Author’s response to reviews

Title: Mechanisms of doxorubicin-induced drug resistance and drug resistant tumour growth in a murine breast tumour model

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Reviewer 2:

1. The following acronyms on first appearance were identified in the abstract (page 1 and 2): mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), epithelial-mesenchymal transition (EMT) and extracellular-signal-regulated kinase (ERK). “MCM2” was changed to “minichromosome maintenance 2”. The following acronyms on first appearance were identified in the main text of the manuscript: Background section (page 3 and 4) - Global Cancer Incidence, Mortality and Prevalence (GLOBOCAN) and alpha smooth muscle actin (α-SMA); Results section (page 5, 6 and 7) - low dose (LD), high dose (HD), tumour control (TC), B-cell lymphoma 2 (Bcl-2), caspase (Casp), microtubule-associated protein light chain 3 (LC3), mammalian target of rapamycin (mTOR), phosphatase and tensin homolog (PTEN) and phosphoinositide-dependent kinase-1 (PDK1); Methods section (page 11 and 13) - fluorescein isothiocyanate (FITC), analysis of covariance (ANCOVA) and analysis of variance (ANOVA). The following acronyms were included in the List of abbreviations section (page 14): ANCOVA, analysis of covariance; GLOBOCAN, global cancer incidence, mortality and prevalence; PDK1, phosphoinositide-dependent kinase-1; PTEN, phosphatase and tensin homolog.

2. The title was changed to “Mechanisms of doxorubicin-induced drug resistance and drug resistant tumour growth in a murine breast tumour model”.

3. In figure 1 the slope of the regression lines of the LD-DXR and HD-DXR groups were significantly different compared to the TC group and not at certain points. The following was stated in the figure caption: “Error bars indicate the standard error of the mean”. The number of
animals per group was changed from “n = 16” to “n = 15 (TC group), n = 16 (LD-DXR and HD-DXR groups)” in the figure caption. This was a single experiment. The mice were sacrificed when tumours reached 400 mm³. Since the DXR groups grew faster, they were euthanized relatively earlier. By day 29 there were only mice in the TC group left.

4. Matrigel was used for both tumour control and doxorubicin treated groups and therefore any contributing effects of the Matrigel on the tumour volume measurements would have been equal (ruled out) between the groups. The Matrigel would therefore not have an influence on the significant differences observed between the groups. Similar previous studies without Matrigel have shown a decrease in tumour volume after doxorubicin treatment. The interaction between Matrigel and doxorubicin might have contributed to the increased tumour volume observed in the treated groups. Since doxorubicin is immunogenic, inflammation might also have contributed to the increased tumour volume observed in these groups. It would, however, be difficult to circumvent these effects, since it is the nature of the drug. We could have investigated the tumour sections after animal sacrifice to compare the presence of immune cells and inflammatory markers between the groups.

5. Triple negative breast cancer is usually more aggressive and resistant towards doxorubicin treatment. The results obtained in this study could possibly indicate what happens in patients with resistant triple negative breast cancer. The E0771 cell line is a spontaneously developing medullary breast adenocarcinoma from C57BL/6 mice. E0771 has been proposed for immunomodulatory studies, because it can be used in mice with a more complete immune system. This type of model is more physiological since the immune system plays a role in almost all cancers.

6. The results have not been reproduced.