Reviewer's report

Title: Tamoxifen Elicited Uterotrophy: Cross-Species and Cross-Ligand Analysis of the Gene Expression Program

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Reviewer: Todd Skaar

Reviewer's report:

Kwekel and colleagues have conducted a series of preclinical studies focused on understanding the uterotrophic effects of tamoxifen. The goals of these studies are to improve our understanding of the effects of tamoxifen on uterine cancer. This is an important therapeutic problem that is worthy of study. For the most part, the studies are designed and described well. However, there are a few points that are major compulsory points that need to be addressed before it would be acceptable for publication.

1. Table 1 and Table 2 have all the same numbers in them. It is hard to believe that the means and errors would be exactly the same. It appears that the same table was mistakenly put in twice. This will need to be corrected and provided for a rereview.

2. This is one study as a step towards the authors presumed overall goal of understanding the uterotrophic effects of tamoxifen in humans. The effects of the drug on rodent uterine activity, per se, is not of any biomedical value, but rather is of significant interest if it can be used to better understand the human biology. Therefore, it needs to be clear that this is going to help understand the effects of tamoxifen in humans. The following comments relate to the justification of their model of the human problem.

It is my opinion that the authors have not made a convincing argument that these rodent studies mimic what happens in humans. For example, since the rodents are ovariectomized, they are presumably modeling of postmenopausal women. This is, of course, an important population for tamoxifen; however, whether or not the “estrogenic” background of a young ovariectomized mouse or rat is similar to that of a postmenopausal women is not discussed. They do make reference to it being a classic model model of estrogenicity, but I'm not sure that that is sufficient evidence that a 3 day treatment for uterine wet weight is a model of the 5 years of treatment of tamoxifen.

Another question remains about the dosing. I do commend the authors for determining the optimal dose by doing the dose response curves and identifying the near max dose for the uterine wet weight; however, if they are trying to mimic the human dosing, there are still many differences. Actually, they may be well below the normal exposure of humans. First, rodents usually metabolize drugs much faster than humans; therefore, if given on the same ug/kg basis, they will
likely have much lower circulating concentrations. Since circulating concentrations of tamoxifen and metabolites were not measured, they have no idea of the exposure relative to humans. Second, since tamoxifen has a long half-life in humans, with daily dosing, it accumulates and does not reach steady state until approximately 1 month. The three day dosing would not allow time for accumulation. Third, tamoxifen is heavily metabolized to a variety of active metabolites; some of these are more active than the parent drug. Also, some metabolites are antiestrogenic and some estrogenic. If they are trying to model the estrogenicity of tamoxifen in humans, it seems critical to know the metabolite profiles of this study. This has not been presented.

I am not trying to say that these studies are not useful; however, based on the way that the article is written, they do not seem to have considered these limitations to their study. Consequently, they do not provide any direction as to how these are going to be useful in understanding the effects of tamoxifen in human endometrial cancer. It appears to be more of an experiment that was done, because they could do it, rather than because it is a really good model of the important problem in using tamoxifen in humans. This needs to be made more convincing before we could say that it is useful for understanding the side effects of tamoxifen. For the reasons described above, data such as circulating plasma tamoxifen and metabolite concentrations seem critical to the interpretation of the data; therefore, they need some convincing argument of how this data is useful in its absence.

Other minor points that need to be addressed:

Is the source of the tamoxifen z or e isomer? What percent of each?

I am not sure that the 2-way ANOVA with tukey’s is appropriate for time course data analysis. Since the data at the neighboring time points are correlated, this may violate one of the assumptions required for that test.

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

**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