Reviewer's report

Title: Factor VII-targeted verteporfin photodynamic therapy for breast cancer in vitro and in vivo in mice

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Reviewer: Mahendra Deonarain

Reviewer's report:

Hu et al describe an innovative procedure to use a natural tissue-factor ligand, Factor VII to target verteporfin (VP) photosensitiser for the application of cancer PDT. The concept is wholly reasonable and the impact of this work important as this work could lead to the development of improved agents for human cancer therapy. PDT is becoming increasingly attractive due to its many advantages, but many photosensitisers, such as VP have limitations. These are addressed here. However, I find this paper unacceptable for publication in BMC cancer for the reasons below. This mainly due to the execution of the research (minor issues) and writing of this paper (major issues). The pages are not numbered making my referencing difficult.

Major Revisions

1. The introduction is too brief and lacks important detail. For example, what is the affinity of FVII for TF-is it approaching or better than that of antibodies? By how much does the K341A mutation reduce the TF co-agulation activity? Is it enough to not be a therapeutic risk? VP is a soluble sensitiser and thus clears rapidly from the circulation. Targeting will certainly alter the pharmacokinetics (PK) in vivo and nothing is mentioned of PK effects, which is a key feature. This point is also relevant to the in vivo results below.

2. These experiments describe the in vitro cell killing of human VECs, and tumour cells, mouse tumour cells but the control is a CHO (hamster) cell line. Either a human or murine negative cell line should be used. The authors should at least justify the choice of such a negative cell line

3. In the methods, the cell viability test described is NOT a clonogenic assay. A true clonogenic assay to measure cell killing involves taking the cells after treatment and seeding a culture to see how many cells grow (forms clones of colonies). This assay should be appropriately renamed.

4. In the in vivo therapy, the authors say that one control mouse had metastases. This is an important observation. Was this seen in one mouse per study or one mouse overall (n=4 ?).

5. The discussion is too brief and lacks many important points. For example, FACSSs is not quantitative (as done here), so any decrease in FVIII affinity is not measured. Does the conjugation method used here alter the binding affinity? The authors do not comment on the quality of the conjugate-the coupling ratio described is around 13:1. Is the all covalent or is there some non-covalently
bound material? The authors cite the work of 2 other groups who have made targetable VP conjugates but do not evaluate their work with this other published work. How does it compare in terms of efficacy? Another key discussion point is the choice of 90 minutes for the drug-light interval. This was also used for the in vitro work, but the PK is completely different in vivo. The authors do not show that the conjugate localizes in vivo or how fast the uptake/clearance is. The rationale behind the in vivo work should be explained and justified in the discussion.

6. Figure 1c: I don't understand what PBS-VP control is. Is this VP-there is no Q-band
7. Figure 2b: Why is the TF receptor not evenly and completely expressed on the stimulated cells?
8. Figure 3b: How were EC50s determined when the killing did not reach 50% for the ntPDT?

Minor revisions

9. The English grammar and formatting needs to be checked. For example, in the methods, the washing description of the confocal imaging paragraph is poor. The use of ‘u’ instead of the proper ‘micro’ symbol is also not right. There are numerous other examples.
10. In the introduction, the VP is ‘laser-activatable’ not ‘activated’, as it is not yet activated!
11. In the methods, what is the relevance of vitamin K1 for post-translational modification?
12. Curve fitting by adding a trendline is not appropriate as true EC50s cannot be determined from this, sigmoidal-regression fitting or equivalent should be used.
13. In the discussion, what is meant by ‘third-level treatment’?
14. Figure 1b: The gel is a poor quality with fuzzy protein bands
15. Figure 4 should use log scale X-axes to show the difference better and not squash the points together
16. Figure 6a: The Y-axis needs a label.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

As well as my academic university position, I am a co-founder and director of a
university spin out company developing targeted PDT. The company is developing targetable prostate cancer PDT using a different approach, so there is no direct conflict. However, given the similarities and small commercial field, this potential conflict should be noted.