Author's response to reviews

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

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Version: 3 Date: 2 January 2007

Author's response to reviews: see over
Berlin, January 2\textsuperscript{nd}, 2007

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

Dear Members of the BioMed Central Editorial Team,

Thank you very much for your last email from December 12, 2006 encouraging us to resubmit a revised manuscript of our above mentioned study. Obviously the reviewer Claus Belka now accepts our manuscript for publication in the previously revised form. However, due to the second report of the reviewer R. Daniel Bonfil this reviewer still has some concerns - he would like us to assess intratumoral microvascular density (MVD) and statistical analysis for further comparison.

To this end, we discussed how to proceed within our group and also with some other researchers of our environment a lot coming to the conclusion not to perform further experiments or other extensive revisions (as already done) in our manuscript for the reasons described in the "Reply to reviewers' report" on the following pages after this letter.

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

A Happy New Year to you and your team.

Yours sincerely

Dr. Axel Sckell
(Responsible and corresponding Author)
Reply to reviewers' report:

Report from R. Daniel Bonfil from September 9, 2006:

In his second reviewer's report, R. Daniel Bonfil stated that still "major compulsory revisions are needed" even though many concerns raised by the reviewers have been addressed.

The authors addressed many of the concerns raised by the reviewers. However, the immunohistochemical analysis of the endothelial antigen CD31 did not include the assessment of intratumoral microvascular density (MVD), a concern indicated in the previous critique. From the new images included as Figures 1C and D it is obvious that the absolute number of CD31 positive structures is lower in intraosseous tumor in the SU6668-treated group. However, this has not been shown in terms of density (MVD) in all the samples for a statistical comparison of the results in both groups. An inset at higher power magnification would be useful to confirm that the CD31 positive structures shown in Figs. 1C and D truly reveal the expected microanatomy. I hope that these modifications would improve the quality of the manuscript and allow its acceptance for publication.

Answer:

To this end, we discussed the above mentioned reviewer's report within our group and also with some other researchers of our environment a lot coming to the conclusion not to perform further experiments or other extensive revisions (as already done) in our manuscript for the reasons described in the following:

1) We already added a complete new set of immunohistochemical experiments using the original tissue samples from the original in vivo experiments to prove qualitatively that in vivo findings correlate with ex vivo immunohistochemistry in terms of that functional vessel density (FVD) correlates with the amount of CD31 staining.

In our opinion, this improved the quality of the manuscript a lot and we are thankful to the suggestions of the first review of R. Daniel Bonfil.

2) FVD shows exclusively vessels being perfused i.e. having a significant in vivo function whereas MVD is a static ex vivo parameter not giving any hint whether a vessel is perfused or not. For this reason, in our opinion it is not relevant to compare in vivo FVD with ex vivo MVD statistically or in our study, to perform quantitative analysis of MVD. Furthermore, in our opinion the surface i.e. the outer tissue areas of a tumor which can be investigated by means of intravital microscopy better represent the vitality and aggressivity of a tumor than the more central located areas where often the typical necrosis of fast growing tumors takes place.

3) Intravital microscopy is a highly sophisticated experimental method being the "golden standard" in analyzing microvascular parameters and not ex vivo immunohistochemistry. This has been proven in many different studies investigating various experimental settings and is generally well accepted in the field of experimental research.
4) The reviewer agrees that "... it is obvious that the absolute number of CD31 positive structures is lower in intraosseous tumor in the SU6668-treated group. However, this has not been shown in terms of density (MVD) in all samples for a statistical comparison of the results in both groups." We demonstrated the lower number of CD31 positive structures in SU6668 for all animals of both experimental groups. We would not expect further important/new information by performing additional time and resource consummating analyzing of MVD. Furthermore, we are not experts in performing this method.

5) CD31 is a marker for endothelial cells. This is well known and generally accepted. Of course higher magnifications of our immunohistochemical samples demonstrate this accordingly. However, the common reader of the manuscript will believe this without having further figures in higher magnifications then over viewing only a very limited area of the whole tissue sample.

Due to the explanations above, we hope that the reviewer and the editorial board will accept our manuscript for publication now without further changes.