induction process. Subcutaneous (s.c.) injection of 4T1 breast tumour cells was made into the mammary gland by gently penetrating the skin.

17 From the Methods it remains unclear the model of xenotransplantation: orthotopic or not? Where the tumor cells were inserted? This has been addressed in the new version of the manuscript.

If you meant “orthotopic”, yes it’s. And for the injection of the 4T1 breast tumour was subcutaneously (s.c.) inoculated in the mammary gland by gently penetrating the skin.

18 Also, not entirely clear after reading of the Discussion why was presented the weekly measurements of cytokines. Besides this, since the volume of blood taken for analysis is unknown it remains unclear how the weekly blood sampling affected on the results.

19 Also, in Discussion will be need to show a more detailed analysis of the results obtained. When considering the data presented in the tables, low values of SD, which are more similar to SE, attract the attention. Despite the fact that this direction of research is actively developing in recent years, no publications younger 2015 in the References was present. The tables have now been turned into graphs while some other tables have been named as supplementary.

20 The References are include mainly the publications 10 years old and more. New references are cited and updated accordingly.

Thank you all for your critical review on this manuscript.