This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

nature portfolio

Peer Review File

Avenues for integrating photothermal therapy in cancer clinic

Corresponding Author: Professor Abhijit De

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
De et al. reviewed the challenges hindering the clinical integration of PTT, including photothermal agent, laser properties and strategies for better application, efficacy and precision.This review is detailed and timely, and I recommend acceptance with some revisions.
1. Photothermal therapy is difficult to translate into clinical practice due to hyperthermia, so now more studies focus on mild photothermal therapy, and the authors should add this part.
2. Photothermal reagents include organic and inorganic materials, especially the clinical transformation of inorganic materials may be more difficult, so the author should focus on the safety and pharmacokinetics of photothermal reagents in vivo.
3. Are there any clinical trials for photothermal therapy? The authors should investigate further or show the progress of clinical treatment research.

Reviewer #2

(Remarks to the Author)
Patnaik et al. reviewed the challenges in translating photothermal therapy (PTT) into clinical applications for cancer treatment. In PTT, two primary factors are crucial: the light source and a photothermal agent (PTA), which converts light energy into heat to destroy cancer cells. Numerous preclinical studies have demonstrated PTT's efficacy in both in vitro and in vivo models. However, to date, only two Phase I clinical trials (ICG and Aurolase) have been reported.
In their review, the authors summarize key considerations for the successful clinical application of PTT. These factors include the physico-chemical properties of PTAs, the characteristics of the light source (laser), tumor targeting, post-treatment pain management, and the clearance of PTAs from the body after therapy. The manuscript is well-written, easy to follow, and provides a strong rationale and background, supported by relevant literature citations. However, a few minor revisions are necessary before it can be considered for publication.
Comments:
1. Could the authors include a paragraph discussing how AI and machine learning could help address some of the key factors?
2. The authors should describe Figure 6 in the main text.
3. The purpose of the two mice images in Figure 2 is unclear. Please provide an explanation either in the figure legend or the main text.
4. It is unclear whether Figure 3 has been previously published. If not, please mention it as unpublished data in the figure legend.

Reviewer #3

(Remarks to the Author)
Review attached.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
1. Although the paper provides a detailed review of the application and challenges of photothermal therapy in cancer

treatment, most of its content is a summary of existing research, lacking novel research findings or breakthrough perspectives. In the current research field, many similar reviews have been published, and this paper fails to significantly distinguish itself from the existing literature.
2. The paper mentions the clinical translation challenges of PTT, but the solutions and suggestions are rather general and lack specific data support or experimental verification. For instance, the discussions on pain management and the clearance of nanomaterials are more theoretical speculations without providing actual clinical cases or experimental data.
3. Although the charts in the paper are helpful for understanding, they lack original data or experimental results. As a review, it would be more convincing if it included more quantitative analyses or comparisons of existing research.
4. The paper is some parts are repetitive, which makes the core viewpoints less prominent. It is suggested to streamline the content and focus on key issues (such as specific bottlenecks and solutions in clinical translation).

Reviewer #2

(Remarks to the Author)
The authors have thoroughly addressed the reviewer's comments, leading to a significant improvement in the manuscript's quality. I recommend the manuscript for publication.

Reviewer #3

(Remarks to the Author)
Review attached.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The author has provided a very satisfactory response to the reviewers' comments, and the manuscript can now be accepted.

Point by point response to review comments

Reviewer #1 (Remarks to the Author):

De et al. reviewed the challenges hindering the clinical integration of PTT, including photothermal agent, laser properties and strategies for better application, efficacy and precision. This review is detailed and timely, and I recommend acceptance with some revisions.

Response: We thank reviewer for summarizing the essence of the review paper.

1. Photothermal therapy is difficult to translate into clinical practice due to hyperthermia, so now more studies focus on mild photothermal therapy, and the authors should add this part.

   Response: We have added as a new paragraph under Newer strategies adopted to improve clinical potential of PTT, 4. Combination treatment for therapy enhancement on page 16-17.

2. Photothermal reagents include organic and inorganic materials, especially the clinical transformation of inorganic materials may be more difficult, so the author should focus on the safety and pharmacokinetics of photothermal reagents in vivo.

   Response: We have added as a new paragraph under Major factors influencing PTT efficiency: Physical properties of PTAs on page 8.

3. Are there any clinical trials for photothermal therapy? The authors should investigate further or show the progress of clinical treatment research.

   Response: We had already incorporated clinical trial information under Applications of PTT as cancer therapeutics on page 5-6.

Reviewer #2 (Remarks to the Author):

Patnaik et al. reviewed the challenges in translating photothermal therapy (PTT) into clinical applications for cancer treatment. In PTT, two primary factors are crucial: the light source and a photothermal agent (PTA), which converts light energy into heat to destroy cancer cells. Numerous preclinical studies have demonstrated PTT's efficacy in both in vitro and in vivo models. However, to date, only two Phase I clinical trials (ICG and Aurolase) have been reported.

In their review, the authors summarize key considerations for the successful clinical application of PTT. These factors include the physico-chemical properties of PTAs, the characteristics of the light source (laser), tumor targeting, post-treatment pain management, and the clearance of PTAs from the body after therapy. The manuscript is well-written, easy to follow, and provides a strong rationale and background, supported by relevant literature citations. However, a few minor revisions are necessary before it can be considered for publication.

Response: We thank reviewer for summarizing the strengths while providing further improvement suggestions to add to the paper.

1. Could the authors include a paragraph discussing how AI and machine learning could help address some of the key factors?

Response: On page 19, we have added as a new point under Newer strategies adopted to improve clinical potential of PTT, 6. Artificial Intelligence and Machine Learning in Photothermal Therapy

2. The authors should describe Figure 6 in the main text.

Response: We have added under Newer strategies adopted to improve clinical potential of PTT on page 14.

3. The purpose of the two mice images in Figure 2 is unclear. Please provide an explanation either in the figure legend or the main text.

Response: We have explained in the figure and figure caption on page 7.

4. It is unclear whether Figure 3 has been previously published. If not, please mention it as unpublished data in the figure legend.

Response: We have mentioned "Unpublished data" in the figure 3 legend on page 9.

Reviewer #3 (Remarks to the Author):

In this manuscript, based on photothermal therapy for cancer, key considerations for photothermal therapy are presented including photothermal agents (PTAs), laser properties, and strategies for better application, efficacy, and precision. Common parameters for PTA evaluation are analyzed including biocompatibility, biodistribution, and efficacy assessment. The potential of photothermal therapy and the difficulties faced in its clinical application are presented. Precision engineering of photonic devices, accuracy assessment, and pain relief are essential in order to emphasize the targeting efficiency of PTA in the clinic and the determination of post-treatment regression. This sub-study provides a viable direction for future photothermal therapies. Nonetheless, some of the analytical discussions in this manuscript need improvement, and the main issues are listed below

Response: We thank reviewer for constructive summary and suggestion to further improve the quality of the paper.

1. The manuscript mentions that nanoparticles of sizes smaller than 40 nm have better absorption effects, while those larger than 40 nm have better light scattering effects. Why would the 5-11 nm gold particles mentioned later have the property of having higher stability and photoacoustic signal than other size particles?

   Response: We have amended this sentence on page 7 by clarifying that “the larger particles (>40nm) exhibiting more light scattering and smaller particles (<40nm) more absorption”, is a generalised scenario for absorption in the visible to NIR-I range.

2. In the section of PTT cancer therapy application, the purpose of combining PTA with other antibodies, polymers, and targeting molecules is to enhance the specificity and targeting effect of PTA, which has nothing to do with enhancing the quality of PTA.

   Response: We have modified the sentence in "Applications of PTT as cancer therapeutics" section (Page 5) to clarify that the focus is on directing the PTA to the tumour, not changing its inherent qualities.

3. In the manuscript, it is mentioned that vitamin E and carbon monoxide, as an example of PTA tumor targeting strategy, can significantly enhance the EPR effect of PTA in targeting tumor models, what is the specific mechanism of action and the binding mode?

   Response: The reviewer is right in suggesting that addition of mechanism of action and binding mode of Vitamin E and CO would be beneficial. Accordingly, we have amended the part in PTA tumor targeting strategy and added the necessary information on page 10-11.

4. The manuscript mentions that the factors affecting laser penetration are laser wavelength, power density, and pulse width, but the text only describes the effect of wavelength on laser penetration and the effect with the material, what is the effect of the remaining two factors?

   Response: We have amended and added the impact of laser power density and pulsed width on therapy efficacy on page 8-9.

5. In response to the problem of uneven distribution of PTA in the tumor site due to direct injection of PTA, cancer cell targeting is used to make the concentration of PTA uniformly distributed in the tumor site, and the example cited is the use of neural stem cells for loading PTA, and the mode of delivery after loading has not been mentioned here, and the problems of delivery and distribution of in situ and intravenous injection of neural stem cells exist, which need to be supplemented.

   Response: We have now amended the manuscript to specify that the PTA-loaded NSCs were delivered by *in situ* administration on page 14.

   We recognize that while *in situ* injection or direct intratumoral injection of NSC or NSC-loaded PTA has obvious lacunae in accessing tumours located deep within the body or in sensitive areas, intravenous or

systemic administration of these agents are also met with not-so-subtle immune clearance mechanisms such as the mononuclear phagocyte system (MPS) and reticuloendothelial system (RES) which would eventually reduce the tumour-availability of the PTA. In the context of NSC-mediated delivery, these cells have shown promise due to their natural tropism for tumour sites, potentially enhancing tumour distribution post in situ administration. However, further research in the field is needed to optimize their delivery and retention at the tumour site.

However, we must bear in mind that the question being addressed in the specific subsection is the efficient directing of PTA delivery to the tumour site and delving deeper into specifically NSC-mediated PTA delivery, would deviate the focus from the question in prerogative.

6. The part on the degradation of PTA materials after treatment is too general and lacks specific degradation methods and examples, which needs to be added.

   Response: We have amended to add detailed PTA degradation methods on page 16.

7. In the part on the combination of chemotherapeutic drugs and PTA in the treatment of cancer, the mechanism and process of chemotherapeutic drugs and PTA in the process of action are not clear, and need to be revised and supplemented.

   Response: We have amended and supplemented on page 17.

8. The therapeutic mechanism and clinical application of PTT are not sufficiently discussed in the manuscript, and it is suggested to add the reference “BMEMat 2023, 1(1), e12009” “EXP.20220161”.

   Response: The therapy mechanism of PTT is already explained in the Introduction section, 2nd paragraph. The clinical application of PTT is already explained in the Applications of PTT as cancer therapeutics section, 2nd paragraph, under table 1.

   We have added the reference “BMEMat 2023, 1(1) on page 7, and e12009” “EXP.20220161” on page 5 table 1 under copper-based nanoformulation for photothermal application.

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

1. Although the paper provides a detailed review of the application and challenges of photothermal therapy in cancer treatment, most of its content is a summary of existing research, lacking novel research findings or breakthrough perspectives. In the current research field, many similar reviews have been published, and this paper fails to significantly distinguish itself from the existing literature.

Response: We thank the reviewer for providing new opinion on our already revised version of the manuscript and understanding that some of the remarks are contradictory to round one. In the revised manuscript submitted, we have addressed all previous comments and suggestions made, and our attempt was to bring in some distinctive perspective by focusing on translational impediments and clinical integration challenges of photothermal therapy (PTT), which are rare find in published reviews. This work therefore offers a comprehensive, clinically oriented roadmap discussions. By integrating these diverse aspects, this review aims, not just to summarize, but to strategically inform future design considerations and clinical development of PTT platforms.

2. The paper mentions the clinical translation challenges of PTT, but the solutions and suggestions are rather general and lack specific data support or experimental verification. For instance, the discussions on pain management and the clearance of nanomaterials are more theoretical speculations without providing actual clinical cases or experimental data.

Response: Respectfully we differ with this comment as introduction of new experimental data is neither within the scope of any review article, nor it was asked in the previous round. We revised the manuscript based on previous comments received from the reviewers. Our primary objective in this review was to highlight the key translational bottlenecks that continue to hinder the clinical progression of photothermal therapy (PTT). Therefore, in this review manuscript, we introduced line of thoughts on practical challenges such as post-therapy pain management and physiological clearance, which are generally under-represented in the published reviews so far. Citations of relevant experimental and clinical references, wherever available, to substantiate the challenges and emerging solutions were provided.

- In the pain management section, we refer to clinical case studies and trial data (e.g., Dowlatshahi et al., and the AuroShell trials), which discuss strategies like water-cooled laser systems and analgesic protocols.
- The discussion on nanomaterial clearance includes references to *in vivo* studies of biodegradable nanoplatforms (e.g., thermolabile liposome and polymer, chitosan, copper sulphide etc), and their associated degradation and excretion profiles.
- In the revised version, we had also in fact added concise contexts on safety and pharmacokinetics of organic and inorganic PTAs under the section “Physical properties of PTAs” as per reviewer’s earlier suggestions.

We have emphasized that many of these solutions are still under preclinical or early translational investigation, which inherently limits the availability of extensive clinical datasets.

3. Although the charts in the paper are helpful for understanding, they lack original data or experimental results. As a review, it would be more convincing if it included more quantitative analyses or comparisons of existing research.

Response: As mentioned before, within the scope of a narrative review article, our focus was to discuss translational barriers to clinical adoption of photothermal therapy (PTT), and the concepts/diagrams were added to visualize key concepts, challenges, and strategic directions which are drawn based on previously published experimental studies, rather than presenting original data. We opted not to include an exhaustive meta-analysis due to the heterogeneous nature of study designs and scope limitations.

However, to emphasize on the efficacy and survival advantage of photothermal therapy in preclinical models, we already had the Figure 3 prepared from our unpublished results. Considering reviewer's suggestion, we have now included one additional data table (**Table 2, page 6**) to clearly bring to attention the quantitative outcomes from preclinical and clinical PTT studies. We hope these enhancements address the reviewer's comment and further strengthen the manuscript's illustrative content.

4. The paper is some parts are repetitive, which makes the core viewpoints less prominent. It is suggested to streamline the content and focus on key issues (such as specific bottlenecks and solutions in clinical translation).

Response: Understanding the reviewer's viewpoint, in this second revision, **we have removed repetitive information to the extent possible as well as edited some parts of the text in page 4-5. We have also incorporated Table 2 on page 6 and removed its contextual write-up**. We would like to impress upon the fact that to stress on the major challenges and the solutions, some of the discussions were consciously reiterated.

Reviewer #2 (Remarks to the Author):
The authors have thoroughly addressed the reviewer's comments, leading to a significant improvement in the manuscript's quality. I recommend the manuscript for publication.

Response: We thank the reviewer for confirmation on properly addressing all review comments and approving the revised manuscript for publication.

Reviewer #3 (Remarks to the Author):

After reviewing the revised version of this manuscript and this strategy mentioned is good, I am pleased to see that the authors have taken the initial review comments seriously and made appropriate modifications to the manuscript. I believe this article meets the standards for publication, and I recommend that the editor accept this revised manuscript.

Response: We thank the reviewer for confirmation on properly addressing all review comments and approving the revised manuscript for acceptance.

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

The author has provided a very satisfactory response to the reviewers' comments, and the manuscript can now be accepted.

Response: We thank the reviewer for confirmation on properly addressing all review comments and approving the revised manuscript for acceptance.

Avenues for integrating photothermal therapy in cancer clinic

In this manuscript, based on photothermal therapy for cancer, key considerations for photothermal therapy are presented including photothermal agents (PTAs), laser properties, and strategies for better application, efficacy, and precision. Common parameters for PTA evaluation are analyzed including biocompatibility, biodistribution, and efficacy assessment. The potential of photothermal therapy and the difficulties faced in its clinical application are presented. Precision engineering of photonic devices, accuracy assessment, and pain relief are essential in order to emphasize the targeting efficiency of PTA in the clinic and the determination of post-treatment regression. This sub-study provides a viable direction for future photothermal therapies. Nonetheless, some of the analytical discussions in this manuscript need improvement, and the main issues are listed below:

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8. Translated with www.DeepL.com/Translator (free version)The therapeutic mechanism and clinical application of PTT are not sufficiently discussed in the

manuscript, and it is suggested to add the reference “BMEMat 2023, 1(1), e12009” “EXP.20220161”.

After reviewing the revised version of this manuscript and this strategy mentioned is good, I am pleased to see that the authors have taken the initial review comments seriously and made appropriate modifications to the manuscript. I believe this article meets the standards for publication, and I recommend that the editor accept this revised manuscript.