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Site-specific pharmaco-laser therapy: a novel treatment modality for refractory port wine stains

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Handling editor:
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1st Editorial decision

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Ref.: Ms. No. JCTRes-D-19-00002
Site-specific pharmaco-laser therapy: a novel treatment modality for refractory port wine stains
Journal of Clinical and Translational Research

Dear Mr. van Raath,

Reviewers have now commented on your paper. You will see that they are generally content your work, but do advise that you revise your manuscript. 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 Apr 20, 2019.

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

Rowan van Golen
Associate Editor
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: This manuscript describes site-specific pharmaco-laser therapy for treatment of port wine stains in a very thorough manner.
Overall this is a very good manuscript which provides the most complete information on SSPLT that I have seen.
The manuscript is somewhat long but this does allow a more complete discussion. Authors might consider whether it could be shortened somewhat.
I was wondering if the authors could address what would happen if vessel rupture occurred with laser treatment as opposed to just vascular occlusion? If this is an issue what monitoring would be done to prevent this?

Abstract: The authors states that clinical outcomes with laser therapy remains poor. This is true for some patients but many patients who get early treatment have very good results. You might soften this statement somewhat.
I may have missed it but is Figure 3 first referenced on page 18?
Is Figure 10 referenced in the manuscript?
Section 3:
On page 15, the authors state that it is surprising that complications of thrombosis or embolization does to occur. I believe this is because these are clots in small vessels in the skin and the very small clots formed are not clinically relevant. I would consider removing this statement.
Section 4:
On page 37, the author says that PDT may be used in China partially because this population is culturally sun-shy. Did this come from one of the references? I think this statement could distract the reader and I would recommend removing.

I generally think the discussion should not introduced new material. As such, it might be best to include the information on photodynamic therapy in the introduction. Limitations of laser therapy and PDL could then be briefly reviewed at the beginning of section 4.

Reviewer #2: Authors are congratulated for a very informative manuscript. I have the following comments aimed at further improving the manuscript:

1) Please clarify if ephrin B2 is a receptor or a ligand. Also it would be useful to add some background on eph receptor B1.

2) Figure 1 is somewhat misleading in that it shown the port wine stain to be on the very surface of skin; whereas what gives rise to the appearance of a stain, as pointed out by the authors, is the hyperdilated capillaries and post-capillary venules in the dermis. Please adjust the Figure as necessary.

3) The indicated minimum temperature of 45 °C seems somewhat low as a denaturing temperature. In caption of Fig. 1, I am not sure if "denaturation of cells" is the correct term. Not clear what the white region with an arrow in it is supposed to represent in Figures. 2b and 2d.

4) I supposed the information in the excel sheets will be in a Table form in the final publication.

5) Some of the bars in Fig. 3 seem to add up to more than 100% based on the scale bar provided. For example, top panel Ref. 39. Or maybe it is just how the bars and the scale bar are represented, requiring some clarification.

6) If the superimposed vessels are non-lesional (i.e., normal vessels), would they also be photothermally destroyed? If so, is that necessarily an issue? Please comment.

7) When referencing mathematical modeling results to indicate damage depth of 0.6-0.9, the wavelength and pulse duration should be provided. Same comment when referencing clinical results a few lines below and elsewhere in the manuscript as necessary.

8) Figure 4 caption: Time is the top x-axis, not the bottom x-axis. "Trace" should probably be replace with "zone."

9) If Figure 5 is from a previously published result, the reference should be provided. What is the point of "releasing the cargo" if it is the heat that induces the damage?

10) I am somewhat confused by the images shown in Figure 5: if fluorescence of CF is quenched (due to its high concentration of 100 mM), why is there a bright spot in the middle panel (Laser Pulse). If CV is then released and diluted in blood to restore fluorescence, why is there not bright spot in the image (1 sec after the pulse). If anything, I would have thought CF would be degraded and turned into a lueco form once irradiated.

11) Please provide some more information about the mechanisms associated with chronic inflammatory state that result in removal of PWS vessels.

12) Why are liposomes "most" advantageous? They themselves could be the subject of recognition by immune cells with subsequent removal, leading to a short circulation time. Other types of delivery particles could potentially include the necessary macromolecule to impede or delay uptake by macrophages. To claim as most advantageous is somewhat premature at this time.

13) A key troubling issue is that size is NOT the only determinant of the uptake particles by MPS. There are other factors (e.g., surface characteristics including zeta potentials, etc.) that play a role. This is one of my major comments.

14) PEG size is also important and needs to be indicated (For example, see B. Bahmani et al, Journal of Biomedical Optics, 2011)

15) p. 24: Please rephrase "good" tissue penetration in a quantitative manner.

16) Functionalization of (ICG-loaded) particles with surface PEGylation has been reported, and should be cited (J. T. Mac, Biomedical Optics Express, 2016; B. Bahmani et al, Lasers in Surgery and Medicine, 2014).

17) Platelets are not the only (blood) cell types with PS (normally confined to the inner leaflet). Red blood cells do as other cell types. Therefore, the statement (p. 32) needs to be corrected.

18) For the PDT studies indicated in China, can you please provide the photosensitizers and irradiation wavelengths in the main text (relevant section in Discussion).

Authors'rebuttal:

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Amsterdam, 29 March 2019

Re: Resubmission Ms. No. JCTRes-D-19-00002

Dear Dr. van Golen,

Please find enclosed the resubmission of our manuscript entitled "*Site-specific pharmacolaser therapy: a novel treatment modality for refractory port wine stains.*" We would like to thank the reviewers for critically reading the manuscript. We have addressed the reviewer's comment in red italics below and used the track changes function in the text to indicate the modifications.

Thank you for the opportunity to resubmit the manuscript.

Kindest regards, also on behalf of the co-authors,

Ingmar van Raath.

Review 1

Authors are congratulated for a very informative manuscript. I have the following comments aimed at further improving the manuscript:
We thank the reviewer for his or her time and thorough review of the paper.

1) Please clarify if ephrin B2 is a receptor or a ligand. Also it would be useful to add some background on eph receptor B1.

Although Ephrin B2 is considered a ligand (for eph receptors), in this case this does not mean unidirectional signaling because the ephrin ligands and their receptors are capable of forward (ligand-to-receptor) and backward (receptor-to-ligand) signaling. We have added this information and some background information on ephrin ligands and receptors to the introduction.

2) Figure 1 is somewhat misleading in that it shown the port wine stain to be on the very surface of skin; whereas what gives rise to the appearance of a stain, as pointed out by the authors, is the hyperdilated capillaries and post-capillary venules in the dermis. Please adjust the Figure as necessary.

We modified this figure to represent the deeper-seated (sub-surface) etiology of PWS. Please let us know if you feel this figure requires further modification.

3) The indicated minimum temperature of 45 °C seems somewhat low as a denaturing temperature. In caption of Fig. 1, I am not sure if "denaturation of cells" is the correct term.

Not clear what the white region with an arrow in it is supposed to represent in Figures. 2b and 2d.

We agree that this is somewhat confusing. 45 ºC is the denaturing temperature for plasma proteins (albumin) (Nilsson et al, reference #147). The exact intravascular isotherm needed for coagulum generation during PWS therapy is unclear. We have removed the temperature (>45 ºC) from page 7 to prevent confusion.

Good point, in the caption of Fig. 2, "denaturation of cells" has been changed to "blood cell thermolysis"

The arrows in Fig. 2 indicate the two potential sequences of events. To clarify this, "*upper pathway*" and "*bottom pathway*" were added to the caption of Fig. 2.

4) I supposed the information in the excel sheets will be in a Table form in the final publication.

In order to preserve readability, the final publication will include the original Excel sheets as supplementary files.

5) Some of the bars in Fig. 3 seem to add up to more than 100% based on the scale bar provided. For example, top panel Ref. 39. Or maybe it is just how the bars and the scale bar are represented, requiring some clarification.

The included papers have classified clinical results in the (color-coded) percentage PWS clearance-classes in the legend. The distribution within the bars represent the proportion of patients (of single studies) in these classes. We have added "PWS clearance" to the captions of Fig. 3 and Fig. 4, and changed the legend title within these figures to "Classifications of percentage PWS clearance" to clarify this matter.

6) If the superimposed vessels are non-lesional (i.e., normal vessels), would they also be photothermally destroyed? If so, is that necessarily an issue? Please comment.

In line with Section 1.2., normal capillaries would not be destroyed as their higher surface-to volume ratio precludes sufficient heating to (supra-)critical temperatures.

7) When referencing mathematical modeling results to indicate damage depth of 0.6-0.9, the wavelength and pulse duration should be provided. Same comment when referencing clinical results a few lines below and elsewhere in the manuscript as necessary.

The 0.6-0.9 mm is the modelled depth up to which dilated vessels contribute to the aberrant appearance and is not related to laser irradiation, therefore there are no relevant optical parameters. For the damage depth of 0.6 mm (described a few lines below) the laser parameters are indicated (i.e., 585 nm with a 0.45 ms pulse). The laser parameters of other references throughout the manuscript were added.

8) Figure 4 (now Fig. 5) caption: Time is the top x-axis, not the bottom x-axis. "Trace" should probably be replace with "zone."

We corrected bottom axis to “top x-axis”.

We believe trace is more appropriate. The black, green, and red traces (lines) represent the thickness of the thermal coagulum, thrombosis, and (hypothetical) thrombosis under SSPLT, respectively, relative to the vessel diameter at each time point. Other than in most line-graphs, the size of each individual component runs vertically until the line superior to it. However, “zone” would incorrectly imply the area-under-the-curve and does not reflect the temporal nature of these components.

9) If Figure 5 (now 6) is from a previously published result, the reference should be provided. What is the point of "releasing the cargo" if it is the heat that induces the damage?

Contrary to Figure 6 (now 7), the data in Figure 5 (now 6) is from unpublished data.
This figure illustrates the laser-induced heat generation that is imperative for both standard laser therapy (based on selective photothermolysis; which is indeed based on heat-induced damage), but also heat-induced cargo (i.e., drug) release in SSPLT. In other words, CF-release is used as proof of intravascular heat-generation and also as evidence for cargo/drug-release by thermosensitive liposomes (as described in section 3.2. and 3.4.). To underscore this, a reference to this figure was placed in section 3.2.

10) I am somewhat confused by the images shown in Figure 5 (now 6): if fluorescence of CF is quenched (due to its high concentration of 100 mM), why is there a bright spot in the middle panel (Laser Pulse). If CV is then released and diluted in blood to restore fluorescence, why is there not bright spot in the image (1 sec after the pulse). If anything, I would have thought CF would be degraded and turned into a lueco form once irradiated.

The bright spot in the middle panel is the actual laser pulse directed onto the vessel.
In the panel “1 sec after the laser pulse” bright spots are visible on the laser-irradiated site, likely due to (unquenched) CF trapped within the thermal coagulum. We have added to the caption of Fig. 5 (now 6): *“Part of the CF is trapped within (or tethered to) the thermal coagulum.”*

11) Please provide some more information about the mechanisms associated with chronic inflammatory state that result in removal of PWS vessels.

Good point, in light of your remark this sentence (page 18) seems confusing and inaccurate.

We have changed this to:
“Thrombus formation is followed by a remodeling phase (thrombus organization). During this process, vascular repair or reperfusion can occur, which impedes therapeutic efficacy and results in posttreatment lesional recurrence.”

And in the same section we have added:
“Note that reperfusion as a result of the same or similar processes also hampers clinical results in completely photocoagulated vessels [160,161].”

12) Why are liposomes "most" advantageous? They themselves could be the subject of recognition by immune cells with subsequent removal, leading to a short circulation time.

Other types of delivery particles could potentially include the necessary macromolecule to

impede or delay uptake by macrophages. To claim as most advantageous is somewhat
premature at this time.

Although we do believe that liposomes currently represent the most advantageous delivery system, in particular because of their ease of manufacturing and manipulatable attributes, other drug carriers could indeed proof to be more advantageous. We have thus softened this sentence to: *"liposomes, […], currently appear to be the most advantageous considering…"*

13) A key troubling issue is that size is NOT the only determinant of the uptake particles by MPS. There are other factors (e.g., surface characteristics including zeta potentials, etc.) that play a role. This is one of my major comments.

We have added a comment on the relevance of surface charge for liposome uptake to section 3.1.1. The (reduced) rate of opsonizing plasma protein adsorption is already mentioned in this section. Because we intend to employ PEGylation (which attenuates the negative effects of phospholipid charge and liposome composition on circulation time), a (more) complete discussion of the effects of liposome composition and surface charge was perceived to be beyond the scope of this review. Instead, we have pointed our (future) readers to several excellent resources on this subject. Please let u know if you feel that specific factors still need to be addressed in the manuscript.

14) PEG size is also important and needs to be indicated (For example, see B. Bahmani et al, Journal of Biomedical Optics, 2011)

We have added an explanatory sentence on page 21: "*The extent of PEG-mediated stealth effects are dependent on the size of the PEG polymers [1761,177].*"

The PEG size (2000 Daltons on average) has been added on page 38.

15) p. 24: Please rephrase "good" tissue penetration in a quantitative manner.

We changed this to "*relatively deep tissue penetration (in the order of millimeters to centimeters)*".

16) Functionalization of (ICG-loaded) particles with surface PEGylation has been reported, and should be cited (J. T. Mac, Biomedical Optics Express, 2016; B. Bahmani et al, Lasers in Surgery and Medicine, 2014).

The mentioned references were added to the manuscript.

17) Platelets are not the only (blood) cell types with PS (normally confined to the inner leaflet). Red blood cells do as other cell types. Therefore, the statement (p. 32) needs to be corrected.

Other cell types indeed carry phosphatidylserine (PS) as well. Section 3.4.2.3. has been modified accordingly.

18) For the PDT studies indicated in China, can you please provide the photosensitizers and irradiation wavelengths in the main text (relevant section in Discussion).

More than ten studies on PDT have been performed in China with a range of photosensitizers
and wavelengths. It is impossible to provide these characteristics in a brief manner which is why we refer readers to Supplemental Table S3 where all this information is provided.

Reviewer 2
This manuscript describes site-specific pharmaco-laser therapy for treatment of port wine stains in a very thorough manner.

Overall this is a very good manuscript which provides the most complete information on SSPLT that I have seen.

The manuscript is somewhat long but this does allow a more complete discussion. Authors might consider whether it could be shortened somewhat.

Thank you very much for your time and feedback which definitely strengthens this manuscript.

I was wondering if the authors could address what would happen if vessel rupture occurred with laser treatment as opposed to just vascular occlusion? If this is an issue what monitoring would be done to prevent this?

An interesting question that impels a “thought experiment”. As we know, purpura is the result of vessel rupture and leakage of red blood cells into perivascular tissue. There is some evidence that the appearance of purpura is associated with good clinical outcomes (Lou & Geronemus, Dermatologic Surgery 2001, Greve & Raulin, Laser in Surgery and Medicine, 2004) but the underlying mechanisms are not clear.

In the event of vessel rupture, local thrombosis occurs. After activation of the drug delivery system in SSPLT the thrombotic response is stimulated and, depending on the timing relative to the onset of purpura, we could expect to observe less hemorrhage/purpura in comparison to standard PDL. This would be beneficial to patients in terms of post-treatment comfort.
It is currently not clear whether the clinical results of ruptured vessels during standard PDL is better or worse than completely occluded (but otherwise intact) PWS vessels inasmuch as subpurpuric settings may correspond to the presence of incompletely occluded PWS vessels. (Ruptured vessels might even be more prone to reperfusion). It is therefore difficult to predict what would happen in case of vessel rupture with SSPLT and whether vessels that have achieved complete occlusion due to SSPLT perform better in terms of blanching versus ruptured vessels.

A possible downside of the use of purpuric laser settings for SSPLT would be that a considerable fraction of drug carriers might be lost from the intravascular compartment, for which a correction in the dosage may need to be applied.

All in all, the effects of vessel rupture/purpura on clinical efficacy is uncertain and would need to be addressed in a separate study, e.g., by comparing purpuric and subpurpuric settings and perhaps corroborating this with laser speckle imaging data. Because the nature of this response to your comment is so speculative we chose not to include this in the manuscript. If you believe this should be included, please let us know.

(The above also elicits the question whether purpuric vs. subpurpuric settings may be
responsible for the discrepancies in clinical results between studies that have employed angiogenesis inhibitors. Unfortunately the vast majority of studies with angiogenesis inhibitors have not disclosed whether purpuric settings were used.)

Abstract: The authors states that clinical outcomes with laser therapy remains poor. This is true for some patients but many patients who get early treatment have very good results. You might soften this statement somewhat.

We have removed the sentence on poor outcomes and rephrased the first sentence on suboptimal outcomes.

I may have missed it but is Figure 3 first referenced on page 18?

Figure 3 is first referenced on page 8.

Is Figure 10 (now 11) referenced in the manuscript?
Figure 10 (now 11) is referenced on page 26 and we have placed an additional reference on page 20.

Section 3:

On page 15, the authors state that it is surprising that complications of thrombosis or embolization does to occur. I believe this is because these are clots in small vessels in the skin and the very small clots formed are not clinically relevant. I would consider removing this statement.

We agree and have rephrased this sentence to: "*The fact that no cases of clinical complications caused by thrombosis or embolization after laser therapy have been reported is probably due to small size of these thrombi*".

Section 4:
On page 37, the author says that PDT may be used in China partially because this population is culturally sun-shy. Did this come from one of the references? I think this statement could distract the reader and I would recommend removing.

We agree; this part of the sentence has been removed.

I generally think the discussion should not introduced new material. As such, it might be best to include the information on photodynamic therapy in the introduction. Limitations of laser therapy and PDL could then be briefly reviewed at the beginning of section 4.

Good point, we have added a dedicated section (1.4.) on photodynamic therapy in the introduction and moved this information there together with the relevant information previously presented in section 3.4.1.3.

2nd editorial decision
Date: 18-Apr-2019

Ref.: Ms. No. JCTRes-D-19-00002R1
Site-specific pharmaco-laser therapy: a novel treatment modality for refractory port wine stains
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.

Your revision is due by May 18, 2019.

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,

Rowan van Golen
Associate Editor
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: I thank the authors for addressing comments and now think this manuscript is ready for acceptance.

Reviewer #2: Thank you for addressing my comments. All have been addressed in a satisfactory manner except for my previous comment 12. I think the authors should soften the language that states that liposomes appear to be the most advantageous. Those attributes can also be provided by other types of particles. I recommend just stating what you believe are some of the important attributes of liposomes without referring to them as being most advantageous.

Authors' rebuttal

M. Ingmar van Raath
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Dr. R. van Golen
Associate editor, *Journal of Clinical and Translational Research*
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2333 ZA Leiden
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Amsterdam, 18 April 2019

Re: Resubmission Ms. No. JCTRes-D-19-00002

Dear Dr. van Golen,

Please find enclosed the resubmission of our manuscript entitled "*Site-specific pharmacolaser therapy: a novel treatment modality for refractory port wine stains.*" We have addressed the reviewer's comment using the track changes function in the text to indicate the modifications.

Thank you for the opportunity to resubmit the manuscript.
Kindest regards, also on behalf of the co-authors,
Ingmar van Raath.

3rd editorial decision
Date: 18-april-2019

Ref.: Ms. No. JCTRes-D-19-00002R2
Site-specific pharmaco-laser therapy: a novel treatment modality for refractory port wine stains
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that, after these final modifications, 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,

Rowan van Golen
Associate Editor
Journal of Clinical and Translational Research