Reviewer’s report

Title: Complex NOTCH1 mutations as prognostic impact factors in paediatric T-cell leukaemia.

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Reviewer: Heike Pfeifer

Reviewer’s report:

The current study analyzes the type and frequency of NOTCH1 mutations and their potential prognostic impact in a cohort of 138 paediatric T-ALL patients. The authors furthermore hypothesized, that the analysis of NOTCH1 in concert with genes with functional parallels with the NOTCH1 pathway would provide more information regarding prognosis than analysis of NOTCH1 alone.

The Notch1 signaling pathway has been shown to be an essential factor in normal and pathologic T-lymphoid development. Although, activating NOTCH1 mutations were reported in roughly 50% of childhood T-ALLs, the incidence and prognostic impact remains unclear. The authors examine the prognostic role of NOTCH1 including the significance of each type of NOTCH mutation and their impact on disease recurrence. To include in this analysis relevant pathway members like TLX3, PTEN is reasonable. In addition, it is useful to investigate this together with additional markers like KRAS, SIL-TAL.

The main finding is that complex mutations and the presence of SIL-TAL have an influence on prognosis.

This is a retrospective analysis of a sizable patient cohort uniformly treated within two prospective clinical trials.

Subjects, demographic and clinical data are described in detail. For analysis of NOTCH1 mutations, exons 26, exon 27 and exon 34 were amplified by PCR as a standard practice for NOTCH1 mutation analysis. Similarly, KRAS exon 1 mutations, PTEN mutations, the presence of SIL-TAL fusion transcripts and the presence of TLX3 were performed according to standard techniques and cover the generally accepted gene sequences.

The paper attempts to extend previous studies on the prognostic impact of NOTCH1 mutations in a similar number of children with T-ALL. Novel findings include the favorable prognostic impact of insertions and deletions (named "complex mutations" by the authors) of NOTCH1 as opposed to NOTCH1 point mutations. In contrast to previously published results, the presence of SIL-TAL fusion transcript was associated with an inferior prognosis.

Minor Comments:

- The genomic DNA extraction methods are included within the paragraph NOTCH1 mutations. It would be better to describe this in a separate paragraph. Apparently 3 different extraction methods were used, but it is not apparent which
and how many samples were prepared by this different extraction methods.

- Was a FICOLL separation performed in advance, what was the percentage of blasts within the samples considered adequate for analysis.

- The definition of the type “complex mutation” is confusing and should be replaced by a more precise definition.

Major Comments:

- The authors state, that they want to perform a NOTCH pathway analysis. If they do not replace this term, it would be necessary to have data on the FBXW7 status. FBXW7 is mutated in approximately 20% of patients with mutated NOTCH.

- The authors wanted to demonstrate the significance of each type of NOTCH mutation regarding disease recurrence (p.3). If the authors really want to answer this question, an analysis of initial response, the disease-free survival, event-free survival and remission duration in relation to the molecular analysis should be provided.

- The discussion and conclusion is somewhat superficial, e.g. there should be a paragraph in which the authors compare their results with the literature added for the SIL-TAL results. The authors should more clearly differentiate between their own data and the literature.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests