Reviewer’s report

Title: The effect of chemotherapeutic agents on tumor vasculature in subcutaneous and orthotopic human tumor xenografts

Version: 2 Date: 29 September 2014

Reviewer: Ryan Roberts

Reviewer’s report:

The authors of this paper present a series of studies evaluating changes in tumor vasculature upon treatment of two different tumor cell lines in both subcutaneous and orthotopic sites with each of three different cytotoxic agents. The paper is easy to read and generally easy to follow. My primary criticism of this paper is that 1) there is a general lack of novelty in their approach and in the results/conclusions they provide and 2) they provide an incomplete evaluation of the process they set out to study.

1. In terms of novelty, they provide a very nice summary of the already-documented effects of cytotoxic therapy on tumor vasculature—an area that has received no small amount of attention already. They then state that they intend to evaluate this same question and make no argument for the novelty of their approach or the value this will bring to the field of study. They do make some argument in the body of the paper for the novelty of comparing “functional” vasculature together with simple CD31 IHC, and one can infer that they intend to compare subcutaneous to orthotopic xenografts, but these concepts are poorly developed and poorly explored throughout the paper.

2. With regard to evaluation, the entirety of the data presented are a series of quantifications of CD31 staining and intravascular dye quantification in two tumor types x two implantation sites x three different treatments. Appropriate controls (such as untreated xenografts at the different timepoints) are not included. Data that might facilitate interpretation of their findings (such as tumor size at each of the time points, stains evaluating tumor cell or endothelial cell apoptosis or proliferation, such as CC3 or Ki-67) are sorely lacking. All data is compared to time 0 for each tumor/site/treatment combination, but there is no attempt made to compare groups to each other, which is the fundamental novel approach taken by the authors. Such comparisons might include comparisons of orthotopic to subcutaneous, which may test the validity of subcutaneous xenografts or identify differences in response due to microenvironment or a comparison of intravascular dye to CD31 IHC, which might identify fundamental differences in the biology critical to evaluation such experiments.

3. Finally, there is no attempt, even at a most basic level, to make any exploration into the mechanisms which might account for these observations. Such explorations may be as simple as including the tumor size at each of the timepoints (i.e., is the increase in tumor vascular density a consequence of lost tumor cell volume secondary to cytotoxic therapy or a consequence of
neovascularization stimulated by factors released by the tumor cells upon
treatment?), an evaluation of apoptosis/proliferation in each of the cellular
compartmentst (tumor/endothelial) at different timepoints of treatment, an in vitro
comparison of the sensitivity of quiescent endothelial cells vs stimulated
endothelial cells vs tumor cells to each of the cytotoxic agents, among myriad
others. Many of the changes seen in day 12 tumors and even day 8 to some
extent likely relate to rebound in the proliferation of tumor cells as the effects of
the cytotoxic therapies wane, but no conclusions can be drawn, because data is
not shown.

MAJOR COMPULSORY REVISIONS
4. Please include tumor size/volume at each of the timepoints.
5. Please rewrite to address the novelties of approach and findings.
6. Please provide some comparison/formal evaluation of groups to each other

MINOR ESSENTIAL REVISIONS
7. Please label each of the graphs (on the graph itself) so that it is clear which
tumor type/site/treatment combination is being tested in each.
8. All statistical tests used in this writeup are inappropriate. All data shown is
collected in a repeated measures fashion, and appropriate statistical tests should
be applied. Please consult a statistician if necessary. In addition, statistical
significance should be more clearly addressed. What does clinical significance
mean in this evaluation? For instance, there are several locations where it
appears that time 0 numbers are significant relative to themselves, which doesn't
make sense.
9. Y axes for all of the graphs should be clearly labeled. In addition, they should
all either be put on the same scale (same max/min) or all be put on a scale that
provided the easiest visualization of the data. As it stands, there is a mix of both.
10. What happens at day 4 in figure 5? This should be explored.

DISCRETIONARY REVISIONS
11. The paper would benefit dramatically from some exploration of mechanism,
even if done at a rudimentary level.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests.