Author's response to reviews

Title: Prevalence of TPMT and ITPA gene polymorphisms and effect on mercaptopurine dosage in Chilean children with acute lymphoblastic leukemia

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

The authors hereby submit a revised version of the manuscript 1742622091919204 entitled “Prevalence of TPMT and ITPA gene polymorphisms and effect on mercaptopurine dosage in Chilean children with acute lymphoblastic leukemia”. In this revised version of the manuscript we have addressed all the concerns and suggestions made by the reviewers.

The response to the reviewers’ comments is detailed below. We are certain that the comments and suggestions have improved the manuscript.

Thank you for your efforts in reviewing this manuscript.

Sincerely yours,

Mauricio J. Farfán, PharmD, PhD
Assistant Professor
University of Chile
Reviewer #1 (Gauri Kapoor)

Comment 1. Overall language and grammar need significant editing in entire manuscript - (some are highlighted in the manuscript)

Response: The manuscript was extensively revised by Dr. Philip O. Anderson, Professor of Pharmacy from the UCSD Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California.

Comment 2. page 7 last few lines: As both groups had similar toxicities authors cannot conclude on the basis of their study that screening will reduce adverse reactions!

Response: We agree with the reviewer’s comment and we have eliminated this statement.
Reviewer #2 (Gabriele Stocco)

Major Compulsory Revisions
Comment#1. The results reported in the abstract do not seem to match those in the manuscript. Please revise.

Response: We agree with the reviewer’s comment and we have addressed this observation in the revised version of the manuscript.

Minor Essential Revisions

Abstract, 2 line: change "interindividual toxicity" with "interindividual variability in toxicity"  Done
Abstract, line 8: add "polymerase" before "chain reaction"  Done
Abstract, line 13: change "patient" with "patients"  Done

in the Abstract and manuscript, please change "accumulative" with "cumulative"

Introduction  Done

In the second sentence, please revise "cumulative toxic plasma concentration of 6MP": 6MP half-life is very short and the prodrug does not accumulate; the active metabolites (i.e. TGN) accumulate and are related to toxicity. The same concept has to be corrected later in the introduction (line with reference 8).  Done
Reviewer #3 (Benigna Oliveira)

Comment #1. From the manuscript it is not clear when the genotyping was done. Was the genotyping done prior to beginning of the treatment? If so, did the results of genotyping influence the treatment in any way?

Response: All the patients enrolled in this study were treated according to the ALL-IC-BFM2002 protocol, in which TPMT genotyping is not included. Hence, 6-MP treatment was not influenced by the TPMT genotyping in our study.

Comment #2. The authors commented: “... a situation which might be explained by the 6-MP dose adjustment, considering the amount of leukocytes and lymphocytes, as the clinical guidelines suggest.” It would be interesting that the authors clarified these parameters (the amount of leukocytes and lymphocytes) used to the 6-MP dose adjustment. Was the accounting of neutrophils not used?

Response: We agree with the reviewer’s comment. In our institution, 6-MP dose adjustment in ALL patients was done considering the absolute neutrophils count (ANC). We have modified this statement in the new version of the manuscript.

Comment #3. It seems that no formal sample size calculation was performed for this study. Did the authors investigate the total number of patients treated over a certain time period? Do the authors consider that the sample size is enough for conclusions? If they have taken the sample size as a limitation, this must be included in the discussion section.

Response: No formal sample size calculation was performed. However, every year there are approximately 120 new cases of ALL in children in Chile. In our study, TPTM genotyping was performed in children with ALL enrolled between June 2009 and March 2010 (10 months). Moreover, we found that allelic frequency of the most relevant TPMT polymorphisms was similar to that found in Chilean blood donors from a previous study. Considering the above, and the sample size of several publications in this field, we strongly believe that the sample size of this study is enough to support our conclusions.

Comment #4. Is there any likelihood that some children genotyping or TPMT activity measurement has been influenced by blood transfusions?

Response: In our institution blood for transfusions are filtered and irradiated, so the influence of blood transfusion in TPMT genotyping is very low. Erythrocyte TPMT activity in the selected patients was measured 30 or more days following the last erythrocyte transfusion. We have included this statement in the Material and Method section of the revised version of the manuscript.
Discretionary Revisions

Comment #5. The reference 14 is not the only study conducted in Brazil on this issue.

Response: We have include reference #15 (Silva et al., 2008) which correspond to another study conducted in Brazil related to this manuscript.