Reviewer's report

Title: The influence of P-glycoprotein expression and its inhibitors on the distribution of doxorubicin in solid tumors

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Reviewer: Vittorio Cristini

Reviewer's report:

This work investigates the effect of PgP expression on distribution of doxorubicin in human and murine breast cancers in a mouse model. The paper is well written and the results are interesting. It finds that there is greater drug uptake by cells near blood vessels in wild-type as compared to PgP-overexpressing tumors, and that pre-treatment with PgP inhibitors verapamil or PSC 833 increased uptake in PgP-overexpressing tumors. There were steeper gradients of decreasing drug in wild-type tumors compared to PgP overexpressing tumors, and treatment of the latter with the inhibitors led to steeper gradients and greater heterogeneity in the distribution of doxorubicin. Thus, the inhibitors increased drug uptake in cells close to blood vessels, had little effect on uptake in cells at intermediate distances, and may decrease uptake into distal cells.

- Minor Essential Revisions

1. Title: since only two cell types were studied, and both were breast cancers, please consider changing the title to reflect this fact.

2. Figure 4C uses a model to calculate Dox distribution without fluorescence interference from neighboring out-of-section blood vessels. This is mentioned in the Results (p. 9). A brief description of this model should be included in the Methods in order to better appreciate this result.

3. Figures 1 and 3 should indicate the meaning of the colors.

4. Graphs 5A and 5B may not be needed. They are somewhat difficult to read and the information conveyed is adequately explained in words in the last paragraph of the Results (p. 9).

5. Page 9: 1st paragraph, last sentence: “no significant difference is observed in doxorubicin uptake between control tumors and tumors pretreated with a PgP inhibitor.” It may be interesting to discuss some other PgP inhibitors (e.g., as in Wartenberg et al., Int J Cancer, 1998), since the effects observed with verapamil and PSC 833 may not hold for all PgP inhibitors. Some like cyclosporin A may significantly increase Dox penetration at areas distal to the vessels.

6. Page 10: 2nd paragraph, last sentence: It is good to mention the limitations of mathematical models of tumor growth and drug response, but please consider expanding this statement by mentioning some of the recent work that has

7. Discussion: the non-orthotopic tumors studied in the mouse model may not represent the conditions for human patients. A cautionary note to this effect may be appropriate.

8. Conclusions: it may be good to discuss clinical implications of the results. How are PgP inhibitors employed in therapy? Do patients commonly receive both Dox and verapamil (or PSC) treatments? How could the results help to guide the drug regimen?

- Discretionary Revisions

1. Background: consider briefly discussing liposomal doxorubicin administration studies (e.g., Wang et al., Biol Pham Bull, 2005), and how an encapsulated drug may behave differently than free drug administration in the context of PgP expression or inhibition.

2. Figure 1B and 3A are the same data. Consider presenting the data of Figures 1A, 1B, and 3B so that the repetition is not needed, e.g., as in six panels vs. eight.

3. Figure 2 and Figure 4 repeat the data for AR1 and BC 19 in panels A and B, respectively. Consider presenting the data for these figures without this redundancy.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing interests.