Author's response to reviews

Title: NOTCH1 mutations influence survival in chronic lymphocytic leukemia patients

Authors:

Kerstin Willander (kerstin.willander@liu.se)
Ravi K Dutta (ravdu401@student.liu.se)
Jonas Ungerbäck (jonas.ungerback@liu.se)
Mats Fredrikson (mats.fredrikson@liu.se)
Mats Linderholm (mats_linderholm@hotmail.com)
Peter Söderkvist (peter.soderkvist@liu.se)

Version: 3 Date: 18 March 2013

Author's response to reviews: see over
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Title: *NOTCH1* mutations influence survival in chronic lymphocytic leukemia patients

Authors:

Kerstin Willander ([kerstin.willander@liu.se](mailto:kerstin.willander@liu.se))
Ravi Kumar Dutta ([ravdu401@student.liu.se](mailto:ravdu401@student.liu.se))
Jonas Ungerböck ([jonas.ungerback@liu.se](mailto:jonas.ungerback@liu.se))
Rebeqa Gunnarsson ([Rebeqa.Gunnarsson@med.lu.se](mailto:Rebeqa.Gunnarsson@med.lu.se))
Gunnar Juliusson ([Gunnar.Juliusson@med.lu.se](mailto:Gunnar.Juliusson@med.lu.se))
Mats Fredrikson ([mats.fredrikson@liu.se](mailto:mats.fredrikson@liu.se))
Mats Linderholm ([mats_linderholm@hotmail.com](mailto:mats_linderholm@hotmail.com))
Peter Söderkvist ([peter.soderkvist@liu.se](mailto:peter.soderkvist@liu.se))
Linköping, March 18, 2013

To the Editor,

Object: MS: 9087256848764120

Thank You for consideration of our manuscript for publication in your journal. We are also thankful for the valuable comments from the reviewers.

We have carefully revised our manuscript according to your reviewers´ comments (below). The gene name in the title has been adjusted to the guidelines of gene nomenclature (Hester M et al., Guidelines for human gene nomenclature, Genomics, 79(4):464-470, 2002., www.genenames.org), and now reads “NOTCH1 mutations influence survival in chronic lymphocytic leukemia patients”.

In response to one of the reviewers question, we got access to experimental data on trisomy 12 on a subset of the CLL patients, and therefore also included the responsible scientists in the author list of the manuscript; Prof. Gunnar Juliusson and Dr. Rebeqa Gunnarsson.

We hope that we have complied the suggested corrections by the reviewers satisfactorily and feel that the manuscript has improved substantially and now will be acceptable for publication in BMC Cancer.

Reviewer’s report

Version: 2 Date: 14 February 2013

Reviewer: Gianluca Gaidano

Reviewer’s report:

Willander et al explore the prognostic significance of NOTCH1 mutations in a relatively large cohort of CLL. NOTCH2 mutations were also investigated in this series. The manuscript is well organized and methodologically sound. I have only minor essential revisions, as listed below.

MINOR ESSENTIAL REVISIONS

1. Did the authors assess whether NOTCH1 mutations predicted Richter syndrome in this specific series? Relevant literature on this topic should be cited (eg: Rossi et al, Br J Haematol 2012)
• Clinical information about Richter syndrome has not been reported in our cohort, and consequently, we cannot assess this interesting question. This has now clearly been stated on page 8, line 9-12.

• In addition, we have completed the reference list with Rossi et al, Br J Haematol 2012, reference #30

2. The authors should assess the relationship between NOTCH1 mutations and +12 in their CLL series

• In a separate analysis, a subset of the samples (n=152) were analyzed for trisomy 12 by means of a BAC microarray. Trisomy 12 was found in 15/152 (10%), but only two of these had mutation in the NOTCH1 gene and this association is non-significant (p=0.56). However, it seems to be an interesting relationship that may be significant in a larger population of patients or in a meta-analysis of CLL cohorts. These results have been added in the Results and Discussion section page 7, line 13-16.

3. The authors fail to quote one of the two seminal papers of NOTCh2 mutations in lymphoid malignancies. The two reports were published back-to-back in the same issue of J Exp Med and should both be cited.

• We apologize for the unintended exclusion of one of these seminal papers, which now have been corrected and added as ref # 23, Rossi et al, J Exp Med 2012.

4. Spelling mistakes should be revised (eg trisomi)
• Done

Reviewer's report

Title: Notch1 mutations influence survival in B-cell chronic lymphocytic leukemia Patients

Version: 2 Date: 15 February 2013

Reviewer: valter VG gattei

Reviewer's report:

The manuscript characterizes the NOTCH1, NOTCH2 and mutational status in a cohort of 209 CLL previously characterized for TP53 and IGHV mutational status. Authors define a short overall survival for NOTCH1 mutated CLL when compared with NOTCH1 wild type CLL. An association with IGHV mutational status and a more aggressive disease is also found. Finally, Authors define significant shorter survivals for CLL cases carrying either TP53 or NOTCH1 mutations when compared with cases wild type for both the two genes.
Major points:

1) The main problem regarding this study is the lack of FISH analyses that represent the gold standard in the prognostic stratification of CLL patients.

- We agree that it had been valuable to have FISH analyses, but FISH analyses was not performed in the clinical routine at the time for collection and we do not have the possibility to examine this retrospectively, since no viable cells are available.

2) The study lacks of multivariate regression models with NOTCH1 mutations and the other clinical and biological features of CLL.

- Since, all clinical data (Binet stage, treatment, IGHV status) are not available for all patients, multiple regression analysis will reduce the number of patients in respective category, and an estimate of HR therefore becomes unstable. Sex and age are the only parameters that do not reduce the number of patients dramatically and the multiple regression analysis for sex and age has been added to table 3. Including additional parameters seems not meaningful due to the wide variation and instability of estimates.

Minor points:

1) Significant associations between clinical and biological features and presence of NOTCH1 mutations in the 209 CLL cases should be added in Table 2. Relative sentences should be added in text, in particular for IGHV status and Binet staging.

- P values are added to Table 2.
- We have added information in the manuscript how IGHV status and Binet stage relate to NOTCH1 mutations. IGHV: Page 7, line 24-25, and page 8, line 1. Binet: Page 8, line 7-9.

2) The background section is not focused and sentences regarding genes that are not investigated in this study should be deleted or, alternatively, heavily reduced.

- We believe that it is valuable to mention other genes than the investigated, which frequently are mutated in CLL. However, we have according to the reviewer’s suggestion reduced information on how alterations of these genes contribute to CLL and this section is now only one sentence, page 3, line 4-7.