Author's response to reviews

Title: Sunitinib-induced severe toxicities in a Japanese patient with the ABCG2 421 AA genotype

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Author's response to reviews: see over
Dear Dr. Solera,

We would like to resubmit our manuscript for publication in *BMC Cancer*. We have revised the manuscript in accordance with the reviewers’ comments; each of these changes is underlined and described below.

English of the revised manuscript has been edited by Edanz, a professional English editing company.

We hope our manuscript will be acceptable for publication in *BMC Cancer*.

I look forward to hearing from you at your earliest convenience.

Sincerely yours,

Yuji Miura M.D.
Thank you for your timely review and comments. We appreciate the opportunity to revise our manuscript. We believe your comments have enabled us to greatly improve the quality of our manuscript.

Response to Reviewer 1

Comment 1
Abstract should be rewritten.

Reply to comment 1:
Thank you for this comment. We are unclear as to whether the reviewer is suggesting that the abstract should be rewritten to improve the English, but we have now had the manuscript copyedited by Edanz, a professional language editing company.

Comment 2
Background part should be extended to include information’s about the investigated genes, their polymorphism and roles in sunitinib toxicities.

Reply to comment 2
According to the reviewer’s suggestion, we have added the following sentence to the Background section of the manuscript (page 7-8, lines 92-96):

“Previous studies suggested that several single-nucleotide polymorphisms (SNPs), including those in genes that are relevant to the pharmacokinetics of sunitinib such as CYP3A5 (cytochrome P450, family 3, subfamily A, polypeptide 5) and ABCG2 (ATP-binding cassette, sub-family G (WHITE), member 2), might be associated with its toxicities [4, 5].”

Comment 3
Authors should discuss role of ABCG2 polymorphism on mechanistic basis. It seems that the results and discussion section are compilation of facts rather than mechanism. It could be because authors are presenting a case study, discussing results in light of mechanistic aspect will strengthen the paper.
According to the reviewer’s suggestion, we have added information regarding the proposed effects of \textit{ABCG2 421C}\textgreater A on the pharmacokinetics of sunitinib (page 14, lines 203-207):

“The reduced protein levels of the AA genotype in \textit{ABCG2 421C}\textgreater A in the apical membranes of small intestinal enterocytes, hepatocytes, and renal proximal tubule epithelial cells might affect intestinal absorption and/or elimination after oral administration of sunitinib, leading to extremely high systemic exposure to sunitinib.”

Response to Reviewer 3

\textbf{Comment 1}

\textit{The authors should add the word “gene/s” when mentioning the gene name (example line 171-172: single-nucleotide polymorphisms (SNPs) in CYP3A5, ABCB1 and ABCG2 genes.)}

Reply to comment 1

We appreciate the helpful comments from the reviewer. We have italicized the gene symbols in the revised manuscript in accordance with HUGO nomenclature. This removes the need to insert the word ‘genes’ after each gene symbol.

\textbf{Comment 2}

\textit{The authors should give the complete gene name when they are mentioning it for the first time for the convenience of the readers.}

Reply to comment 2

Following the reviewer’s suggestion, the full name of each gene has been defined at the first mention of each gene symbol, as follows:
1. **CYP3A5** (cytochrome P450, family 3, subfamily A, polypeptide 5) (page 7, lines 94-95)
2. **ABCB1** (ATP-binding cassette, sub-family B (MDR/TAP), member 1) (page 11, lines 154-155)
3. **ABCG2** (ATP-binding cassette, sub-family G (WHITE), member 2) (page 5, lines 63-64 in the abstract and page 7-8, lines 95-96 in the text)

**Comment 3**

*Please mention the method by which the authors isolated DNA from formalin fixed paraffin embedded tissue samples. If they used a kit, please mention the source.*

Reply to comment 3

Following the reviewer’s suggestion, we have added details of the method of DNA extraction (page 11, lines 154-161):

“Genotyping of seven SNPs in **CYP3A5** (6986A>G), **ABCB1** (ATP-binding cassette, sub-family B (MDR/TAP), member 1; 1236C>T, 2677G>T/A, and 3435C>T), and **ABCG2** (34G>A, 376G>A, and 421C>A) that are potentially relevant to the pharmacokinetics of sunitinib [7-11] was performed using a genomic DNA sample extracted from formalin-fixed paraffin-embedded normal renal tissue blocks with a QIAamp DNA FFPE Tissue Kit (QIAGEN, Germany), because blood was not available for DNA extraction owing to this being a posthumous investigation.”

**Comment 4**

*Line 201- line 205: Please reframe the sentence. The result in Phase I dose-escalation……plasma concentration # 100ng/ml.*

Reply to comment 4

Thank you for this helpful comment. We have had the manuscript copyedited by a professional language editing company, Edanz, and have changed the sentence as follows (page 13, lines 186-190):
“A phase I dose-escalation study of sunitinib administered daily on a 4-weeks-on, 2-weeks-off schedule indicated that most patients with a dose-limiting toxicity (DLT), including thrombocytopenia, hypertension and asthenia, had a combined (sunitinib plus SU012662) trough plasma concentration of ≥100 ng/mL [12].”

Comment 5
Line 213-214: “that causes sunitinib higher exposure” does the authors mean high concentration of plasma levels of sunitinib? If yes please reframe the sentence.

Reply to comment 5
We appreciate this helpful comment from the reviewer. According to the reviewer’s suggestion, we reworded the sentence as follows (page 14, lines 198-199).

“.... that cause higher plasma concentrations of sunitinib.”

Replies to Reviewer 4

Comment 1
In the title author should provide the full gene name ABCG2 (ATP-binding cassette, sub-family G (WHITE), member 2)

Reply to comment 1
We appreciate the insightful comments from the reviewer. As suggested by the reviewer, the “Instructions for authors” of BMC Cancer indicates that abbreviations within the title should be avoided. However, to avoid redundancy and for the readers’ convenience, it is better to provide a well-known gene symbol, ABCG2, but not the full gene name in the title.

Comment 2
on page 5; para 2; line75 put a day after 12.
Reply to comment 2
Thank you for the helpful comment. In accordance with the reviewer’s suggestion, we have changed the relevant sentence as follows: (page 4, lines 54):

“Sunitinib treatment was discontinued on day 12;”

Comment 3
Page 6; conclusion section: please clarify how much fold differences in the ABCG2 frequency in non-Asians.

Reply to comment 3
We have revised the appropriate sentence of the Conclusion section of the Abstract, as follows (page 5, lines 69-70):

“The minor allele frequencies of ABCG2 421C>A are approximately three-fold higher in Asians than in Caucasians.”

Comment 4-1
Page 12, para 2; line 172 as this is the first time mentioning about the SNPs, it will be nice if they clarify the how many SNPs from each gene type.

Reply to comment 4-1
We thank the reviewer. In accordance with this comment, we have defined the full name of each gene in this paragraph. Moreover, we have amended the following sentence of the Case Presentation section (page 11–12, lines 154–161):

“Genotyping of seven SNPs in CYP3A5 (6986A>G), ABCB1 (ATP-binding cassette, sub-family B (MDR/TAP), member 1; 1236C>T, 2677G>T/A, and 3435C>T), and ABCG2 (34G>A, 376G>A, and 421C>A) that are potentially relevant to the pharmacokinetics of sunitinib [7-11] was performed using a genomic DNA sample extracted from formalin-fixed paraffin-embedded normal
renal tissue blocks with a QIAamp DNA FFPE Tissue Kit (QIAGEN, Germany), because blood was not available for DNA extraction owing to this being a posthumous investigation.”

**Comment 4-2**

*In the discussion It is not clear how the severe toxicities is linked to ABCG2 421 AA genotype*

Reply to comment 4-2

According to the reviewer’s suggestion, we have added details regarding the potential effects of ABCG2 421C>A on the pharmacokinetics of sunitinib (page 14, lines 203-207):

“The reduced protein levels of the AA genotype in ABCG2 421C>A in the apical membranes of small intestinal enterocytes, hepatocytes, and renal proximal tubule epithelial cells might affect intestinal absorption and/or elimination after oral administration of sunitinib, leading to extremely high systemic exposure to sunitinib.”

**Comment 4-3**

*Authors should also discuss about the other factors associated with The pathogenesis of sunitib and SU12662, in terms of SNPs.*

Reply to comment 4-3

Following the reviewer’s suggestion, we have added the following sentences to the revised manuscript (page 16, lines 228-232):

“In the present study, the genotypes of SNPs in CYP3A5 and ABCB1, which are potentially related to the pharmacokinetics of sunitinib [7-11], were heterozygous for the variant alleles, indicating that these SNPs might also be involved in the high systemic exposure of sunitinib to the patient.”

**Comment 4-4**
Is there any report showing no adverse drug reaction with non Asian treated with sunitib with this SNP.

Reply to comment 4-4
Previously, two research groups reported no significant association of ABCG2 421C>A with adverse drug reactions induced by sunitinib in Caucasian patients (van Erp NP et al. J Clin Oncol. 2009 Sep 10;27(26):4406-12; Garcia-Donas J et al. Lancet Oncol. 2011 Nov;12(12):1143-50). However, the low minor allele frequency of this SNP in Caucasians may cause a loss in power, resulting in these negative associations.