Author's response to reviews

Title: Notch1 mutations influence survival in B-cell 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)

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Author's response to reviews: see over
Dear Editor,

Enclosed, please find the requested changes of the manuscript entitled “Notch1 mutations influence survival in B-cell chronic lymphocytic leukemia patients” for consideration in the methods section.

Hope the ms now meets the format criteria for the review process.

(1) Methods

Page 5, line 1-4: In this study, peripheral blood from 209 CLL patients (145 men and 64 women) was collected between 1996 and 2006 at Department of Hematology, Linköping University Hospital. Mononuclear cells were isolated by Ficoll-Paque gradient centrifugation and genomic DNA was extracted by proteinase K digestion and stored frozen until used as earlier described [17].

Page 6, line 3-12: TP53 and IGHV gene status detection

TP53 gene mutation analysis was performed for exons 5-8 (the DNA binding domains) by the PCR-single strand conformation analysis (SSCA) technique and samples displaying mobility shifts were sequenced with the dideoxy termination method to confirm the nucleotide changes.

The IGHV gene mutational status was performed by PCR amplification on genomic DNA by using specific VH/JH primers [20], followed by DNA sequencing of both forward and reverse strands. To determine the IGHV gene identity the sequences were aligned by using the IMGT/V-QUEST database (http://imgt.org), ≥ 98% identity to the corresponding germline sequence was considered as an unmutated IGHV gene.

(2) and (3) Ethics and consent statement

Page 5, line 12-14: Informed consent was obtained from the patients and the study was approved by the regional ethical committee (Dnr 02-459) in Linköping and conducted in accordance with the ethical guidelines of the Helsinki Declaration.
Sincerely,

Kerstin Willander

Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden.