Author's response to reviews

Title: Tyrosine kinase inhibitor SU6668 represses chondrosarcoma growth via antiangiogenesis in vivo

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Author's response to reviews: see over
Berlin, December 6, 2006

Manuscript: MS: 1315524864100251 - Tyrosine kinase inhibitor SU6668 represses chondrosarcoma growth via antiangiogenesis in vivo.

Dear Members of the BioMed Central Editorial Team,

Thank you very much for your emails from October 13, 2006, including the reviewers' comments encouraging us to resubmit a revised version of the aforementioned manuscript, and from November 16, 2006, extending the deadline for the revision to December 12, 2006. The latter enabled us to add new experiments with immunohistochemical analysis.

We were most appreciative of the reviewers' suggestions and corrections and feel that the manuscript has now been improved a lot.

On the following pages after this letter, please find a point-by-point description of the changes made in general as well as responding to each reviewer's report.

Thank you again for your consideration. We look forward to hearing from you.

Happy Santa Claus to your team!

Yours sincerely

Dr. Axel Sckell
(Responsible and corresponding Author)
General revisions independent from reviewers' reports

General:
Control was written with a capital "C" as first letter in all cases.

Reply to reviewers' reports:

Report from Claus Belka from October 13, 2006:
The reviewer described our manuscript as a "nice descriptive paper" and suggested that the "discussion part should be shortened significantly".

For this reason, the discussion part has been shortened from approximately 4 pages in the first version to less than 3 pages in the actual resubmitted version:

Apart from this, the reviewer suggested to accept the manuscript "after minor essential revisions" and stated that it is "an article of importance in its field".

Report from R. Daniel Bonfil from September 9, 2006:
The reviewer stated that "the results obtained are interesting”. However, "major compulsory revisions are needed":

1.) The non-parametric Mann-Whitney test should be used to compare results obtained in SU6668-treated and control mice at every time point.

Answer:
As suggested by the reviewer, the Mann-Whitney test is now used for statistical analysis of the results obtained in SU6668 treated and control mice at any time point.

2.) The authors did not state clearly why they chose 250 mg/kg of SU6668. To strengthen the results, the study should include at least two experimental groups treated with different doses of SU6668, so that a dose-dependent effect could be observed.

Answer:
SU6668 has been used as an anti-angiogenic agent in a number of tumor xenograft animal models for primary tumors. The substance can be administered orally and by i.p. or s.c. injection. We have decided to inject SU6668 subcutaneously in order to achieve a reproducible and save administration. As intraperitoneal injections are associated with the risk of puncture and injection into the intestine, subcutaneous injections have a reduced risk of complications compared to repeated intraperitoneal injections.

The dose-dependent effects of SU6668 on angiogenesis and tumor growth have been investigated previously with doses ranging from 4 mg/kg/day to 400 mg/kg/day. Anti-tumor and antiangiogenic effects can be achieved with dosages between 75 mg/kg/day and 400 mg/kg/day [1-8]. In order to reduce the stress accompanied with the injections of the drug we have chosen an application interval of 48 hours with a dosage of 250 mg/kg. This dosage is in accordance with the dosages for animal trials reported in the literature and represents the lower range of dosages that were shown to be effective against primary tumors.
In "Methods" under the section "SU6668 treatment", we have now added a paragraph concerning the chosen dose of SU6668 including appropriate citations from the literature.

The reviewer argues that the study should include at least two experimental groups treated with different doses of SU6668 in order to describe dose-dependent effects. However, the dose-dependency of the anti-angiogenic and anti-tumor effects of SU6668 have been well described in tumor xenograft models for primary tumors [2]. After the establishment of a dose-dependency of the antitumor effects of SU6668 which has been widely accepted as the work refers to in terms of SU6668 dosing, recent publications did not establish additional dose-dependencies for each individual tumor / model used. The present study was not designed to establish a dose-dependency of the antitumor effects in SW1353 chondrosarcomas but was performed to investigate the effects of SU6668 in primary bone tumors. That is why we have chosen a dosage at the lower end of the anti-tumor effectiveness. As the chosen dosage is already very effective it can be assumed that higher dosages will also be effective but will also carry a higher risk of side-effects.

Additionally, we have chosen this approach to minimize the number of animals needed for the experiments which is accordance with the principle of 3R (reduce, refine, replace).

For these reasons, we kindly ask to re-consider the requirement of another experimental group to be treated with a different dose of SU6668.

Additional references mentioned in the present answer:


3.) Immunohistochemical analysis of intratumoral microvascular density should be performed to confirm the results reported in *in vivo* studies for functional microvessel density.

**Answer:**

As suggested by the reviewer, immunohistochemical analysis of the intratumoral microvascular density was performed additionally. By doing so, the results reported in the *in vivo* studies for functional microvessel density have been confirmed.

For this reason, in "Methods" the section "Histopathologic assessment", was renamed in "Histopathologic and immunohistochemical assessment" and expanded by a new paragraph describing the method which was used for immunohistochemical analysis to identify endothelial cells. Furthermore, in "Results" and "Discussion" the new data have been included. Fig. 1 was enlarged by two images showing CD31 immunohistochemistry of representative tissue samples of Controls (Fig. 1C) and SU6668 (Fig. 1D).

**Minor essential revisions:**

1.) In Material and Methods, Statistics, it is mentioned that data are presented as median with 25% and 75% quartiles. However, Figure 1A and B show median and individual values

**Answer:**

Figure 1A and 1B now show median values with 25% and 75% quartiles as mentioned in the “Statistics” subsection of “Methods”.

Additionally, two images from the CD31 immunohistochemistry have been added to Figure 1 (Fig. 1C and 1D).

2.) Figure 2 should be improved if, as shown in the manuscript, seems to be very small and the contrast is low.

**Answer:**

Figure 2 has been improved.