Dear Editor and Reviewers,

We would like to express our sincere thanks to you and the reviewers for a thorough review of our paper, for the excellent suggestions which we feel strengthened the quality of the paper, and for the opportunity to submit a revised manuscript.

We have carefully revised the manuscript in response to the reviewers' concerns and believe it is significantly strengthened as a result, and hope you agree. Please see below for our responses in italics to each reviewer's individual comments.

Reviewer #1:

Major Comments

1. Page 4, line 25: A more recent paper than refs 17 and 18 that describes influx and efflux transporters related to Pt compounds is Burger et al. (Drug Resist Updat. 2011 Feb;14(1):22-34).

Response:

We agree with the reviewer and subsequently included the abovementioned reference (20) and discussed the currently identified platinum drug transporters in more detail.

2. Page 5, line 20: It is stated that Pt has a long half-life in human tissue. How long exactly and how this tissue half-life does relate to its pharmacokinetics (half-life in plasma)?

Response:

The reviewer's suggestion is pertinent to this paper, however the half-life of platinum in tissue is yet to be quantified and there have been no studies to correlate platinum pharmacokinetics in tissue versus blood. We have described in detail the current understanding of this concept in the paper with references.

3. Page 6, III. Copper transporters, line 17: it is not true that none of the pumps and transporters implicated in Pt transport is well-characterized (see again Burger et al. Drug Resist Updat. 2011 Feb;14(1):22-34). For example SLC22A2 is well characterized as Pt. influx transporter

Response:

We agree with the reviewer and in the revised manuscript now state "some of which are well characterized". We did not elaborate further on these transporters as our emphasis is on CTR1 in the manuscript.

4. Page 7, line 1: It is stated that "Higher CTR2 levels correlated with Pt resistance in ovarian cancer cell lines" is this correct, if so I do not understand as CTR2 activity may lead to increased influx of Pt compounds. Please explain /more clearly address this discrepancy.

Response:

CTR2 initially described as a copper influx transporter is considered to be a platinum efflux transporter based on current available literature. The manuscript has been modified to clearly state this and avoid any confusion.

5. Page 7, line 10-11: As ATP7B is not located in the plasma membrane one could say that in addition to efflux also sequestration (in Golgi network and/or vesicles) may play a role in resistance.

Response:

The manuscript now states "whereas ATP7B located in the Trans Golgi network mediates Pt drug efflux via a process that involves its transport into vesicles involved in the secretory pathway." And a new reference added.

6. 6. Page7, line 14-15: It is stated that "ATP7B silencing results in enhanced cisplatin sensitivity and increased DNA adducts formation in cisplatin-resistant cells" I do not understand you would expect less DNA adducts in cisplatin-resistant cells not more adducts! Please check.

Response:

ATP7B is postulated to be a platinum efflux transporter. With the silencing of the gene, the expression levels of ATP7B would be lower, which in turn translates to lower efflux of platinum and thus increased platinum -DNA adduct formation.

7. In the context of this review a critical question would be whether Pt compounds are taken up by transporters (e.g. CTR1) or diffusion. This may be different for the various Pt compounds. Please discuss.

Response:

We agree with the reviewers that there still remains a question regarding the different mechanism involved in platinum transport. Hence we have added

"Ivy et al. also noted that higher intracellular Pt correlated with higher CTR1 levels in human embryonic kidney cells and mouse embryonic fibroblasts. However contrary to other studies, the investigators noted that in ovarian tumor cells uptake of Pt was linear and non-saturable, suggesting that there could be other mechanisms besides CTR1 involved in Pt transport, including proteins involved in copper homeostasis."

"Pt drug influx has been attributed to both non-saturable as well as energy-dependent active transport processes."

Minor comments (responses are in italics)

1. Give in the text – at least once – the official gene names for the transporters that are discussed e.g. CTR1 = SLC31A1

   Manuscript now states official names of genes

2. Page 3, I. introduction, line 15-16: The main adverse event related to oxaliplatin is actually neurotoxicity. Please mention this.

   We now mention this

3. Page 3, I. introduction, third paragraph: I miss the referral to ovarian cancer as the standard treatment for this cancer is a combi of carboplatin and paclitaxel. Moreover experiments relating to ovarian cancer are discussed several times throughout the manuscript.

   We complete agree with the reviewer and now state that Pt is used in ovarian cancer as well.

4. Page 4, line 9: "…with up to an 80 percent response rates…" remove "an"

   Done

5. Page 4, line 23: "infux" should be "influx"

   Changed

6. Page 6, line 23: "These transporters possess…" I guess with transporters you mean ATP7A and ATP7B?

   Yes and in order to avoid confusion we now state " All of the above discussed transporters possess"

7. Page 7, line 8: Note that the KB-3-1 was recently identified as a derivative of HELA (cervix carcinoma)

   Incorporated into the manuscript

8. Page 7 and page 8: There are two chapters IV.

Formatting has been changed to correct the numbering

9. Page 11: Chapter VII is missing.

Formatting has been changed to correct the numbering

9. Page 10, line 11: what is meant by Pt-based doublet chemotherapy?

The manuscript now states "Pt based combination chemotherapy" to avoid any confusion

10. Page 11, line 15: What is meant by "different rs10981694 genetic polymorphisms"?

Reference single nucleotide polymorphism and the revised manuscript states this

11. Reference 29: please give year in which this ASCO abstract was published

In the updated manuscript is now reference 31 and reflects the year

12. Reference 57: remove capitals

Changed

Reviewer #2:

Major Comments:

1. I would suggest to consider also the following ones, if believed of interest by Authors: Liang et al., Regulation of the high-affinity copper transporter (hCtr1) expression by cisplatin and heavy metals, J Biol Inorg Chem. 19(1): 17–27, 2014. doi: 10.1007/s00775-013-1051-z [which focuses on the regulation of hCTR1 expression by Pt and other heavy metal ions through Sp1].

   Response:

   Reference 80 added with appropriate statement in the text as below

   "Also in IGROV1 and SKOV-3 cells treated with different concentrations of Zn, and Ag, there was a concentration-dependent increase in expression of CTR1 and Sp1"

2. Also the paper by Ivy & Kaplan (A Re-Evaluation of the Role of hCTR1, the Human High-Affinity Copper Transporter, in Platinum-Drug Entry into Human Cells, Mol Pharmacol 83:1237–1246, 2013. doi: 10.1124/mol.113.085068) might be quoted, since it considers data on the fact that hCTR1 is not the main route for entry of Pt drugs; moreover, the copper transporter is not internalized in response to extracellular drug, indicating that hCTR1 mechanism is not saturable and not protein-mediated

   Response:

   Reference 65 added with appropriate text in the manuscript as below

   Ivy et al. also noted that higher intracellular Pt correlated with higher CTR1 levels in human embryonic kidney cells and mouse embryonic fibroblasts. However contrary to other studies, the

investigators noted that in ovarian tumor cells uptake of Pt was linear and non-saturable, suggesting that there could be other mechanisms besides CTR1 involved in Pt transport, including proteins involved in copper homeostasis.

3. Regarding the pharmacological modulation of CTR1 a very recent paper showed that also compounds of natural origin could be useful [Wang X, Jiang P, Wang P, Yang CS, Wang X, Feng Q (2015) EGCG Enhances Cisplatin Sensitivity by Regulating Expression of the Copper and Cisplatin Influx Transporter CTR1 in Ovary Cancer. PLoS ONE 10(4): 0125402. doi:10.1371/journal.pone.0125402] and therefore may deserve quotation in order to widen the spectrum of intervention.

Response:

Added a paragraph with reference- the manuscript now states

More recently, in ovarian cancer cells and xenograft mice, (-)-epigallocatechin-3-gallate (EGCG), a major polyphenol from green tea was noted to increase CTR1 m RNA and protein expression. These findings translated into EGCG enhancing the sensitivity of ovarian cancer SKOV3 and OVCAR3 cells to Pt through increased Pt accumulation and DNA-Pt adducts.

4. Also, a new paper has considered the contemporary modulation of CTR1 and ATP7A targets by D-penicillamine, in order to improve the therapeutic efficacy of Pt drugs in oxaliplatin-resistant tumors: Chen SJ et al., Mechanistic basis of a combination D-penicillamine and platinum drugs synergistically inhibits tumor growth in oxaliplatin-resistant human cervical cancer cells in vitro and in vivo. Biochem Pharmacol. 95(1):28-37, 2015. doi: 10.1016/j.bcp.2015.03.006.

Response:

The above reference has been added and the manuscript has been revised to now state

"Similarly, in oxaliplatin-resistant cell lines derived from human cervical carcinoma, D-penicillamine in combination with cisplatin and oxaliplatin overcomes resistance through increased CTR1 expression by up regulation of Sp1"

Minor comments

Minor points: Introduction, page 3, line 6: "Escherichia coli" must appear as italics Page 10, line 5, 11, 16; page 11 line 8: "Pt-based" Page 12 line 8: "Copper" Page 12 line 23: "penicillamine"

Response:

All the above valuable comments were addressed appropriately in the revised manuscript.

We again thank you for the opportunity to resubmit the manuscript. Please do not hesitate to contact us with any concerns

Sincerely,

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