Author's response to reviews

Title: Effectivity of pazopanib treatment in orthotopic models of human testicular germ cell tumors

Authors:

Mercè Juliachs (mjuliachs@iconcologia.net)
August Vidal (avidal@csub.scs.es)
Xavier Garcia del Muro (garciaelmuro@iconcologia.net)
Josep M Piulats (jmpiulats@iconcologia.net)
Enric Condom (ecm@csub.scs.es)
Oriol Casanovas (ocasanovas@iconcologia.net)
Mariona Graupera (mgraupera@idibell.cat)
Jose R Germa (jgerma@iconcologia.net)
Alberto Villanueva (avillanueva@iconcologia.net)
Francesc Vinals (fvinals@iconcologia.net)

Version: 2 Date: 26 July 2013

Author's response to reviews: see over
Dear Sirs:

We would like to resubmit our manuscript entitled “Effectivity of pazopanib treatment in orthotopic models of human testicular germ cell tumors” (old title “Synergistic effect of anti-angiogenic and anti-ErbB S treatment in orthotopic models of human testicular germ cell tumors”) to BMC, addressing the specific criticisms raised by the reviewers. In this letter we have included point-by-point responses to issues raised by reviewers and modifications have been included in the revised paper.

Thank you for considering our paper for resubmission.

Sincerely yours,

Francesc Viñals, Ph.D.
Translational Research Laboratory
Catalan Institute of Oncology and University of Barcelona, IDIBELL,
Hospital Duran i Reynals,
Avda Gran Via s/n km 2,7,
L’Hospitalet de Llobregat (Barcelona), SPAIN
Response to reviewers

Reviewer: Friedemann Honecker
Reviewer's report:
Minor Essential Revisions

The manuscript contains novel and interesting data on an unresolved issue - cisplatin resistance in germ cell cancer. Experiments and methods are clearly described, the results are presented in an appropriate way, and the list of references includes all important data that the work is either building on or extends. The main criticism is that there are several spelling mistakes and stylistic flaws, which could be resolved by a native English speaker.

We sent our manuscript to an English spoken reviewer.
Reviewer: Michael Höpfner  
Reviewer's report:  
Juliachs et al, BMC Cancer-2013:  
“Synergistic effect of anti-angiogenic and anti-ErbBS treatment in orthotopic models of human testicular germ cell tumors”  
The study of Juliachs et al. evaluated the antitumoral and antiangiogenic effects of two small molecule inhibitors in two different models of human testicular germ cell tumors that were orthotopically grown in nude mice. Applied as single drugs the two inhibitors pazopanib and lapatinib exhibited antitumoral and antiangiogenic activity. Based on additive effects of a combination of both agents in one of the two mouse models the authors concluded that the pazopanib and its combination with lapatinib might be an interesting new approach for the treatment of cisplatin-sensitive and especially cisplatin-insensitive germ cell tumors. Despite of some minor grammatical and typing errors the text is well written and the authors’ hypothesis is clear. The set of experiments chosen to explore the effects of these inhibitors is logical and well-chosen. Thus, the paper will be interest for the readers of BMC Cancer.

However, some criticisms should be taken into account, before publication can be recommended:
1. The title of the paper doesn’t fit to the actually presented data, as the combination of antiangiogenic and anti-ErbBS treatment was only investigated in CDDP-sensitive TGT38 mice. Moreover, it appears that the main goal of the paper was to demonstrate pazopanib as a promising novel agent for the treatment of CDDP-refractory GCTs. This is reflected in the conclusion and should thus be also reflected in the title. In our opinion the reviewer is right. So we changed the title of our manuscript in order to highlight pazopanib effects and remove the combination treatment of it.

2.) Fig. 2 shows a boxplot diagram on the effects of pazopanib in CDDP-refractory TGT44 mice. The CDDP-treatment shows rather high deviation, overlapping with the effects shown for pazopanib. As only n=3 (control) or n=4 (CDDP, pazopanib) individuals were treated in each group, the data
are rather weak. A higher number of individuals tested in each group (e.g. like in the TGT38 tests> n=12 to13) is required to clearly demonstrate CDDP-insensitivity and pazopanib’s effectiveness.

Concerning this point, the reviewer is right, the number of control or treated TGT44 implanted animals is low. But we must stress that TGT44 testicular cancer model is an interesting but very difficult model to work. The time to growth is very long (3 months) and the growth success after implantation is very low (about 50%). In fact in our experiment we implanted pieces of this tumor in 24 independent animals, but only 10 finally grewed. These animals were distributed as indicated in 3 control, 3 CDDP and 4 pazopanib treated. But even the number is limitated, in our opinion the information we can extract is sufficient informative to reach the conclusions presented in our manuscript.

3.) CD31 staining depicted in figure 3A is hardly legible. Even in a high resolution print out only black boxes with light hazes of green can be seen. The figure should be overworked or removed as the respective data are also depicted as a diagram in Fig. 3B.

The reviewer is right, we decided to remove the picture and maintain the diagram.

4.) Figure 5A: Bar: 4000µM ?

The reviewer is right, we have corrected this point (40 µM). The bar scale was labelled as 40.00, but to make it more clear we removed the decimals.

5.) Reference 31 is incomplete

The reviewer is right and we have corrected this reference.

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Needs some language corrections before being

We sent our manuscript to an English spoken reviewer.
Reviewer: Marco Barchi
Reviewer's report:
The paper from Juliachs M et al., describe the effect in vivo of lapatinib and Pazopamib on an orthotopic model of TGCT intrinsically sensitive (TGT38) and resistant (TGCT44) to cisplatin treatment.
The paper is overall very clear, well written and results straightforward.
Few comments are shown below:

Major points:
Page 7 row 22. The authors state that drugs treatment do not have significant effect on body weight. Please provide a table in supplementary. In addition, it is require to show whether lapatinib and pazopanib single and combined treatments have a toxic effect on specific sensitive organs. It is mandatory to show wether drug-induced apoptotic effects are induced in liver, kidney and GI tract.
Concerning the possible toxic effects of drug combination, first as suggested we included a figure in Additional data with lack of effect of drugs on body weight during treatment time (new Additional. Fig. 1A). Second, in order to discard a toxic non specific effect on non tumoral organs, we analyzed the most drug-sensitive organ, liver, and we performed TUNEL to analyze possible apoptosis induced by drugs. As is shown in Additional. Fig. 1B, we did not detect any effect on apoptosis by pazopanib alone or in combination with lapatinib.

Minor points:
Page 7, row 8th. Please specify how pazopanib is orally administrated (gavages? Drink water?)
By gavage, we specified this point in the text.

Page 7, row 12, please specify the number of mice in the “control group”
The number of mice for the control group has been incorporated in row 9.
Page 11, row 19, please use a different nomenclature for each picture within figure 1A e.g. Fig1AI, Fig1All…Fig1AXV or alternatively use italic letters (Fig1A a., b…..o)
As suggested by the reviewer we changed the nomenclature in Fig. 1A to italic letters.

Page 24 and 25, please in Figure legends of Figures 2-5 specify whether the Mann-Whitney U Test is one tail or two-tail test
We specified this point in the text as suggested by the reviewer.