Reviewing process: Short Note sn20260821-15r

Modeling Patient-Derived Breast Cancer in three dimensions: Patient-derived tumorospheres as a promising ex vivo Experimental Model

Peer-review Report
Short Notes applies an anonymous, free-of-charge peer-review process conducted by experts in the relevant field. After acceptance, reviewers' reports, authors' responses and revisions, and the editorial decision are published, and reviewers may choose to disclose their identity.

Peer-reviewers:

- Reviewer 1 : Ulysse PEREIRA, CS FC3R
- Reviewer 2 : choose not to disclose their identity

Executive Editor: Marc Le Bert, FC3R

Course of the Short Note sn20260821-15r

Short Note submitted on: March 23, 2026

1st editorial response: May 12, 2026

Dear Author,

Following peer review, your Short Note entitled "Modeling Patient-Derived Breast Cancer in three dimensions: Patient-derived tumorospheres as a promising ex vivo Experimental Model" requires revision.

Please submit a substantially revised version of your manuscript, together with updated point-by-point responses to the reviewers and to the editor in the review-tracking space. This will initiate a second round of review with the same reviewers.

When responding in the interface, please explain clearly how each comment has been addressed in the revised Short Note. To facilitate evaluation, please indicate for each change either the relevant line number or the beginning of the modified text passage (or the corresponding figure/table legend, where applicable). If you disagree with a comment, please provide a brief justification.

QUALITY OF WRITING _____

Reviewer 1 ticked: yes
Reviewer 1 comment:

The manuscript is overall clearly written and well-structured. The logic flows naturally from the clinical rationale to model development, histological and biochemical characterization, and finally functional drug testing. The abstract is concise and accurately reflects the content of the paper. The introduction provides a rapid and well-balanced overview of existing preclinical models (PDX, PDTOs) and clearly identifies the unmet need that PDTs are designed to address. The topic is fully aligned with the objectives and scope of FC3R Short Notes.

However, some conclusions appear to overstate the strength of the evidence in view of the limited number of samples analyzed in depth. The authors are therefore encouraged to adopt more cautious and nuanced language, particularly with respect to the Masson's trichrome and SHG analyses. These results would be more appropriately framed as promising preliminary observations rather than definitive conclusions.
Authors' response:

Reviewer 2 ticked: yes, no comment

QUALITY OF FIGURES AND ADDITIONAL DOCUMENTS

Reviewer 1 ticked: no
Reviewer 1 comment :
Both figures are informative and visually well-organized.
Figure 1 provides a comprehensive multimodal characterization of PDTs, while Figure 2 addresses biochemical heterogeneity and drug response.

However, several specific issues should be addressed to improve accuracy and interpretability. It should also be noted that the current pagination causes some information to be truncated when printed; the layout should be revised accordingly.
Authors' response:

- Figure 1
Panel A: if the schematic illustrations were extracted from an external software or image library, the source must be explicitly credited in the figure legend or acknowledgements section.
Authors' response:

Panel B: the scale bars are too small to be readable. They should be made larger and bolder to meet standard publication requirements.Panel C (diameter over time): error bars must be added to reflect inter-spheroid variability, which is an important indicator of PDT homogeneity.
Authors' response:

Panel D: the bar chart illustrating the 94% success rate across 32 patients adds limited informational value beyond what is already stated in the text. It could be removed to free up space for more informative content.
Authors' response:

Panel E- F the scale bar is illegible in its current form. Since the scale is already provided in the legend, the text label on the bar should be removed and the bar itself made thicker and more visible.
Panel F the sentence "showing preserved collagen architecture" should be removed from the legend.
Authors' response:

- Figure 2
Panel A (K-means colors): the cluster colors are randomly assigned and differ between patients 1 and 2. Although this is explained in the Methods, the figure legend must include an explicit note warning the reader that colors are not comparable between patients, to prevent misinterpretation.
Authors' response:

Panel B (cytotoxicity): this panel is currently difficult to interpret. It combines too much information simultaneously -- four patients, fifteen concentration levels, three timepoints, and statistical annotations -- making it hard to understand which comparisons are being made and between which groups. A major revision of this figure is required.
Authors' response:

Reviewer 2 ticked: no
Reviewer 2 comment:
The Figures are well presented for the most part.

However, I have an issue with the way that Figure 2B is presented. The graphs are trying to present too much information and could have been simplified so that the dose and time dependency of the treatment is more clear. This could be done by pooling data between samples and also presenting the statistical analysis in the form of trend tests. The graphs need to start at 0. The statistical comparisons are not clear - how were these done? What was compared with what in the statistical tests?
Authors' response:

QUALITY OF THE EXPERIMENTAL DESIGN _____

Reviewer 1 ticked: no
Reviewer 1 comment:
The overall objective of this work is to validate a 3D culture model (PDTs) for breast cancer that preserves the tumor microenvironment without the use of animal-derived matrices or exogenous growth factors.

However, the culture medium contains recombinant EGF (50 ng/mL). The authors are recommended to reframe this claim from the outset as follows: "without the addition of exogenous growth factors, with the exception of EGF".
Authors' response:

However, the approach is well-conceived and the combination of complementary characterization methods provides a multi-dimensional validation framework. The 94% formation rate across 32 patients is a notable result. But, several design issues limit the strength of the conclusions that can currently be drawn.
Authors' response:

Definition of "success rate": the 94% formation rate is remarkable, but the criteria used to define a successful PDT are not clearly stated. Does success refer to spheroid formation alone? To a minimum viability threshold? To a minimum culture duration? To the capacity to reflect the tumor tissue composition? These criteria must be precisely defined, along with the number of replicates per patient used to establish this rate.
Authors' response:

Sample selection for in-depth analyses: while 32 patients were included for PDT formation, subsequent analyses were performed on only 2 patients (FTIR, SHG) or 4 patients (drug response). This dramatic reduction in sample size substantially limits the scope of the study. The criteria for selecting these specific patients are not provided and must be justified. In particular, it should be clarified whether the selected samples are representative of the different tumor molecular subtypes present in the cohort. This information is critical given the stated objective of reflecting inter-patient variability. The authors are strongly encouraged to link Supplemental Table 1 to the specific samples analyzed in each experiment.
Authors' response:

Scope and power of the study: the current design would benefit greatly from being extended to a larger number of patients across all experimental endpoints, which would provide stronger evidence that the PDT method captures patient diversity. If such an extension is not feasible, the authors should consider repositioning this manuscript more explicitly as a preliminary methodological study, focusing on the promising aspects of the model and honestly acknowledging its current limitations Missing variability data: error bars reflecting inter-spheroid variability are absent from the size tracking data (Panel 1C) Homogeneity of spheroids is a critical quality parameter for a 3D model.

Authors' response:

Statistical analysis: the cytotoxicity data are analyzed using a two-way ANOVA, but no post-hoc test or correction for multiple comparisons is specified.
Authors' response:

Reviewer 2 ticked: no
Reviewer 2 comment:
The aim of this Short Report is to validate PDTs as models for breast cancer and in particular to overcome the limitations of other models in accounting for diversity in patient samples. It is impressive that so many samples are processed for PDTs in this report. However, the experimental analysis falls short in a few areas:

1. In the analysis undertaken, a few PDT samples are selected for analysis rather than analysis of the whole cohort being undertaken. Therefore the impact of diversity in patient samples cannot be accounted for - obviating one of the goals of the study as mentioned in the Introduction. It would be better to have provided an overview of the staining (Fig 1E-G), infrared spectroscopy (Fig 2A) and paclitaxel response (Fig 2B) from the cohort as a while, although I appreciate this would require a lot more work. However, it would give the reader a lot more insight into the heterogeneity in PDTs across a large cohort of samples. Without this, only anecdotal evidence of a few samples is presented.
Authors' response:

2. The generation of PDTs requires tumour deconstruction using mechanical enzyme dissociation and agitation. These are very harsh treatments that are notorious in resulting in high levels of cell loss. It would be crucial to understand how much attrition there is of cells in this process - so some data on viability of cells pre and post processing would be important to validate PDTs as reflective of original tumour composition.
Authors' response:

3. Some indication of viability of PDTs over longer time frames would be valuable.
Authors' response:

QUALITY OF THE REPORTING _____

Reviewer 1 ticked : yes
Reviewer 1 comment:
The reporting is generally good. The Methods section is detailed -- reagents are identified with catalog numbers, instruments are specified, and the FTIR preprocessing pipeline is described with adequate precision. Ethics approval and patient consent procedures are clearly stated. H

owever the Matlab code used for FTIR data processing is described as "in-house developed". In line with FC3R's open science principles, the authors are encouraged to deposit this code.
Authors' response:

Reviewer 2 ticked: yes, no comment

FINAL REVIEWERS DECISIONS _____

Reviewer 1 final decision: to be discussed
Reviewer 1 final comment:
This manuscript introduces a promising 3D culture methodology for breast cancer research that is well-aligned with the scope and philosophy of FC3R Short Notes. The PDT model initial results are encouraging. The approach could potentially be extended to other cancer types. The work is appreciated however, several major revisions are required before this manuscript can be considered for publication.
Authors' response:

Reviewer 2 final decision: to be discussed
Reviewer 2 final comment:
I have indicated a few points above with regard to the experimental design and methodology linked to this study, which need addressing.
I have one other major consideration: Although PDTs may serve as an alternative to PDOs as a preclinical model platform, they also suffer from the drawback of being generated from deconstructed tumours. As such the original tumour architecture and microenvironment are not maintained. While they may have some of the cells that were components of the microenvironment, the cell-cell relationships that were present in the original tumours are not maintained, which is a major drawback to this platform. As a result this manuscript exaggerates the value of PDTs as preclinical models and would be better served by presenting a more balanced view of their advantages and limitations compared to other breast cancer models.
In particular, the manuscript also fails to mention the growing interest in patient-derived explants which represent the culture of live tumour tissue which maintain architecture and the microenvironment.
Authors' response:

Thank you for your work and for your careful consideration of the reviewers' comments. We appreciate your contribution to the Short Notes initiative and look forward to receiving your revised manuscript.

Best regards,

Athanassia Sotiropoulos
in behalf of the Short Notes' editorial board

Author's first response: July 03, 2026

Response to the Editor:
We sincerely appreciate the Editors' and Reviewers' rigorous assessment, constructive comments, and recognition of our work's alignment with the objectives and scope of FC3R Short Notes. We have addressed all concerns through a revised version of our manuscript with the apparent modifications in blue. Line numbers have been added to facilitate the point-by-point responses to reviewers. Our responses to reviewers are marked in black in this document.
Reviewer 1 ticked: yes
Reviewer 1 comment:
The manuscript is overall clearly written and well-structured. The logic flows naturally from the clinical rationale to model development, histological and biochemical characterization, and finally functional drug testing. The abstract is concise and accurately reflects the content of the paper. The introduction provides a rapid and well-balanced overview of existing preclinical models (PDX, PDTOs) and clearly identifies the unmet need that PDTs are designed to address. The topic is fully aligned with the objectives and scope of FC3R Short Notes. However, some conclusions appear to overstate the strength of the evidence in view of the limited number of samples analyzed in depth. The authors are therefore encouraged to adopt more cautious and nuanced language, particularly with respect to the Masson's trichrome and SHG analyses. These results would be more appropriately framed as promising preliminary observations rather than definitive conclusions.

Response: We thank the reviewer for his/her constructive comments. We have now nuanced our conclusions, as suggested (lines 181-199).
Reviewer 2 ticked: yes, no comment

QUALITY OF FIGURES AND ADDITIONAL DOCUMENTS _____
Reviewer 1 ticked: no
Reviewer 1 comment :
Both figures are informative and visually well-organized. Figure 1 provides a comprehensive multimodal characterization of PDTs, while Figure 2 addresses biochemical heterogeneity and drug response. However, several specific issues should be addressed to improve accuracy and interpretability. It should also be noted that the current pagination causes some information to be truncated when printed; the layout

should be revised accordingly.

Response: We have slightly modified the figure and legend layouts, hoping that no information will be truncated when printed.

- Figure 1
Panel A: if the schematic illustrations were extracted from an external software or image library, the source must be explicitly credited in the figure legend or acknowledgements section.
Panel B: the scale bars are too small to be readable. They should be made larger and bolder to meet standard publication requirements.
Panel C (diameter over time): error bars must be added to reflect inter-spheroid variability, which is an important indicator of PDT homogeneity.
Panel D: the bar chart illustrating the 94% success rate across 32 patients adds limited informational value beyond what is already stated in the text. It could be removed to free up space for more informative content.
Panel E- F the scale bar is illegible in its current form. Since the scale is already provided in the legend, the text label on the bar should be removed and the bar itself made thicker and more visible.
Panel F the sentence "showing preserved collagen architecture" should be removed from the legend.
- Figure 2
Panel A (K-means colors): the cluster colors are randomly assigned and differ between patients 1 and 2. Although this is explained in the Methods, the figure legend must include an explicit note warning the reader that colors are not comparable between patients, to prevent misinterpretation.
Panel B (cytotoxicity): this panel is currently difficult to interpret. It combines too much information simultaneously -- four patients, fifteen concentration levels, three timepoints, and statistical annotations -- making it hard to understand which comparisons are being made and between which groups. A major revision of this figure is required.

Response: We thank the reviewer for the helpful comments.
Figure 1:
Panel A: We have now mentioned in figure legend 1 and in the acknowledgment section that Fig 1A was created with Biorender,
Panel B: All scale bars have been modified and are now readable.
Panel C: Error bars were added as required. The number of replicates was added in the figure legend. Panel D was removed as suggested.
Panel E, F: scale bars were modified and the sentence "showing preserved collagen architecture" removed from the legend.
Figure 2:
Panel A: we have added in the legend that "Cluster colors were randomly assigned independently for each patient"
Panel B: We thank the reviewer for this comment. We have now proposed a more

interpretable graph by pooling all patients, with 10 concentrations of paclitaxel. We have adapted the text and statistics in the figure legend, and in the materiel and method section (lines 324-333).

Reviewer 2 ticked: no
Reviewer 2 comment:
The Figures are well presented for the most part. However, I have an issue with the way that Figure 2B is presented. The graphs are trying to present too much information and could have been simplified so that the dose and time dependency of the treatment is more clear. This could be done by pooling data between samples and also presenting the statistical analysis in the form of trend tests. The graphs need to start at 0. The statistical comparisons are not clear - how were these done? What was compared with what in the statistical tests?

Response: We thank the reviewer for this comment. We agree that interpretation was particularly difficult. As suggested, we have simplified the graph by pooling all 4 patients, with 10 concentrations of paclitaxel. The graph starts at 0. We have clarified the statistical analysis in both the Methods section (lines 324-333) and figure legend. Briefly, technical replicates were averaged for each patient sample and each patient was considered as one independent biological replicate (n=4). Data were analyzed using a repeated-measures two-way ANOVA with concentration and time as within-subject factors, followed by post-hoc comparisons of each concentration against untreated control at each time point.

QUALITY OF THE EXPERIMENTAL DESIGN _____
Reviewer 1 ticked: no
Reviewer 1 comment:
The overall objective of this work is to validate a 3D culture model (PDTs) for breast cancer that preserves the tumor microenvironment without the use of animal-derived matrices or exogenous growth factors. However, the culture medium contains recombinant EGF (50 ng/mL). The authors are recommended to reframe this claim from the outset as follows: "without the addition of exogenous growth factors, with the exception of EGF".
However, the approach is well-conceived and the combination of complementary characterization methods provides a multi-dimensional validation framework. The 94% formation rate across 32 patients is a notable result. But, several design issues limit the strength of the conclusions that can currently be drawn.
Definition of "success rate": the 94% formation rate is remarkable, but the criteria used to define a successful PDT are not clearly stated. Does success refer to spheroid formation alone? To a minimum viability threshold? To a minimum culture duration? To the capacity to reflect the tumor tissue composition? These criteria must be precisely defined, along with the number of replicates per patient used to establish this rate.

Response: We have reframed the EGF presence in the manuscript, as asked, lines 98,

113 and 169. Regarding the PDT formation rate of 94%, this was evaluated from day-to-day (from day 0 to day 20) visual monitoring of every spheroid formation (from 10 to over 100 PDTs par patient). Among 32 patients, 30 successfully assembled in a cohesive manner, forming spheroid-like structures (this was added line 95,96 in the result and discussion section). We observed no dissociation of the formed PDT over time. We randomly attributed PDTs to IHC for concordance with parental tissues as now added in the supplemental table. A pool size of at least 20 PDTs was necessary for sectioning (added line 236-237). Although it was impossible to verify every single PDT viability and cohesion, IHC stainings confirm the success of spheroid formation and similarity to parental tissues.

Sample selection for in-depth analyses: while 32 patients were included for PDT formation, subsequent analyses were performed on only 2 patients (FTIR, SHG) or 4 patients (drug response). This dramatic reduction in sample size substantially limits the scope of the study. The criteria for selecting these specific patients are not provided and must be justified. In particular, it should be clarified whether the selected samples are representative of the different tumor molecular subtypes present in the cohort. This information is critical given the stated objective of reflecting inter-patient variability. The authors are strongly encouraged to link Supplemental Table 1 to the specific samples analyzed in each experiment.
Response: The utilization of patient-derived PDTs is now reported in supplemental table 1 as suggested. The 32 tissue samples were used either for protocol development, model characterization (IHC, IF, SHG, FTIR), or to assess response to treatment. We did not establish any selection criteria for the samples to be used in our tests. We were limited by the number of PDTs obtained for each sample.
We agree that the number of patients for FTIR, SHG and drug response experiments is low. Regarding FTIR and SHG, the low number on patients is due to limited access to the imaging platform. For drug response experiments, we had planned to include 5 patients (as noted in supplemental table 1); unfortunately, one biopsy was too small to generate enough PDTs for comparing dose responses.

Scope and power of the study: the current design would benefit greatly from being extended to a larger number of patients across all experimental endpoints, which would provide stronger evidence that the PDT method captures patient diversity. If such an extension is not feasible, the authors should consider repositioning this manuscript more explicitly as a preliminary methodological study, focusing on the promising aspects of the model and honestly acknowledging its current limitations Missing variability data: error bars reflecting inter-spheroid variability are absent from the size tracking data (Panel 1C) Homogeneity of spheroids is a critical quality parameter for a 3D model.
Statistical analysis: the cytotoxicity data are analyzed using a two-way ANOVA, but no post-hoc test or correction for multiple comparisons is specified.
Response: We thank the reviewer for this comment. Error bars reflecting inter-spheroid variability with mean ± SD were added in figure 1C. We have clarified the statistical analysis in both the Methods section and figure legend. Briefly, technical replicates

were averaged for each patient sample and each patient was considered as one independent biological replicate (n=4). Data were analyzed using a repeated-measures two-way ANOVA with concentration and time as within-subject factors, followed by post-hoc comparisons of each concentration against untreated control at each time point.
We have rewritten the discussion by focusing on the promising aspects of the model and acknowledging its current limitations (lines 81-199).

Reviewer 2 ticked: no
Reviewer 2 comment:
The aim of this Short Report is to validate PDTs as models for breast cancer and in particular to overcome the limitations of other models in accounting for diversity in patient samples. It is impressive that so many samples are processed for PDTs in this report. However, the experimental analysis falls short in a few areas:

1. In the analysis undertaken, a few PDT samples are selected for analysis rather than analysis of the whole cohort being undertaken. Therefore the impact of diversity in patient samples cannot be accounted for - obviating one of the goals of the study as mentioned in the Introduction. It would be better to have provided an overview of the staining (Fig 1E-G), infrared spectroscopy (Fig 2A) and paclitaxel response (Fig 2B) from the cohort as a while, although I appreciate this would require a lot more work. However, it would give the reader a lot more insight into the heterogeneity in PDTs across a large cohort of samples. Without this, only anecdotal evidence of a few samples is presented.

2. The generation of PDTs requires tumour deconstruction using mechanical enzyme dissociation and agitation. These are very harsh treatments that are notorious in resulting in high levels of cell loss. It would be crucial to understand how much attrition there is of cells in this process - so some data on viability of cells pre and post processing would be important to validate PDTs as reflective of original tumour composition.

3. Some indication of viability of PDTs over longer time frames would be valuable.

Responses:
1.We thank the reviewer for raising these critical points patient number and method limitations. The utilization of patient-derived PDTs is now reported in supplemental table 1. The 32 tissue samples were used either for protocol development, model characterization (IHC, IF, SHG, FTIR), or to assess response to treatment. We did not establish any selection criteria for the samples to be used in our tests. As now mentioned, lines 236-237, a pool size of at least 20 PDTs was necessary for sectioning, which limited our capacity to perform multiple analysis on one same patient. We fully agree that the number of patients for each experiment is low; unfortunately, we are currently limited in the access of human fresh material for this study. In consequence, we have now rewritten part of the manuscript discussion to position our

work more clearly as a preliminary methodological study, highlighting the potential of the proposed model while acknowledging its current limitations and the need for further validation (lines 181-199).

2. Regarding the enzymatic dissociation kit, to our knowledge, there is no evidence in the literature suggesting that its use leads to reduced cell viability. On the contrary, this dissociation method is widely used by multiple research groups working with organoid models. For instance, the OrgAPred platform routinely uses this kit for the dissociation of organoids derived from various cancer types. As an example, this dissociation approach was successfully used in organoid-based studies such as Functional miRNA Screening Identifies Wide-ranging Antitumor Properties of miR-15/16 in Organoid Models (Mol. Cancer Ther. 19(7):1506-1518. doi: 10.1158/1535-7163.MCT-19-1059).

In addition, immunostainings for E-cadherin and beta-catenin indicate that key components of adherens junctions are still present after dissociation, suggesting that cell-cell adhesion structures are not overtly disrupted by the procedure (Supplemental Figure 1 - Immunohistochemical showing E-cadherin (left) and beta-catenin (right) in PDT, in attachment).

3. It was impossible to verify every single PDT viability, however, immunohistochemistry and H&E staining performed after 3 weeks of culture suggest that the overall structural integrity of the PDTs is preserved.

QUALITY OF THE REPORTING _____
Reviewer 1 ticked : yes
Reviewer 1 comment:
The reporting is generally good. The Methods section is detailed -- reagents are identified with catalog numbers, instruments are specified, and the FTIR preprocessing pipeline is described with adequate precision. Ethics approval and patient consent procedures are clearly stated. However the Matlab code used for FTIR data processing is described as "in-house developed". In line with FC3R's open science principles, the authors are encouraged to deposit this code.

Response: We have now indicated in the method section that the code will be available upon request from the developer, Dr. Cyril Gobinet (line 319-320).

Reviewer 2 ticked: yes, no comment

FINAL REVIEWERS DECISIONS _____
Reviewer 1 final decision: to be discussed
Reviewer 1 final comment:
This manuscript introduces a promising 3D culture methodology for breast cancer research that is well-aligned with the scope and philosophy of FC3R Short Notes. The PDT model initial results are encouraging. The approach could potentially be extended to other cancer types. The work is appreciated however, several major revisions are

required before this manuscript can be considered for publication.

Response: we thank reviewer 1 for his/her helpful comments. We hope the manuscript will be suitable for publication as a short note.

Reviewer 2 final decision: to be discussed
Reviewer 2 final comment:
I have indicated a few points above with regard to the experimental design and methodology linked to this study, which need addressing.

I have one other major consideration: Although PDTs may serve as an alternative to PDOs as a preclinical model platform, they also suffer from the drawback of being generated from deconstructed tumours. As such the original tumour architecture and microenvironment are not maintained. While they may have some of the cells that were components of the microenvironment, the cell-cell relationships that were present in the original tumours are not maintained, which is a major drawback to this platform. As a result this manuscript exaggerates the value of PDTs as preclinical models and would be better served by presenting a more balanced view of their advantages and limitations compared to other breast cancer models.
In particular, the manuscript also fails to mention the growing interest in patient-derived explants which represent the culture of live tumour tissue which maintain architecture and the microenvironment.

Response: We thank the reviewer for this highly relevant comment and fully acknowledge the validity of the criticism raised. We agree that our initial presentation of PDTs was overly favorable and did not adequately address the inherent limitations of this model, particularly regarding the loss of the native tumor microenvironment and original tissue architecture. We have now modified our discussion (lines 181-199).
During their establishment, PDTs lose some components of the tumor microenvironment (TME), which are known modulators of tumor response to therapeutic treatment as well as disease progression. Even if PDTs lack an intact microenvironment they conserve major cell populations of TME as endothelial cells,and immune cells, as well as ECM components (elastin and collagen I) which recapitulate their capacity to resume the heterotypic cell-cell and cell-ECM interactions observed in tumor tissues.
PDEs involve the ex vivo culture of fragments of freshly resected human tumors that retain the histological features of the original tumors. Although PDE methodology for anti-cancer drug testing has been in existence for many years, the platform has not been widely adopted in translational research facilities, despite strong evidence for its clinical predictivity. (Front. Oncol. 11:767697. doi: 10.3389/fonc.2021.767697).
PDOs offer key practical advantages: scalability, biobanking capacity, compatibility with high-throughput drug screening, and relative cost-effectiveness compared to in vivo models. They also better preserve intratumoral genetic heterogeneity than conventional 2D cultures. PDOs lack an intact microenvironment including ECM, endothelial cells, cancer associated fibroblasts, and immune cells, which limits their capacity to

recapitulate heterotypic cell-cell interactions observed in tumor tissues. Moreover, clonal selection may occur during in vitro culture, resulting in overgrowth of specific subclones and loss of representation of the original tumor.(J Natl Cancer Cent. 2022 Oct 22;2(4):263-276.doi: 10.1016/j.jncc.2022.10.001)
We have now introduced PDE advantages and limitations in the introduction section (lines 63-67)

Revised version of the Short Note submitted on: July 03, 2026

2nd editorial response: August 21, 2026

Dear authors,

I am pleased to inform you that your manuscript, SN015 - "Modeling Patient-Derived Breast Cancer in three dimensions: Patient-derived tumorospheres as a promising ex vivo Experimental Model", has been accepted for publication with minor revisions.

Congratulations and thank you for carefully addressing the reviewers' and editorial requests, and for your commitment to sharing previously unpublished results. This contributes to greater clarity, accessibility, and transparency, thereby strengthening the robustness of science.

Both reviewers have now accepted the revised manuscript, and no further formal review round will be required. The remaining minor corrections, together with the preparation of the publication-ready files, will therefore be handled directly with the Short Notes Editorial Board by email before the final version and its supplementary materials are deposited on HAL.

Could you send me the final PDF and Word versios for post-editing? Your Short Note will first be deposited in HAL and assigned a DOI. It will then also be made available on the Short Notes platform, together with the peer-review document annexed to the publication. You will be notified at the end of this editorial process and provided with the corresponding links.

Please also remember to apply to the OPAL Short Notes Challenge to claim the prize offered by the OPAL association. Being selected for this prize may also provide an opportunity for your Short Note to be highlighted during the online FC3R 3Rs Day event on 2 July 2026.

To prepare the publication-ready version, I have noted below a few editorial and reviewers points and clarifications that we can finalize together by email.

With best regards,
Marc Le Bert
Short Notes Editorial Team

A- Editorial check:

1. Scientific interpretation and wording

Please ensure that the Abstract, Results and Discussion, as well as the figure titles and legends, remain fully consistent with the statistical results of the paclitaxel experiment.

In the version assessed by the reviewers, the analysis reported:

- a significant effect of time: p - no significant effect of paclitaxel concentration: p = 0.8212;
- no significant time × concentration interaction: p = 0.6565.

This analysis therefore does not, by itself, support a conclusion of a dose-dependent response or demonstrated sensitivity to paclitaxel. In addition, the increase in Cytotox Green signal over time is also visible in the untreated control condition, which may reflect background cell death associated with culture duration.

Note: Following a preliminary exchange with authors, you proposed an additional analysis in which treated values are normalized to the untreated control at each corresponding time point, in order to account for background cell death during culture. According to the preliminary analysis communicated to the Editorial Board, this results in a significant effect (p = 0.0028).

Since this additional normalization and analysis were not part of the version assessed by the reviewers, one possibility would be to retain the reviewed Figure 2B in the main manuscript, with a cautious interpretation, and present the control-normalized analysis as an additional Supplementary Figure, with a brief reference to it in the Results. If you choose this option, please describe clearly the normalization procedure and the corresponding statistical analysis, including what the reported p = 0.0028 refers to.

Providing the underlying source data for the cytotoxicity experiment as Supplementary Data would also be welcome and would further strengthen transparency and reproducibility.

For Figure 2, the current title could also be revised so that it does not state that PDTs are "sensitive to chemotherapy". One possible formulation would be:

"Spectroscopic comparison of PDTs and parental tissues and exploratory assessment of paclitaxel-associated cytotoxicity."

More generally, please continue to use cautious wording when describing the extent to which PDTs "recapitulate" the parental tumor, its architecture, or its microenvironment. The study supports the retention of selected cellular markers and extracellular-matrix-associated signals, but the preservation of the original cellular proportions, spatial relationships, viability, and complete tumor architecture was not directly demonstrated.

The manuscript should therefore remain clearly positioned as a preliminary methodological study establishing the feasibility and initial characterization of the PDT model.

2. Final manuscript

For the publication-ready version, please provide a clean manuscript:

- without line numbering;

- without tracked changes, comments, or colored text;

- with Figures 1 and 2 and their complete legends incorporated into the main manuscript at the appropriate locations;

- with all citations and references presented according to the Short Notes guidelines;

- with three keywords that do not already appear in the title or Abstract;

- after a final English-language and typographical check.

Please leave sufficient blank space at both the top and bottom of the first page to allow the FC3R Short Notes branding, logos, and publication information to be added.

3. Supplementary Table 1

It would be helpful to assign an anonymous identifier, for example P01-P32, to each patient so that the samples used in the different analyses can be clearly linked to Supplementary Table 1.

If possible, please also indicate in the table or its legend which samples were used for immunohistochemistry, SHG imaging, FTIR analysis, and the paclitaxel assay.

It would also be useful to clarify:

- which four samples were included in the final paclitaxel analysis;
- which additional sample had initially been allocated to this experiment but could not be analyzed because of insufficient material;
- whether the non-malignant breast sample was included in the PDT establishment-rate calculation and/or in the paclitaxel analysis;
- the precise definition used for "successful PDT establishment".

These clarifications would make the relationship between the cohort and the different experimental analyses easier for readers to follow.

4. Supplementary Figure 1

For Supplementary Figure 1, please provide a sufficiently detailed, self-contained legend so that the E-cadherin and beta-catenin staining can be understood independently of the main text.

In particular, please include, where relevant, the day of PDT culture, the patient or number of independent patient samples represented, the staining method, the magnification or objective, and the scale bars.

The corresponding antibody information and methodological details can be included in the Materials and Methods section, which is not subject to the Short Note word limit, or, if more appropriate, in the Supplementary Methods.

A possible starting point for the legend would be:

Supplementary Figure 1. Representative immunohistochemical staining of E-cadherin (left) and beta-catenin (right) in patient-derived tumorospheres. Brown staining corresponds to DAB immunolabelling, and nuclei were counterstained with hematoxylin. Scale bars: 50 µm.

5. Supplementary paclitaxel analysis and source data

If you choose to include the additional control-normalized paclitaxel analysis discussed above, please provide the finalized graph as a separate supplementary figure together with a complete legend.

The graph should make clear that each treated value has been normalized to the untreated control at the corresponding time point, and the legend should describe the statistical analysis used.

If possible, the source data underlying the cytotoxicity experiment could also be provided as a separate Excel or CSV file. Ideally, this would include the anonymized patient identifier, technical replicate, paclitaxel concentration, time point, raw Cytotox

Green values, corresponding untreated-control values, and normalized values used for the additional analysis.

These could, for example, be identified as:

- Supplementary Figure 2. Control-normalized analysis of paclitaxel-associated cytotoxicity in PDTs.

- Supplementary Data 1. Source data underlying the paclitaxel cytotoxicity analyses.

6. Supplementary video and striking image

Please also provide the video as a separate supplementary file for publication alongside the final Short Note on HAL.

The video represents a three-dimensional reconstruction of a representative PDT displayed through a complete 360° rotation. Please provide a self-contained legend including the information needed to interpret the reconstruction, in particular the day of culture and experimental condition, the markers shown and their corresponding colors, the imaging method and objective, the scale or dimensions of the reconstructed PDT, and the software used for the three-dimensional reconstruction.

For example:

Supplementary Video 1. Three-dimensional reconstruction of a representative patient-derived tumorosphere. The video shows a complete 360° rotation of a PDT measuring approximately [...] µm and cultured for [...] days. [...] is shown in [...], [...] in [...], and nuclei in [...]. Images were acquired using [...] microscopy with a [...] objective. Three-dimensional reconstruction was performed using [...].

As discussed, the selected striking image extracted from this three-dimensional reconstruction can be provided as a separate high-resolution file, together with a short caption identifying the markers and their colors and indicating that it is a still image extracted from Supplementary Video 1.

7- Graphical abstract

If you wish, you are welcome to prepare a simple graphical abstract summarizing the main concept of the study. This could be used to promote the Short Note on LinkedIn and other FC3R communication channels.

B- Final reviewers comments

QUALITY OF WRITING _____
Reviewer 1 ticked : yes
Reviewer 1 comment :
The manuscript is overall clearly written and well structured. The logic flows naturally from the clinical rationale to model development, followed by the histological and biochemical characterization, and finally the functional drug testing. The abstract is concise and accurately reflects the content of the paper. The introduction provides a rapid and well-balanced overview of existing preclinical models (PDX, PDTOs) and clearly identifies the unmet need that PDTs are designed to address. The topic is fully aligned with the objectives and scope of FC3R Short Notes. In addition, the authors have taken into account the recommendations made during the review process by adopting a more nuanced discussion of their model and a more cautious interpretation of their results, which strengthens the overall scientific rigor of the manuscript.

=> Authors' response:

Reviewer 2 ticked : yes

QUALITY OF FIGURES AND ADDITIONAL DOCUMENTS _____
Reviewer 1 ticked : yes
Reviewer 1 comment :
The authors have satisfactorily addressed all recommendations concerning the figures The revised figures are now fully compliant with the requirements and are considerably more readable, substantially improving the clarity of the manuscript.

=> Authors' response:

Reviewer 2 ticked : yes

QUALITY OF THE EXPERIMENTAL DESIGN _____
Reviewer 1 ticked : yes
Reviewer 1 comment :
The authors have adequately addressed the reviewers' concerns by revising the claims and narrowing the scope of the study. The manuscript now presents PDTs as a promising preclinical model rather than as an established one, an interpretation that is more appropriate given the limited cohort size and the exploratory nature of the data.

This more cautious and balanced positioning enhances the overall robustness and credibility of the study.

=> Authors' response:

Reviewer 2 ticked : yes

QUALITY OF THE REPORTING _____
Reviewer 1 ticked : yes
Reviewer 2 ticked : yes

FINAL REVIEWERS DECISIONS _____
Reviewer 1 final decision : yes
Reviewer 1 final comment :
This manuscript introduces a promising 3D culture methodology for breast cancer research that is well aligned with the scope and objectives of *FC3R Short Notes*. The initial results obtained with the PDT model are encouraging and suggest that this approach could potentially be extended to other cancer types. The authors have satisfactorily addressed the reviewers' comments by moderating their claims, refining the scope of the study, and improving the clarity of the manuscript. In its revised form, the manuscript is suitable for publication

=> Authors' response:

Reviewer 2 final decision : yes
Reviewer 2 final comment :
The authors have adequately addressed my comments and I confirm final acceptance

=> Authors' response:

Author's 2nd response: November 30, -0001

Revised version of the Short Note submitted on: August 21, 2026

Final editorial response: August 26, 2026

Short Note 015 - Final acceptance

Dear Stéphane Potteaux and authors,

I am pleased to confirm that I have now checked all the documents you submitted. They are complete and fully address the requests made by the reviewers and the editor.

Your Short Note is therefore definitively accepted for publication.

It will now enter the post-acceptance process, including final formatting and branding, deposit in HAL, and DOI registration.

As soon as the Short Note is online, I will send you its DOI together with the links to the publication on HAL and in the FC3R Short Notes library.

Congratulations, and many thanks for your work throughout the review and revision process.

Best regards,

Marc Le Bert
The Short Notes editorial team

Final validation and publication: August 21, 2026