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Aluminium (III) phthalocyanine chloride tetrasulphonate is an effective photosensitizer for the eradication of lung cancer stem cells

Anine Crous and Heidi Abrahamse

Article citation details
R. Soc. open sci. **8**: 210148.
http://dx.doi.org/10.1098/rsos.210148

Review timeline
Original submission: 28 January 2021
Revised submission: 13 July 2021
Final acceptance: 19 August 2021

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSOS-210148.R0 (Original submission)

Review form: Reviewer 1

Is the manuscript scientifically sound in its present form?
No

Are the interpretations and conclusions justified by the results?
No

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
Yes

Recommendation?
Major revision is needed (please make suggestions in comments)

Comments to the Author(s)
This manuscript by Crous et al. investigates a photosensitizer (AIPcS4CL) to kill lung cancer stem cells. Following purification/characterisation of lung cancer stem cells the authors proceed to investigate the potency and potential mechanism of action of the photosensitiser, showing that it effectively kills cancer cells. Overall the work is timely, interesting and well performed. However, there are few expts. that in my opinion have been over interpreted, require additional work or toning down of conclusions, these are detailed further.

- Figure 4 - due to the poor quality of the images (and inherent low resolution with conventional IF) it really is impossible to state that the PS is in mitochondria and/or lysosomes, I think the most that can be stated with any certainty is that it (PS) is in the cell, though ideally a non PS incubated cell should be included as negative control for the red channel. Conclusions from this expt. should be toned down.

- Figure 6. statistics - the LDH assay states an N=3, its difficult (near impossible) to consider these data as biological replicates given the extremely small error bars, are these technical replicates ? - if so the text requires modification and some indication of the reproducibility of the expts should be provided.

- Figure 5/Figure 10, cells with PS plus light don't look apoptotic by morphology - does caspase inhibition prevent or at least slow the extent of cell death ? I think this is necessary to conclude the type of cell death is apoptotic.

- Figure 9. Loss of mitochondrial membrane potential invariably occurs during cell death, authors nicely demonstrate loss of mitochondrial membrane potential but its frankly impossible to state that this is causative of cell death - the text should be modified accordingly

- title should be changed to encompass the authors' findings not the purpose/question of the study

Review form: Reviewer 2

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept as is

Comments to the Author(s)
This MS describes the photosensitizing properties of AlPcS4Cl for targeting CSCs in lung cancers as they are considered responsible for the cell proliferation, cancer recurrence, metastasis, and resistance of cancer to drugs.
Various cell biology methodologies have been used to demonstrate, with regularly positive results, the efficacy of a combination of the new PS at 20 uM plus red light (10 J/cm2).
Although the idea of this study and the experiments are not so innovative, I think this is a good MS, well written, with good figures and explanations, and more importantly, the results could be useful for the palliative treatment of lung cancer with AlPcS4Cl-PDT.

Decision letter (RSOS-210148.R0)

We hope you are keeping well at this difficult and unusual time. We continue to value your support of the journal in these challenging circumstances. If Royal Society Open Science can assist you at all, please don't hesitate to let us know at the email address below.

Dear Dr Crous,

The Editors assigned to your paper RSOS-210148 "Photodynamic Therapy for Lung Cancer Stem Cell Eradication – Is $AlPcS_4Cl$ an Effective Photosensitizer?" have now received comments from reviewers and would like you to revise the paper in accordance with the reviewer comments and any comments from the Editors. Please note this decision does not guarantee eventual acceptance.

We invite you to respond to the comments supplied below and revise your manuscript. Below the referees' and Editors' comments (where applicable) we provide additional requirements. Final acceptance of your manuscript is dependent on these requirements being met. We provide guidance below to help you prepare your revision.

We do not generally allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Editors, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available, we may invite new reviewers.

Please submit your revised manuscript and required files (see below) no later than 21 days from today's (ie 28-Jun-2021) date. Note: the ScholarOne system will 'lock' if submission of the revision is attempted 21 or more days after the deadline. If you do not think you will be able to meet this deadline please contact the editorial office immediately.

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Thank you for submitting your manuscript to Royal Society Open Science and we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Kind regards,
Royal Society Open Science Editorial Office
Royal Society Open Science
openscience@royalsociety.org

on behalf of Dr Simon Cook (Associate Editor) and Catrin Pritchard (Subject Editor)
openscience@royalsociety.org

Associate Editor Comments to Author (Dr Simon Cook):

Your manuscript has now been reviewed. The manuscript is considered to be of interest to the readership of Open Science. However, it is felt that some of your conclusions are premature or over-interpreted.

Please in particular consider the comments of Reviewer 1 below in preparing a revised version of your manuscript

- Figure 4 - due to the poor quality of the images (and inherent low resolution with conventional IF) it really is impossible to state that the PS is in mitochondria and/or lysosomes, I think the most that can be stated with any certainty is that it (PS) is in the cell, though ideally a non PS incubated cell should be included as negative control for the red channel. Conclusions from this expt. should be toned down.

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- title should be changed to encompass the authors' findings not the purpose/question of the study

I look forward to receiving your revised manuscript

Reviewer comments to Author:
Reviewer: 1
Comments to the Author(s)
This manuscript by Crous et al. investigates a photosensitizer (AIPcS4CL) to kill lung cancer stem cells. Following purification/characterisation of lung cancer stem cells the authors proceed to investigate the potency and potential mechanism of action of the photosensitiser, showing that it effectively kills cancer cells. Overall the work is timely, interesting and well performed. However, there are few expts. that in my opinion have been over interpreted, require additional work or toning down of conclusions, these are detailed further.

- Figure 4 - due to the poor quality of the images (and inherent low resolution with conventional IF) it really is impossible to state that the PS is in mitochondria and/or lysosomes, I think the most that can be stated with any certainty is that it (PS) is in the cell, though ideally a non PS incubated cell should be included as negative control for the red channel. Conclusions from this expt. should be toned down.

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Reviewer: 2
Comments to the Author(s)
This MS describes the photosensitizing properties of AlPcS4Cl for targeting CSCs in lung cancers as they are considered responsible for the cell proliferation, cancer recurrence, metastasis, and resistance of cancer to drugs.
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Although the idea of this study and the experiments are not so innovative, I think this is a good MS, well written, with good figures and explanations, and more importantly, the results could be useful for the palliative treatment of lung cancer with AlPcS4Cl-PDT.

===PREPARING YOUR MANUSCRIPT===

Your revised paper should include the changes requested by the referees and Editors of your manuscript. You should provide two versions of this manuscript and both versions must be provided in an editable format:
one version identifying all the changes that have been made (for instance, in coloured highlight, in bold text, or tracked changes);
a 'clean' version of the new manuscript that incorporates the changes made, but does not highlight them. This version will be used for typesetting if your manuscript is accepted.

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===PREPARING YOUR REVISION IN SCHOLARONE===

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Attach your point-by-point response to referees and Editors at Step 1 'View and respond to decision letter'. This document should be uploaded in an editable file type (.doc or .docx are preferred). This is essential.

Please ensure that you include a summary of your paper at Step 2 'Type, Title, & Abstract'. This should be no more than 100 words to explain to a non-scientific audience the key findings of your research. This will be included in a weekly highlights email circulated by the Royal Society press office to national UK, international, and scientific news outlets to promote your work.

At Step 3 'File upload' you should include the following files:
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2) A 'clean' version of the new manuscript that incorporates the changes made, but does not highlight them.
-- An individual file of each figure (EPS or print-quality PDF preferred [either format should be produced directly from original creation package], or original software format).
-- An editable file of each table (.doc, .docx, .xls, .xlsx, or .csv).
-- An editable file of all figure and table captions.
Note: you may upload the figure, table, and caption files in a single Zip folder.
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-- If you are requesting a discretionary waiver for the article processing charge, the waiver form must be included at this step.
-- If you are providing image files for potential cover images, please upload these at this step, and inform the editorial office you have done so. You must hold the copyright to any image provided.
-- A copy of your point-by-point response to referees and Editors. This will expedite the preparation of your proof.

At Step 6 'Details & comments', you should review and respond to the queries on the electronic submission form. In particular, we would ask that you do the following:
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-- If you have uploaded ESM files, please ensure you follow the guidance at https://royalsociety.org/journals/authors/author-guidelines/#supplementary-material to include a suitable title and informative caption. An example of appropriate titling and captioning may be found at https://figshare.com/articles/Table_S2_from_Is_there_a_trade-off_between_peak_performance_and_performance_breadth_across_temperatures_for_aerobic_scope_in_teleost_fishes_/3843624.

At Step 7 'Review & submit', you must view the PDF proof of the manuscript before you will be able to submit the revision. Note: if any parts of the electronic submission form have not been completed, these will be noted by red message boxes.

Author's Response to Decision Letter for (RSOS-210148.R0)

See Appendix A.

Decision letter (RSOS-210148.R1)

We hope you are keeping well at this difficult and unusual time. We continue to value your support of the journal in these challenging circumstances. If Royal Society Open Science can assist you at all, please don't hesitate to let us know at the email address below.

Dear Dr Crous,

It is a pleasure to accept your manuscript entitled "$AlPcS_4Cl$ is an Effective Photosensitizer for the Eradication of Lung Cancer Stem Cells" in its current form for publication in Royal Society Open Science. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

===COVID-SPECIFIC TEXT -- WILL ONLY BE ADDED TO COVID-PAPERS BY THE EDITORIAL OFFICE===

COVID-19 rapid publication process:
We are taking steps to expedite the publication of research relevant to the pandemic. If you wish, you can opt to have your paper published as soon as it is ready, rather than waiting for it to be published the scheduled Wednesday.

This means your paper will not be included in the weekly media round-up which the Society sends to journalists ahead of publication. However, it will still appear in the COVID-19 Publishing Collection which journalists will be directed to each week (https://royalsocietypublishing.org/topic/special-collections/novel-coronavirus-outbreak).

If you wish to have your paper considered for immediate publication, or to discuss further, please notify openscience_proofs@royalsociety.org and press@royalsociety.org when you respond to this email.

===END OF COVID-SPECIFIC TEXT -- WILL BE REMOVED AS NECESSARY BY THE EDITORIAL OFFICE===

Please ensure that you send to the editorial office an editable version of your accepted manuscript, and individual files for each figure and table included in your manuscript. You can send these in a zip folder if more convenient. Failure to provide these files may delay the processing of your proof. You may disregard this request if you have already provided these files to the editorial office.

You can expect to receive a proof of your article in the near future. Please contact the editorial office (openscience@royalsociety.org) and the production office (openscience_proofs@royalsociety.org) to let us know if you are likely to be away from e-mail contact -- if you are going to be away, please nominate a co-author (if available) to manage the proofing process, and ensure they are copied into your email to the journal.

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Thank you for your fine contribution. On behalf of the Editors of Royal Society Open Science, we look forward to your continued contributions to the Journal.

Kind regards,
Royal Society Open Science Editorial Office
Royal Society Open Science
openscience@royalsociety.org

on behalf of Dr Payam Gammage (Associate Editor) and Catrin Pritchard (Subject Editor)
openscience@royalsociety.org

Associate Editor Comments to Author (Dr Payam Gammage):
Associate Editor
Comments to the Author:
(There are no comments.)

Reviewer comments to Author:

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Appendix A

13 July 2021

To: Dr Simon Cook (Associate Editor) and Catrin Pritchard (Subject Editor)
Title: " $AlPcS_4Cl$ is an Effective Photosensitizer for the Eradication of Lung Cancer Stem Cells"
Authors: Dr Anine Crous and Prof Heidi Abrahamse

CORRECTIONS TO MANUSCRIPT: RSOS-210148

Dear Dr Simon Cook

Thank you for the opportunity to strengthen the manuscript Titled: "**$AlPcS_4Cl$ is an Effective Photosensitizer for the Eradication of Lung Cancer Stem Cells**" as suggested by the reviewers. The reviewers provided informative feedback and recommendations. All statements were addressed, and revisions were made for publishing in the ***Royal Society Open Science***.

Please find below the reports of the reviewers, together with the comments and corrections made, as shown in red.

I trust these corrections will meet with your approval.
Thank you for your support.

Sincerely,

Dr Anine Crous (PhD)
Postdoctoral Fellow
Laser Research Centre
Faculty of Health Sciences
University of Johannesburg
South Africa
Email: acrous@uj.ac.za

RESPONSE TO REVIEWERS: RSOS-210148

Associate Editor:

"Your manuscript has now been reviewed. The manuscript is considered to be of interest to the readership of Open Science. However, it is felt that some of your conclusions are premature or over-interpreted. Please in particular consider the comments of Reviewer 1 below in preparing a revised version of your manuscript. I look forward to receiving your revised manuscript."

Comment and correction:

The authors would like to thank the editor for their feedback and the opportunity to revise and improve the manuscript as suggested by reviewer 1.

Reviewer #1:

This manuscript by Crous et al. investigates a photosensitizer (AlPcS4CL) to kill lung cancer stem cells. Following purification/characterisation of lung cancer stem cells the authors proceed to investigate the potency and potential mechanism of action of the photosensitiser, showing that it effectively kills cancer cells. Overall the work is timely, interesting and well performed. However, there are few expts. that in my opinion have been over interpreted, require additional work or toning down of conclusions, these are detailed further.

Comment and correction:

The authors would like to thank the reviewer for their positive feedback, the manuscript was revised where overinterpretation of experiments and conclusions were toned down as seen below.

- Figure 4 - due to the poor quality of the images (and inherent low resolution with conventional IF) it really is impossible to state that the PS is in mitochondria and/or lysosomes, I think the most that can be stated with any certainty is that it (PS) is in the cell, though ideally a non PS incubated cell should be included as negative control for the red channel. Conclusions from this expt. should be toned down.

Comment and correction:

Figure 4. Image processed using Image J, where backgrounds were subtracted to enhance the FL images.

Conclusions from intracellular localisation of the PS toned down:

Page 9; line 283-285: Results: "Results show AlPcS$_4$Cl is embedded in the cytosol and perinuclear region, that upon photoactivation can cause peripheral photodamage to the cell membrane and plasma membranes of various intracellular organelles including mitochondria and lysosomes."

Page 17; line 456: Discussion:" …where the PS localizes in the cytosol and around vital intracellular organelles…"

- Figure 6. statistics - the LDH assay states an N=3, its difficult (near impossible) to consider these data as biological replicates given the extremely small error bars, are these technical replicates ? - if so the text requires modification and some indication of the reproducibility of the expts should be provided.

Comment and correction:

Figure 6. An axial break (Y-axis) was included in the graph for ease of viewing experimental errors. All experimental groups were performed 3 times (n=3) (biological replicates). Furthermore, all spectroscopy assays were performed in duplicate (technical replicates).

- Figure 5/Figure 10, cells with PS plus light don't look apoptotic by morphology - does caspase inhibition prevent or at least slow the extent of cell death ? I think this is necessary to conclude the type of cell death is apoptotic.

Comment and correction:

Morphological findings seen and described motivate for the apoptotic and necrotic cell death conclusions and were edited as below. Furthermore, the accumulated experimental results specifically, mitochondrial membrane potential, and Annexin VPI cell death mechanisms indicate apoptotic and necrotic cell death.

Page 10; line 312 – 313: Results: "…including, condensation, fragmentation, apoptotic bodies and vacuolization indicative of apoptosis, cell swelling and lysis was also noted indicative of necrosis…"

Page 1; line 317-318: Figure 5 Caption: "…d) Lung CSCs that received PDT using 20 µM AlpcS$_4$Cl and 10J/cm^2 irradiation, show indications of apoptotic (green) and necrotic (red) cell death."

- Figure 9. Loss of mitochondrial membrane potential invariably occurs during cell death, authors nicely demonstrate loss of mitochondrial membrane potential but its frankly impossible to state that this is causative of cell death - the text should be modified accordingly

Comment and correction:

The authors are not aware of the above statement: "los of MMP is caused by cell death". The authors checked that the correct statement referring to 'loss of MMP **leads** to cell death' was used throughout the text.

- title should be changed to encompass the authors' findings not the purpose/question of the study

Comment and correction:

The title was changed to encompass the research findings: "AlPcS$_4$Cl is an Effective Photosensitizer for the Eradication of Lung Cancer Stem Cells."

Reviewer #2:

This MS describes the photosensitizing properties of AlPcS4Cl for targeting CSCs in lung cancers as they are considered responsible for the cell proliferation, cancer recurrence, metastasis, and resistance of cancer to drugs. Various cell biology methodologies have been used to demonstrate, with regularly positive results, the efficacy of a combination of the new PS at 20 uM plus red light (10 J/cm2). Although the idea of this study and the experiments are not so innovative, I think this is a good MS, well written, with good figures and explanations, and more importantly, the results could be useful for the palliative treatment of lung cancer with AlPcS4Cl-PDT.

Comment:

The authors would like to thank the reviewer for their time in reviewing the manuscript and the overall positive comments regarding the research.