Reviewer's report

Title: FIP1L1-PDGFRA molecular analysis in the differential diagnosis of eosinophilia

Version: 1 Date: 3 October 2008

Reviewer: Christoph Walz

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Major Compulsory Revisions:

1. Background: It is clear by now that the prevalence of the FIP1L1-PDGFRA fusion gene in HES is much lower than 60% and is currently estimated at 10-15%. E.g., in a series of 81 patients with primary eosinophilia, the FIP1L1-PDGFRA fusion was detected in 11 patients (14%) (Pardanani et al., Blood 2004). The sentence should be changed accordingly.

2. The mutation within c-Kit in patients with systemic mastocytosis is D816V and not D618V as stated throughout the text.

3. The abstract claims that 14 patients were investigated. However, the text describes 15 patients. What number is correct?

4. Patient data: For how long was the eosinophilia present in the 15 patients? For longer than 6 months?

5. The complete text has to be carefully checked and re-phrased by a native speaker. Also, the overall structure is confusing to me. What is the focus of this study, what are the exact objectives? Is it more a clinical study of patients with HES, a prevalence study or a genetic study characterizing the FIP1L1-PDGFRA fusion gene? Based on the low number of patients examined (14?, 15?), I think it is not valid to make claims about the FIP1L1-PDGFRA prevalence as done in the discussion.

6. Was the mutational analysis of c-Kit carried out using PB or BM material? The use of PB and conventional sequencing is not optimal for the detection of D816V.

7. The Methods/Patients section contains a whole paragraph with results that should be moved/deleted.

Minor Essential Revisions:

1. Chusid et al. should be cited in the background section since they postulated the three criteria for the HES.

2. FIP1L1 is alternative spliced extensively. Therefore, when using FIP1L1 exon numbering (as exon 8 or 8a), it should be listed which FIP1L1 transcript variant was used (e.g., www.ensembl.org).

3. The references should be checked for errors (e.g., ref #5) and uniformity.

4. Either use “15” or “fifteen”.

5. All gene names should be upper case and in italics, also BCR-ABL.

6. What was the median time of response to imatinib (7-50 months)?

6. The expression “…FIP1L1-PDGFRA fusion tyrosine kinase signal” in the results section is misleading and should be rephrased.

7. The importance of the disruption of the juxtamembrane domain was investigated by Stover et al. (PNAS, 2006). This work should be cited in the discussion.

8. Vitamin B12 serum levels are often elevated in FIP1L1-PDGFRA positive patients. Have the patients from the manuscript been tested for this marker? If not, it should be mentioned in the text.

Discretionary Revisions

1. It could be mentioned that nested RT-PCR can be false negative in the detection of FIP1L1-PDGFRA and that the use of FISH as a complementary technique is possible and recommended.

2. The FIP1L1-PDGFRA positive patients that were treated with imatinib became negative for the fusion gene by nested-RT PCR. It would be interesting to know what the sensitivity of this PCR assay was.

**Level of interest:** An article of limited interest

**Quality of written English:** Not suitable for publication unless extensively edited

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