EMBO Molecular Medicine

Decreased serum level of sphingosine-1-phosphate: a novel predictor of clinical severity in COVID-19

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Editor: Zeljko Durdevic

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

2nd Oct 2020

Dear Dr. Marfia,

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now received feedback from the three reviewers who agreed to evaluate your manuscript. As you will see from the reports below, the referees acknowledge the interest of the study and are overall supporting publication of your work pending appropriate revisions.

Addressing the reviewers' concerns in full will be necessary for further considering the manuscript in our journal, and acceptance of the manuscript will entail a second round of review. EMBO Molecular Medicine encourages a single round of revision only and therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. For this reason, and to save you from any frustrations in the end, I would strongly advise against returning an incomplete revision.

I look forward to receiving your revised manuscript.

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System for Author):

This work is very timely and clinically significant. However, the methodology used to measure S1P is not optimal. LC/MS/MS methods are more acuurate and the ELISA method employed is not as accurate.

Referee #1 (Remarks for Author):

This work is very timely and important. Sample collection and analysis seems to be done properly. The key weakness is that the ELISA method to measure S1P is not validated by a more rigorous method such as LC/MS/MS. I recommend that the authors validate some of the key findings in limited samples and show a concordancy between the two methods.

Referee #2 (Remarks for Author):

The paper by Marfia et al. reports that the serum level of sphingosine-1-phosphate could be a predictor of the severity of Covid-19. Overall, the data is well presented and discussed. The population is well selected and analyzed. Statistic anaysis is appropriate. Few points are below.

1. ICU patients have low S1P level than no-ICU. Unclear how the authors claim that S1P is a "predictor" of ICU admission. If so, it should be lower in patients before being transferred/admitted to the ICU. A stratification of these patients is required.
2. ICU patients with low S1P have higher mortality (data provided in Figure 5). These data are difficult to interpret. It will be helpful to show a mortality rate of the ICU patients having less than 0.60 micromolar S1P and the mortality rate of the patients having more than 0.60 micromolar. This analysis should start from the day of ICU admission and not from the hospital admission, as the authors do not know the S1P level before the ICU admission in the same patient.
3. The lack of sequential analysis is a major limitation of this study (also acknowledged by the authors).
4. Figure 6 is highly complex. It should be modified to show the key findings.
5. That S1P level is low in Covid-19 patients is not novel. It has been already reported (see PMID:32610096), although that paper compares healthy versus Covid-19 patients and does not address severity.
6. Low level of S1P could simply be an epiphenomenon of host tissue damage and not directly linked to the damage caused by the SARS-CoV-2. In fact, low serum level of S1P has been suggested to predict mortality in patients with liver cirrhosis (PMID: 28334008); low serum level of S1P is associated with peripheral artery disease (PMID: 27973607), severity of septic patients (PMID: 31019718), in diabetic patients

Referee #3 (Remarks for Author):

1. These data are interesting and important.
2. Other health systems have been talking about this but no-one has laid out the data yet, so this is a first.
3. S1P receptor agonists (bothfingolimod and ozanimod) are in clinical trials for Covid
4. The published data on cytokine storm in H1N1 2009 from Oldstone, Kawaoka and colleagues, shows that S1P receptor agonists protect from cytokine storm, while antagonists are deleterious. These data have been demonstrated on human plasmacytoid dendiritic cells and the auto amplification loop for IFNa. Furthermore, IFNAR1 is downmodulated through s1PR1. These papers should perhaps be cited as supporting the mechanisms underlying predictive elements seen here.
5. I would expedite revision and publication

1.
S1PR1-mediated IFNAR1 degradation modulates plasmacytoid dendritic cell interferon-α autoamplification.
Teijaro JR, Studer S, Leaf N, Kiosses WB, Nguyen N, Matsuki K, Negishi H, Taniguchi T, Oldstone MB, Rosen H.
Proc Natl Acad Sci U S A. 2016 Feb 2;113(5):1351-6. doi: 10.1073/pnas.1525356113. Epub 2016 Jan 19.
PMID: 26787880 Free PMC article.

2.
Cytokine storm plays a direct role in the morbidity and mortality from influenza virus infection and is chemically treatable with a single sphingosine-1-phosphate agonist molecule.
Oldstone MB, Rosen H.
Curr Top Microbiol Immunol. 2014;378:129-47. doi: 10.1007/978-3-319-05879-5_6.
PMID: 24728596 Free PMC article. Review.
3.
Protection of ferrets from pulmonary injury due to H1N1 2009 influenza virus infection: immunopathology tractable by sphingosine-1-phosphate 1 receptor agonist therapy.
Teijaro JR, Walsh KB, Long JP, Tordoff KP, Stark GV, Eisfeld AJ, Kawaoka Y, Rosen H, Oldstone MB.
Virology. 2014 Mar;452-453:152-7. doi: 10.1016/j.virol.2014.01.003. Epub 2014 Jan 31.
PMID: 24606692 Free PMC article.
4.
Mapping the innate signaling cascade essential for cytokine storm during influenza virus infection.
Teijaro JR, Walsh KB, Rice S, Rosen H, Oldstone MB.
Proc Natl Acad Sci U S A. 2014 Mar 11;111(10):3799-804. doi: 10.1073/pnas.1400593111. Epub 2014 Feb 26.
PMID: 24572573 Free PMC article.
5.
Sphingosine-1-phosphate and its receptors: structure, signaling, and influence.
Rosen H, Stevens RC, Hanson M, Roberts E, Oldstone MB.
Annu Rev Biochem. 2013;82:637-62. doi: 10.1146/annurev-biochem-062411-130916. Epub 2013 Mar 18.
PMID: 23527695 Review.
6.
Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection.
Teijaro JR, Walsh KB, Cahalan S, Fremgen DM, Roberts E, Scott F, Martinborough E, Peach R, Oldstone MB, Rosen H.
Cell. 2011 Sep 16;146(6):980-91. doi: 10.1016/j.cell.2011.08.015.
PMID: 21925319 Free PMC article.
7.
Suppression of cytokine storm with a sphingosine analog provides protection against pathogenic influenza virus.
Walsh KB, Teijaro JR, Wilker PR, Jatzek A, Fremgen DM, Das SC, Watanabe T, Hatta M, Shinya K, Suresh M, Kawaoka Y, Rosen H, Oldstone MB.
Proc Natl Acad Sci U S A. 2011 Jul 19;108(29):12018-23. doi: 10.1073/pnas.1107024108. Epub 2011 Jun 29.
PMID: 21715659 Free PMC article.

Authors' Response to Reviewers

Referee #1

This work is very timely and clinically significant. However, the methodology used to measure S1P is not optimal. LC/MS/MS methods are more acuurate and the ELISA method employed is not as accurate.

Referee #1 (Remarks for Author):

This work is very timely and important. Sample collection and analysis seems to be done properly. The key weakness is that the ELISA method to measure S1P is not validated by a more rigorous method such as LC/MS/MS. I recommend that the authors validate some of the key findings in limited samples and show a concordancy between the two methods.

We thank the reviewer for recognizing the importance of our work and also for his/her recommendation.

We recognize that, as all methods, ELISA may have pitfalls. However, if this method is performed in proper conditions, it provides values consistent with those obtained by LC/MS/MS. In agreement, as we added to the text (pag. 7, lines 179 and 180), the levels of serum S1P in the control group were comparable with those reported by a recent study performed by LC/MS/MS on 174 healthy blood donors (Daum et al. 2020. Determinants of Serum- and Plasma Sphingosine-1-Phosphate Concentrations in a Healthy Study Group TH Open. 4(1):e12-e19, ref. n. 17). In addition, while LC/MS/MS is more rigorous, it should be noted that accurate quantification of serum S1P levels still poses many difficulties to LC/MS/MS technology, mainly due to variable effects of different biological matrices, as well as the lack of proper matrices free of analytes or samples with known concentrations of analytes (Tang et al. 2020. Validated LC-MS/MS method of sphingosine 1-phosphate quantification in human serum for evaluation of response to radiotherapy in lung cancer. Thoracic Cancer 11:1443-1452). Moreover, LC/MS/MS is significantly expensive and labor-intensive.

Despite we were unable to perform LC/MS/MS (the triple quadrupole mass spectrometer, required for S1P quantification, is not readily available in our laboratories), based on your recommendation, we performed new analyses to validate the serum level of S1P measured by ELISA. We verified our findings by enzymatic derivatization, which was reported to provide values that are very similar to those obtained by MS (Edsall L, Vann L., Milstien S., et al. Enzymatic measurement of sphingosine 1-phosphate, Methods Enzymol. 2000;312:9–16, ref n. 15). The results showed concordance between the two methods. The methodology of the enzymatic assay has been added to the text (pag. 5, lines 132-138).

Referee #2 (Remarks for Author):

The paper by Marfia et al. reports that the serum level of sphingosine-1-phosphate could be a predictor of the severity of Covid-19. Overall, the data is well presented and discussed. The population is well selected and analyzed. Statistic analysis is appropriate. Few points are below.

We thank the reviewer for his/her positive evaluation of the manuscript.

1. ICU patients have low S1P level than no-ICU. Unclear how the authors claim that S1P is a "predictor" of ICU admission. If so, it should be lower in patients before being transferred admitted to the ICU. A stratification of these patients is required.

We thank the reviewer for her/his queries, which allowed us to clarify that all blood samples were collected on the first day of hospitalization. This clarification has been added at page 4, line 114.

COV patients were then followed up and monitored for all their hospitalization time and when ICU admission was required they were classified into the ICU cohort, while the other patients who did not require ICU admission were categorized into the noICU group. A sentence was added to the text on pages 8,9, lines 207-209).

2. ICU patients with low S1P have higher mortality (data provided in Figure 5). These data are difficult to interpret. It will be helpful to show a mortality rate of the ICU patients having less than 0.60 micromolar S1P and the mortality rate of the patients having more than 0.60 micromolar. This analysis should start from the day of ICU admission and not from the hospital admission, as the authors do not know the S1P level before the ICU admission in the same patient.

Thank you for your suggestion, which allowed us to strengthen the relationship between S1P and patient mortality. As proposed, we stratified our COV population into patients with low (< 0.60 μM) and high (≥ 0.60 μM) levels of S1P. The results are reported on page 10, lines 242-245.

3. The lack of sequential analysis is a major limitation of this study (also acknowledged by the authors).

As we acknowledged in the discussion, a limitation of this study is the lack of sequential analyses. When we started this investigation, no data were available on S1P in COVID-19, and we concentrated on S1P levels at the time of admission. We recently started evaluating S1P at different time points in the same, new patients, and this will hopefully provide new insights, but it requires additional time.

4. Figure 6 is highly complex. It should be modified to show the key findings.

We simplified Figure 6, focusing on the main findings, Accordingly, the figure legend was also modified (page 13, lines 272-281).

5. That S1P level is low in Covid-19 patients is not novel. It has been already reported (see PMID:32610096), although that paper compares healthy versus Covid-19 patients and does not address severity.

We thank you for your comment and recent reference indication. The findings of this very recent paper were inserted in the first paragraph of the Discussion session (pag. 11, lines 254-257). As briefly commented in the text, we should note that the paper you cited did not reported S1P in molar concentrations, but as Intensities, preventing the comparison of data across independent studies. Notwithstanding our study appears consistent with this study, it provides novelty in addressing severity and mortality (as you acknowledge).

6. Low level of S1P could simply be an epiphenomenon of host tissue damage and not directly linked to the damage caused by the SARS-CoV-2. In fact, low serum level of S1P has been suggested to predict mortality in patients with liver cirrhosis (PMID: 28334008); low serum level of S1P is associated with peripheral artery disease (PMID: 27973607), severity of septic patients (PMID: 31019718), in diabetic patients.

Thank you for this comment. We agree with the Referee that we cannot exclude that the decreased levels of S1P could be an epiphenomenon of host tissue damage. However, taking into account the pleiotropic effects of S1P on multiple organs, and that S1P plays crucial roles in endothelial dysfunction, as well as in immunity, we think reasonable the possibility that low S1P levels contributes to COVID-19 complications. The evidence that low serum level of S1P predicts mortality in patients with the different diseases (you reported) appear to support a role of S1P in the progression of different diseases, as endothelial and immune alterations which are

common to these diseases, as well as COVID-19. According to reviewer's suggestions, we added a new piece of text, mentioning the studies recommended by the reviewer in the discussion section (pages 15,16, lines 348-349, 352-356, references n. 14, 31, 32).

Referee #3 (Remarks for Author):

1. These data are interesting and important.
Thank you for your comment.

2. Other health systems have been talking about this but no-one has laid out the data yet, so this is a first.
Thank you for recognizing the novelty of our study.

3. S1P receptor agonists (both fingolimod and ozanimod) are in clinical trials for Covid.
4. The published data on cytokine storm in H1N1 2009 from Oldstone, Kawaoka and colleagues, shows that S1P receptor agonists protect from cytokine storm, while antagonists are deleterious. These data have been demonstrated on human plasmacytoid dendiritic cells and the auto amplification loop for IFNa. Furthermore, IFNAR1 is downmodulated through s1PR1. These papers should perhaps be cited as supporting the mechanisms underlying predictive elements seen here.
Thank you for the suggestion. According to it, we enriched the discussion by reporting about the controversial effect of the modulation of S1P receptor by Fingolimod in viral infection (page 16, lines 368-371, references n. 35,36).

5. I would expedite revision and publication
Thank you. We appreciated your positive evaluation.

1st Revision - Editorial Decision 27th Oct 2020

27th Oct 2020

Dear Dr. Marfia,

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

Please implement all adjustments suggested by the referee #1. No additional experiments are required.

***** Reviewer's comments *****

Referee #1 (Remarks for Author):

The authors have addressed my comments in part by using a classical enzymatic method to assess S1P, in which S1P is extracted, phosphorylated and estimated by radioactive phosphate incorporation and TLC analysis. The results, which are not shown, apparently are in concordance with the ELISA methods. While this may be so, the authors should acknowledge that this classic method is prone to inaccuracies, such as inefficient lipid extraction at multiple steps, misidentification of other lipids that run at the same Rf value as S1P on the TLC plate, etc. The state of the art technique that is the most accurate to quantify S1P in biological samples is one step extraction followed by LC/MS/MS. Acknowledging this is important for the scientific literature. The authors should at the very least acknowledge this in the limitations of their study. Ideally, they should send a few of their samples (high and low S1P) to a LC/MS/MS core facilities or metabolomics centers and show correlation between the two. This would strengthen the study tremendously.

Referee #2 (Comments on Novelty/Model System for Author):

Highly relevant study that may help in the stratification of the severity of the COVID 19 disease.

Referee #2 (Remarks for Author):

The authors responded to my comments in a satisfactory manner

Referee #3 (Remarks for Author):

The revisions have improved the readability and clarity of the manuscript. I have no further suggestions

2nd Authors' Response to Reviewers 9th Nov 2020

The authors performed the requested changes.

2nd Revision - Editorial Decision 12th Nov 2020

The authors performed the requested changes.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Giovanni Marfia
Journal Submitted to: EMBO Molecular Medidine
Manuscript Number: EMM-2020-13424-V2

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- ➔ the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ➔ figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- ➔ graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- ➔ if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- ➔ Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- ➔ a specification of the experimental system investigated (eg cell line, species name).
- ➔ the assay(s) and method(s) used to carry out the reported observations and measurements
- ➔ an explicit mention of the biological and chemical entity(ies) that are being measured.
- ➔ an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- ➔ the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- ➔ a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- ➔ a statement of how many times the experiment shown was independently replicated in the laboratory.
- ➔ definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	The power size was calculated considering a power of 80%, type I error rate of 5% and an effect size of 50%.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Data acquisition was performed blindly.
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes, a description of the statistical analysis ifor each measurement is provided in the method section.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Blood parameters were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests, and when normally distributed, the two conditions were compared by the two-tailed Students's t-test.
Is there an estimate of variation within each group of data?	We performed an estimation of variation coefficient.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repor

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumo

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	Yes, the variance was similiar between the groups analyzed.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	NA
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	COST Action n.2020/ST/057
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	All subjects were recruited after written informed consent, conformed to principles in the WMA Declaration of Helsinki.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA