



Life Science Alliance

Dimerization of kringle 1 domain from HGF/SF provides a potent minimal MET receptor agonist

Giovanni De Nola, Bérénice Leclercq, Alexandra Mougel, Solenne Taront, Claire Simonneau, Federico Forneris, Eric Adriaenssens, Herve Drobecq, Luisa Iamele, Laurent Dubuquoy, Oleg Melnyk, Ermanno Gherardi, Hugo de Jonge, and Jérôme Vicogne

DOI: <https://doi.org/10.26508/lsa.202201424>

Corresponding author(s): Jérôme Vicogne, University of Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 - CILL - Center for Infection and Immunity of Lille and Hugo de Jonge, University of Pavia

Review Timeline:

Submission Date:	2022-02-25
Editorial Decision:	2022-04-19
Revision Received:	2022-06-20
Editorial Decision:	2022-06-27
Revision Received:	2022-07-01
Accepted:	2022-07-06

Scientific Editor: Novella Guidi

Transaction Report:

(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. The original formatting of letters and referee reports may not be reflected in this compilation.)

April 19, 2022

Re: Life Science Alliance manuscript #LSA-2022-01424-T

Dr. Jérôme Vicogne
University of Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 - CIIL - Center for Infection and Immunity of Lille
1 rue du Pr Calmette
Lille 59021
France

Dear Dr. Vicogne,

Thank you for submitting your manuscript entitled "Dimerization of kringle 1 domain from HGF/SF provides a potent minimal MET receptor agonist" to Life Science Alliance. The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revised manuscript addressing the Reviewer comments.

To upload the revised version of your manuscript, please log in to your account: <https://lsa.msubmit.net/cgi-bin/main.plex>

You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

While you are revising your manuscript, please also attend to the below editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

The typical timeframe for revisions is three months. Please note that papers are generally considered through only one revision cycle, so strong support from the referees on the revised version is needed for acceptance.

When submitting the revision, please include a letter addressing the reviewers' comments point by point.

We hope that the comments below will prove constructive as your work progresses.

Thank you for this interesting contribution to Life Science Alliance. We are looking forward to receiving your revised manuscript.

Sincerely,

Novella Guidi, PhD
Scientific Editor
Life Science Alliance

A. THESE ITEMS ARE REQUIRED FOR REVISIONS

-- A letter addressing the reviewers' comments point by point.

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure, supplementary figure and video files uploaded as individual files: See our detailed guidelines for preparing your production-ready images, <https://www.life-science-alliance.org/authors>

-- Summary blurb (enter in submission system): A short text summarizing in a single sentence the study (max. 200 characters including spaces). This text is used in conjunction with the titles of papers, hence should be informative and complementary to the title and running title. It should describe the context and significance of the findings for a general readership; it should be written in the present tense and refer to the work in the third person. Author names should not be mentioned.

-- By submitting a revision, you attest that you are aware of our payment policies found here: <https://www.life-science-alliance.org/copyright-license-fee>

B. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, <https://www.life-science-alliance.org/authors>

We encourage our authors to provide original source data, particularly uncropped/-processed electrophoretic blots and spreadsheets for the main figures of the manuscript. If you would like to add source data, we would welcome one PDF/Excel-file per figure for this information. These files will be linked online as supplementary "Source Data" files.

IMPORTANT: It is Life Science Alliance policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Reviewer #1 (Comments to the Authors (Required)):

The paper of de Nola et al is a very interesting study, which describes the development of a novel MET agonist, namely K1K1, derived from the natural HGF ligand. The HGF is a well-recognized promoter of cell migration and tissue regeneration, thereby the described recombinant molecule could potentially represent a tool for translational medicine applications. I am not able to review and discuss the structural data of K1K1 and the proposed mechanism of 1:2 K1K1: MET configuration. However, I find very convincing the data showing that the new molecule has 1) low affinity binding for heparin and 2) high potency on MET activation. The in vivo efficacy of K1K1 highlights the potentially promising perspective of this new molecule.

The manuscript is well written, technically sound and the results are very convincing. The experiments are carefully performed and the results are clearly presented. It is well-grounded on the literature, which is well referenced. I would recommend the publication of this paper essentially as it is.

Reviewer #2 (Comments to the Authors (Required)):

In this manuscript, a novel agonist of HGF (Hepatocyte Growth Factor) and its characterisation is reported. It consists in a dimer of the first Kringle domain of HGF, called K1K1. The rationale for its design and the determination of its structure are presented. Its role in binding MET and in in vitro functional assays and in vivo (as treatment of alcoholic steatohepatitis) was determined. In vitro, comparisons were made with HGF and K1K1 mutated in its the low-affinity heparin binding sites (K1K1S2 and K1K1S4). It was concluded that K1K1 is a potent agonist of HGF, suggesting it could be used for therapeutic purpose in the future for various diseases in the liver or other diseases such as COPD.

This is an interesting manuscript with potential important clinical implication. The paper is clearly written and presented and experiments appear well performed.

Minor comments:

Figure 5B: The difference in 3D morphogenesis between 10nM of K1K1S2 and 10nM of K1K1 are not clear. It seems that K1K1S2 is still able to stimulate the 3D morphogenesis. Is there a way to provide quantitative data of this assay?

Figure 5C: It would be good to obtain statistical results from at least 3 independent experiments in this assay as the scattering and the morphogenesis assay are not quantitative.

It would be important to discuss the possible adverse effects of treating patients with such a MET agonist, such as promotion of cancer development or progression.

Dear editor,

We thank the reviewers for their very positive evaluations of our work and we are pleased to propose to LSA a revised version of our manuscript taking into consideration the comments from Reviewer#2. In particular we have added a quantitative assay (Fig. 5C). All modification/addition have been highlighted within the text.

- *Figure 5B: The difference in 3D morphogenesis between 10 nM of K1K1S2 and 10 nM of K1K1 are not clear. It seems that K1K1S2 is still able to stimulate the 3D morphogenesis. Is there a way to provide quantitative data of this assay?*

In this morphogenesis assay, we have selected the “most branched” observable structure for each condition. Indeed, it is very difficult to make quantification in a thick Matrigel layer since only a small volume of the well is accessible for imaging. In addition, in agreement with the scattering (Fig. 5A) and migration assays (Fig. 5C), K1K1S2 at doses >10 nM still induced a significant tubulogenesis, even if less pronounced than HGF/SF and K1K1, whereas the K1K1S4 is ineffective with a net proliferation (spheres) but no tubulogenesis. This point has been added in the results section.

- *Figure 5C: It would be good to obtain statistical results from at least 3 independent experiments in this assay as the scattering and the morphogenesis assay are not quantitative.*

We have performed three independent experiments with each condition present five times and averaged. To allow comparison between the independent assays, we made the migration induced by 0.1 nM HGF/SF - which is the maximum observed in each assay - 100% and used this to calculate the percentage of migration for all other conditions. For statistical analysis, we used the Anova Dunnett's test and marked the conditions that significantly differed with an asterisk.

- It would be important to discuss the possible adverse effects of treating patients with such a MET agonist, such as promotion of cancer development or progression.

We have added this point at the end of the discussion section with the corresponding references.

The manuscript and figures have been edited to fit the LSA guidelines. All source data have been collected and uploaded together with supplementary information.

June 27, 2022

RE: Life Science Alliance Manuscript #LSA-2022-01424-TR

Dr. Jérôme Vicogne
University of Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 - CIIL - Center for Infection and Immunity of Lille
1 rue du Pr Calmette
Lille 59021
France

Dear Dr. Vicogne,

Thank you for submitting your revised manuscript entitled "Dimerization of kringle 1 domain from HGF/SF provides a potent minimal MET receptor agonist". We would be happy to publish your paper in Life Science Alliance pending final revisions necessary to meet our formatting guidelines.

Along with points mentioned below, please tend to the following:

- please add ORCID ID for secondary corresponding author-they should have received instructions on how to do so
- please add a conflict of interest statement to the main manuscript text
- please consult our manuscript preparation guidelines <https://www.life-science-alliance.org/manuscript-prep> and make sure your manuscript sections are in the correct order
- please add your supplementary figure and table legends to the main manuscript text and provide your table files in editable doc or excel file format
- please add a callout for Figure 2A & C; Figure 3B; and Figure 4C to your main manuscript text
- please upload your main figures as single files; these will be displayed in-line in the HTML version of your paper, so please provide them as single page files (Figure 1 currently spans 2 pages); we do not have a limit on the number of main figures and these can be split if necessary for space
- 3 secondary Corresponding Authors are listed; it is LSA's policy to limit the number of Corresponding Authors to 2

Figure Check:

- The scale bars in Figures 7, S4 and S7 are hard to see

If you are planning a press release on your work, please inform us immediately to allow informing our production team and scheduling a release date.

LSA now encourages authors to provide a 30-60 second video where the study is briefly explained. We will use these videos on social media to promote the published paper and the presenting author (for examples, see <https://twitter.com/LSAjournal/timelines/1437405065917124608>). Corresponding or first-authors are welcome to submit the video. Please submit only one video per manuscript. The video can be emailed to contact@life-science-alliance.org

To upload the final version of your manuscript, please log in to your account: <https://lsa.msubmit.net/cgi-bin/main.plex>
You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. FINAL FILES:

These items are required for acceptance.

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure, supplementary figure and video files uploaded as individual files: See our detailed guidelines for preparing your production-ready images, <https://www.life-science-alliance.org/authors>

-- Summary blurb (enter in submission system): A short text summarizing in a single sentence the study (max. 200 characters including spaces). This text is used in conjunction with the titles of papers, hence should be informative and complementary to the title. It should describe the context and significance of the findings for a general readership; it should be written in the present tense and refer to the work in the third person. Author names should not be mentioned.

B. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, <https://www.life-science-alliance.org/authors>

We encourage our authors to provide original source data, particularly uncropped/-processed electrophoretic blots and spreadsheets for the main figures of the manuscript. If you would like to add source data, we would welcome one PDF/Excel-file per figure for this information. These files will be linked online as supplementary "Source Data" files.

****Submission of a paper that does not conform to Life Science Alliance guidelines will delay the acceptance of your manuscript.****

****It is Life Science Alliance policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.****

****The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements.****

****Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.****

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7 days.

Thank you for this interesting contribution, we look forward to publishing your paper in Life Science Alliance.

Sincerely,

Novella Guidi, PhD
Scientific Editor
Life Science Alliance

Reviewer #2 (Comments to the Authors (Required)):

I am satisfied with the revised manuscript and recommend publication

July 6, 2022

RE: Life Science Alliance Manuscript #LSA-2022-01424-TRR

Dr. Jérôme Vicogne
University of Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 - CIIL - Center for Infection and Immunity of Lille
1 rue du Pr Calmette
Lille 59021
France

Dear Dr. Vicogne,

Thank you for submitting your Research Article entitled "Dimerization of kringle 1 domain from HGF/SF provides a potent minimal MET receptor agonist". It is a pleasure to let you know that your manuscript is now accepted for publication in Life Science Alliance. Congratulations on this interesting work.

The final published version of your manuscript will be deposited by us to PubMed Central upon online publication.

Your manuscript will now progress through copyediting and proofing. It is journal policy that authors provide original data upon request.

Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.

IMPORTANT: If you will be unreachable at any time, please provide us with the email address of an alternate author. Failure to respond to routine queries may lead to unavoidable delays in publication.

Scheduling details will be available from our production department. You will receive proofs shortly before the publication date. Only essential corrections can be made at the proof stage so if there are any minor final changes you wish to make to the manuscript, please let the journal office know now.

DISTRIBUTION OF MATERIALS:

Authors are required to distribute freely any materials used in experiments published in Life Science Alliance. Authors are encouraged to deposit materials used in their studies to the appropriate repositories for distribution to researchers.

You can contact the journal office with any questions, contact@life-science-alliance.org

Again, congratulations on a very nice paper. I hope you found the review process to be constructive and are pleased with how the manuscript was handled editorially. We look forward to future exciting submissions from your lab.

Sincerely,

Novella Guidi, PhD
Scientific Editor
Life Science Alliance