Journal of Clinical and Translational Research
Peer review process file 07.202106.010

High affinity monoclonal antibody targeting Siglec-15 for cancer immunotherapy

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Review timeline:

Received: 26 April, 2021
Editorial decision: 10 May, 2021
Revision received: 14 June, 2021
Editorial decision: 17 September, 2021
Published online: 16 November, 2021

1st Editorial decision
10-May-2021

Ref.: Ms. No. JCTRes-D-21-00062
High Affinity Monoclonal Antibody Targeting Siglec-15 for Cancer Immunotherapy
Journal of Clinical and Translational Research

Dear Dr. wang,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript and conduct additional experiments. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Jun 09, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission

record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: Siglec-15 has been identified to be targeted for normalization cancer immunotherapy (Nat Med. 2019 Apr;25(4):656-666. Clin Cancer Res. 2021 Feb 1;27(3):680-688.). The authors in this manuscript a monoclonal antibody (mAb) against Siglec-15, which may show its neutralizing function in vivo. However, the authors did not provide the functional analysis of this mAb. The following should be addressed.
1, Page 6, line 26-27, the authors used a commercially available anti-Siglec-15 antibody 5G12 (ThermoFisher Scientific) as a positive control in their work without the detailed information about this antibody such as the catalog number and the source;
2, The source and the cloning strategy of the plasmids Pmax1-Siglec-15-mIgG and Pmax1-Siglec-15-his are missing;
3, The authors may provide the data of at least one negative control except for the blank control, such as the mIgG; Is there any special consideration of mIgG Fc tag in antigen preparation? Should be mentioned in the discussion section;
4, More detailed figure legends may be provided, such as in Figure 5A, the authors may double check the size of the mAb;
5, The authors may update the references they cited such as Wang et al., 2019 would be Nat Med. 2019 Apr;25(4):656-666. doi: 10.1038/s41591-019-0374-x. Epub 2019 Mar 4.
6, Functional assay of their anti-Siglec-15 mAb may be provided in T cell responses in vitro and in vivo.

Authors’ response

To the Editor-in-Chief, Journal of Clinical and Translational Research

Re: Responses to the editor’s Comments of JCTRes-D-21-00062

Dear Editor,
Please find our responses to the reviewers’ comments, marked in blue. The main revisions in the manuscript text are highlighted in blue for clear and easy identification.

Reviewers' comments:
Reviewer #1: Siglec-15 has been identified to be targeted for normalization cancer immunotherapy (Nat Med. 2019 Apr;25(4):656-666. Clin Cancer Res. 2021 Feb 1;27(3):680-688.). The authors in this manuscript a monoclonal antibody (mAb) against Siglec-15, which may show its neutralizing function in vivo. However, the authors did not provide the functional analysis of this mAb. The following should be addressed.

1, Page 6, line 26-27, the authors used a commercially available anti-Siglec-15 antibody 5G12 (ThermoFisher Scientific) as a positive control in their work without the detailed information about this antibody such as the catalog number and the source;

Response: Thanks for the reviewer. We checked the source of 5G12 and found that we confused it with another antibody, so we corrected it in the revised manuscript. The anti-Siglec-15 antibody 5G12, also known as NC318, was produced by Kyinno Biotechnology (Beijing) Co., Ltd with the patent NO. CN110035769A (PCT, WO2018057735). This has been added in the revised manuscript in Section 2.1 and labeled blue.

2, The source and the cloning strategy of the plasmids Pmax1-Siglec-15-mIgG and Pmax1-Siglec-15-his are missing;

Response: Thanks for the reviewer. The plasmid PmaxGFP was bought from BioVector NTCC Inc. (Beijing, China). The Siglec-15-mIgG and Siglec-15-his genes were synthesized by Sangon Biotech Co., Ltd (Shanghai, China). Restriction enzymes Eco47III and Nhe1 were purchased from New England Biolabs (Beijing) LTD. The plasmid PmaxGFP and exogenous genes Siglec-15-mIgG or Siglec-15-his were cut by restriction enzymes *Eco*47III and *Nhe*1 and linked by T4 DNA Ligase. This has been added in Sections 2.1 and 2.3 in the revised manuscript and labeled blue.

3, The authors may provide the data of at least one negative control except for the blank control, such as the mIgG; Is there any special consideration of mIgG Fc tag in antigen preparation? Should be mentioned in the discussion section;

Response: Thanks for the reviewer. The negative control has been added in Section 3.2 and Figures 1 and 2C in the revised manuscript (labeled blue therein).

Antigen Siglec-15-mIgG, containing mFc tag, was used for immunization. The addition of mFc tag increases the mass and volume of the antigen, therefore elevates the half-life period of antigen in vivo. The longer half-life period enhances the performance of antigen and improves the titer of antibody. Besides, the mFc tag facilitates the purification of Siglec-15-mIgG by an affinity chromatography with G-protein columns (Hitrap ProteinG HP column) (Nikolayenko I V et al., ukrainica bioorganica acta, 2005, 3-11). This has been added in Sections 3.2 in the revised manuscript and labeled blue.

4, More detailed figure legends may be provided, such as in Figure 5A, the authors may double check the size of the mAb;

Response: Thanks for the reviewer. We have added more detailed figure legends in the revised manuscript (see Figure captions in the revised manuscript labeled blue therein).

We also double checked the size of mAb, and found that we mislabeled the protein ladder. We have corrected it in the revised manuscript. Therefore, according to the figure, the heavy chain of the mAb is 50 kDa and the light chain is 27 kDa. These have been modified in Figure

5A and Section 3.4 in the revised manuscript (labeled blue therein). Thanks again for pointing out the error.

5, The authors may update the references they cited such as Wang et al., 2019 would be Nat Med. 2019 Apr;25(4):656-666. doi: 10.1038/s41591-019-0374-x. Epub 2019 Mar 4.

Response: Thanks for the reviewer. We have updated our references according to the Journal's standards in the revised manuscript (Ref. 15).

6, Functional assay of their anti-Siglec-15 mAb may be provided in T cell responses in vitro and in vivo.

Response: Thanks for the reviewer. Actually, the in vitro and in vivo verification are now ongoing, including flow cytometry, western blotting, quantitative immunofluorescence, and so on. We are also optimizing the gene sequence of Siglec-15-mIgG to further upgrade the titer of anti-Siglec-15 mAb. However, this work is conducted collaboratively with Kyinno Biotechnology (Beijing) Co., Ltd, and the company requires a confidentiality agreement about the information. So we did not provide the information in the revised manuscript.

2nd Editorial decision
14-Jun-2021

Ref.: Ms. No. JCTRes-D-21-00062R1
High Affinity Monoclonal Antibody Targeting Siglec-15 for Cancer Immunotherapy
Journal of Clinical and Translational Research

Dear author(s),

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at http://www.jctres.com/en/author-guidelines/ under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by Jul 14, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger
Editor-in-Chief

Journal of Clinical and Translational Research

Reviewers' comments:

Dear authors,

Thank you for implementing the changes per suggestions of the reviewer. The manuscript can now exit the peer review process and is deemed suitable for publication pending a few minor issues.

1) Please proofread the manuscript, preferably with the help of a native speaker. There are numerous grammar/spelling errors that must be eliminated in accordance with our guidelines on academic level English (https://www.jctres.com/en/author-guidelines/). The syntax errors require perusal and correction by a native speaker. If you do not have access to this option, our journal has professional proofreaders available who could perform a deep dive correction for a fee. Please contact me (m.heger@jctres.com) if you need assistance.

2) Please indicate the protocol number that the institutional review board approved with respect to the animal study.

3) The figures should be self-explanatory in a standalone manner, which is why the reviewer requested more elaboration in the legends. Although you made some modifications, the information is not sufficient to understand the figures without having to consult the text. For example, in Figure 1 it is not clear which primary and secondary antibody were used to stain the cells, which fluorophore was used, how many events were counted, etc. And these are the nitty gritty details. Your study is divided into key steps, ranging from the development of target protein-expressing cell lines (Figure 1), recombinant expression of the IgG and tagged protein in 293F cells to develop the antibody (Figure 2), etc. The figure legends should reflect each step clearly to provide logical flow in your data and systematic approach. Finally, please ensure that the legend is constructed in such a way that readers do not have to look for explanatory information in the main text (for example, in Fig. 3 the significance of mouse F1, F2 and W1, W2, etc. should be explained). Kindly modify the legends before text corrections are implemented to ensure that any additions are written in correct English. We do not have a word limit on the legends, so please use as much space as you need to make the figures clear and unequivocal.

4) Please make sure that the text in the figures is clearly legible. Some of the fonts used are too small, which makes the figures difficult to read.

5) I understand that some of the steps in the preparation protocols and validation procedures fall under IP, but I think that some additional validation data that you mention in your rebuttal letter can be exempted from this argument. It would benefit the paper if the following could be included:

- a map of the vectors/plasmids that were used
- the sequence of the Siglec-15-mIgG and Siglec-15-his genes that were inserted into the plasmids
- data showing T cell proliferation suppression in vitro
- immunofluorescence of Siglec-15 staining in Siglec-15-overexpressing human tumor xenografts grown in mice (can be histological sections) or human tumors from clinical

biobanks.

These results have nothing to do with IP in that they do not reveal any IP-related material beyond what is currently presented in the manuscript, especially since the sequences are being optimized. Moreover, they would provide a compelling argument for the use of the newly established antibody over the standard antibody (5G12) given that the difference in affinity towards its cognate ligand is rather small (EC50 = 1.3 ug/mL versus 1.6 ug/mL). You could demonstrate advantage of its utility and possibly target selectivity and specificity.

6) Please present all the Singlet-15 expression data in the CHO-K1 cells for the pspax2, pMD2G, and pLVXIRES-Siglec-15-Puro vectors, possibly in a supplemental document for the inferior vectors (i.e., those that did not make the cut). Do this for all the other data obtained but not reported.

Thank you,

Michal Heger
Editor

Authors' response

To the Editor-in-Chief, Journal of Clinical and Translational Research

Re: Responses to the editor's Comments of JCTRes-D-21-00062R1

Dear Editor,
Please find our responses to the reviewers' comments, marked in blue. The main revisions in the manuscript text are highlighted in blue for clear and easy identification.

Reviewers' comments:

Dear authors,

Thank you for implementing the changes per suggestions of the reviewer. The manuscript can now exit the peer review process and is deemed suitable for publication pending a few minor issues.

1) Please proofread the manuscript, preferably with the help of a native speaker. There are numerous grammar/spelling errors that must be eliminated in accordance with our guidelines on academic level English (https://www.jctres.com/en/author-guidelines/). The syntax errors require perusal and correction by a native speaker. If you do not have access to this option, our journal has professional proofreaders available who could perform a deep dive correction for a fee. Please contact me (m.heger@jctres.com) if you need assistance.

Response: Thanks for the editor. We have proofread the revised manuscript with the help of a professional proofreader. If you have any questions, please do not hesitate to contact us.

2) Please indicate the protocol number that the institutional review board approved with respect to the animal study.

Response: The protocol number is 2018S016, and this information has been included in Section of Human and animal rights in the revise manuscript.

3) The figures should be self-explanatory in a standalone manner, which is why the reviewer requested more elaboration in the legends. Although you made some modifications, the information is not sufficient to understand the figures without having to consult the text. For example, in Figure 1 it is not clear which primary and secondary antibody were used to stain the cells, which fluorophore was used, how many events were counted, etc. And these are the nitty gritty details. Your study is divided into key steps, ranging from the development of target protein-expressing cell lines (Figure 1), recombinant expression of the IgG and tagged protein in 293F cells to develop the antibody (Figure 2), etc. The figure legends should reflect each step clearly to provide logical flow in your data and systematic approach. Finally, please ensure that the legend is constructed in such a way that readers do not have to look for explanatory information in the main text (for example, in Fig. 3 the significance of mouse F1, F2 and W1, W2, etc. should be explained). Kindly modify the legends before text corrections are implemented to ensure that any additions are written in correct English. We do not have a word limit on the legends, so please use as much space as you need to make the figures clear and unequivocal.

Response: Thanks for the editor. We have deeply modified the figure captions and legends according to your suggestions as possible as we can in the revised manuscript. The modifications were labelled blue in Section of Figure captions in the revised manuscript. If you have any further requirement, please do not hesitate to contact us. Thanks for the valuable advice again.

4) Please make sure that the text in the figures is clearly legible. Some of the fonts used are too small, which makes the figures difficult to read.

Response: Thanks for the editor. We have modified the text in the figures to make them clearly legible. The modifications were seen in the figures in the revised manuscript.

5) I understand that some of the steps in the preparation protocols and validation procedures fall under IP, but I think that some additional validation data that you mention in your rebuttal letter can be exempted from this argument. It would benefit the paper if the following could be included:

- a map of the vectors/plasmids that were used

- the sequence of the Siglec-15-mIgG and Siglec-15-his genes that were inserted into the plasmids

- data showing T cell proliferation suppression in vitro

- immunofluorescence of Siglec-15 staining in Siglec-15-overexpressing human tumor xenografts grown in mice (can be histological sections) or human tumors from clinical biobanks.

These results have nothing to do with IP in that they do not reveal any IP-related material beyond what is currently presented in the manuscript, especially since the sequences are being optimized. Moreover, they would provide a compelling argument for the use of the newly established antibody over the standard antibody (5G12) given that the difference in affinity towards its cognate ligand is rather small (EC50 = 1.3 ug/mL versus 1.6 ug/mL). You could demonstrate advantage of its utility and possibly target selectivity and specificity.

Response: Thanks for the editor. The map of the vectors/plasmids, the sequence of the Siglec-15-mIgG and Siglec-15-his genes that were inserted into the plasmids, the data showing T cell proliferation suppression in vitro, and influence on tumor proliferation in vivo were added in the revised manuscript (see Figures S3 and S4, Figure 6, Section of 2.5 and 3.5 for detailed information).

The difference of the anti-Siglec-15 mAb 3D6 and 5G12 in affinity is small, but the efficacy of 3D6 is better than 5G12, according to our in vitro and in vivo experiments. As shown in Figure 6A&B, the efficacy of 3D6 is better than that of 5G12 on the blocks the Siglec-15-mediated suppression of T cell (p <0.05). In a tumor xenograft mouse model, the tumor suppression efficacy of 3D6 is also better than 5G12 (100 μg, p <0.05; 200 μg, p < 0.01) (Figure 6C&D). These results collaboratively suggested that the anti-Siglec-15 mAb 3D6 has an advantage over 5G12 on cancer immunotherapy.

6) Please present all the Singlet-15 expression data in the CHO-K1 cells for the pspax2, pMD2G, and pLVXIRES-Siglec-15-Puro vectors, possibly in a supplemental document for the inferior vectors (i.e., those that did not make the cut). Do this for all the other data obtained but not reported.

Response: Thanks for the editor. Siglec-15 expression data in the CHO-K1 cells for the pspax2, pMD2G, and pLVXIRES-Siglec-15-Puro vectors were added in Figures S1 and S2 in the supplemental document in the revised manuscript.

3rd Editorial decision
29-Sep-2021

Ref.: Ms. No. JCTRes-D-21-00062R2
High Affinity Monoclonal Antibody Targeting Siglec-15 for Cancer Immunotherapy
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers: