Author's response to reviews

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

Authors:

Krupa J Patel (krupa.patel@utoronto.ca)
Ian F Tannock (Ian.Tannock@uhn.on.ca)

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Author's response to reviews: see over
Dear Editors and Reviewers of BMC Cancer,

Thank you for reviewing our manuscript entitled “The influence of P-glycoprotein and its inhibitors on the distribution of doxorubicin in murine and xenograft breast cancer models”. Your comments were very helpful in allowing us to revise and improve the quality of our manuscript. Below are the details of the revisions.

Reviewer: Vittorio Cristini
Comments/Revisions:
Minor essential revisions

1. We have changed the title to reflect that we have studied breast cancer. The title of the manuscript is “The influence of P-glycoprotein and its inhibitors on the distribution of doxorubicin in breast tumors”

2. The model shown in Figure 4C has been described in the Methods section (page 7, par. 3). As well, we have elaborated on the description of the model in the Results section (page 9; par. 3).

3. The meaning of the colors is now within the captions of Figures 1 and 3.

4. The results shown in Figure 5 illustrate the consequence of the hypothesis of our study; that PgP overexpression and its inhibitors alter drug distribution in solid tumors and that this may be an important contributing factor to the failure of many PgP inhibitors in clinical trials. While it is described in the text, we feel that the graphs emphasize the idea that drug distribution and other factors relating to the tumor microenvironment need to be strongly considered when examining modest and no drug effects on tumor growth delay and evaluating new options for drug development.

5. In the discussion section (page 11), we elaborated on studies done in spheroids to examine the effects of PgP inhibitors on doxorubicin fluorescence and the limitations of these studies such as spheroid depth and uniform PgP expression.

6. In the discussion section (page 13; par.3), we have considered a variety of computational models that have been used to evaluate drug accumulation, distribution, pharmacodynamics and response and its relevance in our study.

7. A cautionary note of the limitations of this non-orthotopic tumor model has been included on page 14.

8. In the conclusions a minor note about the clinical relevance of this data was added, however we are cautious about suggesting clinical implications because of the multifactorial nature of drug resistance.
**Discretionary revisions**

1. In the Background section (page 4; par 1) we have briefly mentioned liposomal doxorubicin administration.

2. We originally considered presenting the data in Figures 1 and 3 together however it affected the flow of the paper because we wanted to keep these results distinct in the results text. Also, it is difficult to appreciate the qualitative differences in doxorubicin distribution immediately, and keeping it all together may make this more difficult.

3. Similarly, we originally combined the graphs in Figures 2 and 4 however there was so much overlap between the wild type and PgP inhibitor distribution curves that it was difficult to distinguish between the two. We feel that by keeping the two separate, we can discuss the two major findings of this manuscript, that (1) PgP overexpression may improve drug distribution and, (2), PgP inhibitors may limit drug distribution, together, as well as independently.

**Reviewer: Yan L**

**Comments/Revisions:**

**Major Compulsory Revisions**

1. Doxorubicin distribution patterns at various time points have been previously studied in our laboratory and we have referenced that work in the manuscript (page 12; par. 1). Doxorubicin is maximally distributed at 10 minutes. Here we try to emphasize that even at maximum time point, doxorubicin distribution is very limited.

2. The linear regressions that were performed were statistically significant. The reason we chose to fit them to a linear model is because many of the individually quantified areas of interests showed linear decay of doxorubicin distribution. However, we took numerous areas of interest and averaged them together, and it is these average plots that appear non-linear. Linear regressions were performed on each individual area of interest and found to be significant. There were also no consistent patterns in the residual plots. The one distribution that appears to be most non-linear is the AR1 tumors treated with PSC 833. However, the linear regressions within this group were also statistically significant and we fit them in this way to make direct comparisons. The model in Figure 4C is fitted to a linear regression under the assumption that there are minimal neighboring blood vessels contributing to doxorubicin fluorescence intensity at farther distances (page 7; bottom and page 10; par. 1). We believe this model is reflects the numerous areas in a tumor that are poorly vascularized and emphasizes the problem of limited drug distribution in solid tumors.

**Minor Essential Revisions**

- we have revised the manuscript for missing labels, spelling mistakes, etc.
Discretionary Revisions

1. We found that there was no difference in doxorubicin distributions in wild-type tumor whether they pretreated with saline or a PgP inhibitor and thus chose not to include these data.

We hope that we have adequately addressed all the revisions given by the reviewers. These comments and revisions have given the manuscript a broader and deeper perspective of P-glycoprotein and doxorubicin distribution. Thank you for your time in giving us very helpful insights into our manuscript. We look forward to hearing from you.

Sincerely

Krupa J. Patel
Ian F. Tannock