Reviewer 4

The manuscript entitled "Radiotherapy Enhancement in Prostate Cancer Treatment by Titanate Nanotubes Engineered with Gold Nanoparticles and Docetaxel" written by Alexis Loiseau and co-authors reports on synthesis and development of novel nano-engineered nanohybrids starting from Titanate Nanotubes, coated with APTES and conjugated with DTDTPA-modified Au nanoparticles and PEG. This hybrid complex system was characterized by several techniques and used for in vivo Bioimaging and in vitro investigations. Authors showed a radiotherapy enhancement by testing them against Prostate Cancers. The paper is well structured and it is surely suitable for Cancers readers but is not yet ready to be published in this form, since it needs substantial revisions in order to reach standard level for Cancers.

A number of issues that authors need to address are listed in the following part:

1) In the introduction authors have overlooked a possible comparison with a competitive type of nanotubes for drug delivery and bioimaging: halloysite clay nanotubes (HNTs). Please revise it and after comparing them with Titanate NTBs

**Answer:** We thank the reviewer for his/her remark and suggestion. Indeed, the studies on HNTs were surprisingly not known from the authors. The manuscript has been updated with some references on them. This valuable comment definitively helps this manuscript to reach a better emphasis on our results and a broader interest to the readership.

2) What about uptake and internalisation study for NTBs inside prostate cancer cells? (eg FACS or CLSM study are suggested to be performed accordingly)

**Answer:** The internalization process and kinetics of TiONts have been previously analyzed in vitro (Mirjolet et al., Radiother. Oncol. 2013). We have highlighted that TiONts were internalized by diffusion or endocytosis processes and stayed inside cells at least 10 days (MET analysis).

3) Release study are also missed. Please update/address this point.

**Answer:** The internalization process and kinetics of TiONts have been previously analyzed in vitro (Mirjolet et al., Radiother. Oncol. 2013). We have highlighted that TiONts were internalized by diffusion or endocytosis processes and stayed inside cells at least 10 days (MET analysis).

In our study, concerning the design of our nanohybrids, we made the decision to have DTX covalently bound to nanotubes in order to be sure that the therapeutic molecule stands exactly in the same place than nanotubes (it does not mean that TiONts are internalized though). It means that the “loading” part corresponds to the grafting of DTX onto the nanohybrid coating at the end of PEG chains presenting thiol functions (the amount of which was determined by TGA). Then, there is no reason that the formed carbon-sulfur bond breaks afterwards in the in vivo environment. That is why a release profile of DTX is not relevant in our case, nanohybrid-bound DTX has been designed to interact while being still attached and accepting a decrease in the therapeutic activity of DTX although still cytotoxic.

By the way, the authors are convinced that the parameters (nature of the coating, PEG length, grafting moiety, etc.) could be tuned to increase the therapeutic activity (lower IC50) but a parametric study in chemistry synthesis was not the objective we wanted to give this study of the biological activity of nanohybrids as proof of concept.
4) What is the rationale in cancer cells choice? A thoroughly discussion about motivations/reasons of it is recommended.

**Answer:** We thank the reviewer for his/her question and comment. This study has been performed using a prostate cancer model because we would like to develop this nanoparticle until clinical situation in association with Iodine 125 seed brachytherapy for locally advanced prostate cancer. During this procedure, radiation oncologists use specific needles to introduce iodine 125 seeds inside prostate gland and may use these same needles to inject nanoparticle at the end of brachytherapy procedure. That is why we have injected our nanohybrids directly inside tumor to be in similar conditions than the final targeted application. The manuscript has been modified to explain this cancer model.

5) For in vivo data it would be good to report tumour volumes variation pictures upon drug loaded-NTBs treatment (what about ex vivo data?)

**Answer:** We would like to thank the reviewer for his/her question. Concerning the presentation of our results, the tumor growth delay is a standard parameter commonly used to evaluate effect of ionizing radiation and to perform statistical analyses. However, if curves of all mice for each treatment group are preferable, they can be found below and have been added to the supplementary information as well.

![Tumor volume variation pictures](https://via.placeholder.com/150)

6) It would be important to discuss/report what is the fate of this NTBs after treatment (e.g. biodegradability is an important issue). Please address this issue.

**Answer:** We thank the reviewer for his/her remark on an important point which is the fate of our nanohybrids. A real advantage of our titanate nanotubes (TiONts) is the stability of them under almost all environments and constraints. However, this advantage becomes a disadvantage when degradability is addressed. A previous study not yet published on the biodistribution of TiONts showed that they are completely excreted from mice organisms within 24h by a renal elimination. In spite of that, the chosen strategy for this study was not systemic injection but intratumoral (IT) injection instead. First because the best possible activity within the tumor is reached with IT injection and second, we would like to develop...
these nanohybrids in association with iodine 125 seeds in brachytherapy for prostate cancer in case of clinical developments. During this procedure, radiation oncologists use specific needles to introduce iodine 125 seeds inside prostate gland and may use these same needles to inject nanohybrids at the end of the brachytherapy procedure. In this case, iodine seeds as well as TiONts will not degrade and will stay inside the prostate gland. This point has been clarified in the text.

7) A moderate English revision by native speaker would also improve the text comprehension and the readability of manuscript itself

**Answer:** Thank you for this remark. The manuscript has been corrected by a native English Speaker (Isabel Gregoire) and hopefully reached an excellent level of comprehension and readability from now on.

8) Finally, a comprehensive revision\update of literature references is also suggested after revision (eg upon updating HNTs contribution and thereby references)

**Answer:** We thank the reviewer for his/her remark and suggestion. Indeed, the studies on HNTs were surprisingly not known from the authors. The manuscript has been updated with some references on them. This valuable comment as well as other addressed questions and remarks definitively helps this manuscript to reach a better emphasis on our results and a broader interest to the readership of Cancers.

Finally, the authors would like to thank the reviewer for his/her valuable questions and comments definitively bringing our manuscript to a finer level of understanding and better emphasis of our results.