Author's response to reviews

Title: Determination of caspase-3 activation fails to predict chemosensitivity in primary acute myeloid leukemia blasts.

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Version: 2 Date: 18 April 2005

Author's response to reviews: see over
Submission of a revised research article:
MS 6353863862186218: “Determination of caspase-3 activation fails to predict chemosensitivity in primary acute myeloid leukemia blasts”

Dear Professor Newark

Thank you very much for giving us the opportunity to modify the manuscript according to the reviewers’ comments. In the following I will systematically answer to the reviewers’ comments and list the pages and lines of the corresponding changes I have made:

Reviewer 1:

Major Revision Points: none

Minor Points:

1. Table 1: For all figures given in percentage, the absolute figures were added in table 1.
2. Table 2: The comment is absolutely correct. Therefore, all maximum Km values of the range in brackets are changed to the suggested maximum tested drug concentration.
3. Figure 1: The figure was adjusted as suggested.
4. Figure 2: No, we did not perform time experiments on patient samples. (see also answer to reviewer 2, major revisions, point 2).
Reviewer 2:

General / Major Revision Points:

1. “major disadvantage of this study is that clinical outcome was not included. 
   …. – include clinical outcome data.”

   In general, since this paper describes a methodological study on the comparison between two in-vitro assays, we did not include clinical outcome data for several reasons:
   
   a. the patient number of n=42 is quite low, so it may be difficult to receive reliable and valid results of a clinical correlation.
   
   b. More importantly, in a large series of AML patients (n=162), we have recently shown an overall predictive accuracy for the DiSC-assay of 98.2% concerning treatment response and proofed the ex-vivo chemosensitivity evaluated by the DiSC-assay as one of the strongest prognostic factors [Staib et al., Br J Haematol 2005, 128:783-791]. This underlines that the DiSC-assay may serve as a valid reference method, which does not need to be validated by clinical correlation again.
   
   c. Since there was not an “almost significant” correlation between the assays, we did not expect a dramatic improvement of a non-significant correlation by consideration of clinical outcome data.

   Changes to the manuscript:
   The reference mentioned above was included into the reference list (No. 34) and, also, integrated in the discussion: page 12, lines 5-8. Accordingly, the comment on CLL was dropped: page 12, lines 15-16.

2. “include experiments on patient samples… concerning stability of Km-values”

   We did not perform time experiments on patient samples, but on three different cell lines. The remarkable points for the Km-values at 6-8 hours (figure 2) demonstrate the rapid onset of the process of apoptosis whose velocity stabilized within 8-14 hours in all experiments. Thus, for patient samples we chose an incubation time of 16 hours which we thought is safe enough. Furthermore, Km-values could be calculated in 92% of the Casp3-tests done on patient material, and the median Km-values were comparable with median LC90-values of the DiSC-assay implicating an adequate incubation period.

   Changes to the manuscript:
   
   a. This issue was addressed in the discussion, page 13, lines 6-11 added.
   
   b. The incubation period of the Casp3-test was mistakenly stated as 18 hours and corrected to 16 hours: page 6, line 23; page 9, line 16.
   
   c. Page 9, line 8: the figure 16 was corrected to 14.
3. “correlation coefficients”
   Changes to the manuscript:
   These were included on page 10, lines 24-25.

4. “even” on page 13 seems disputable..
   The word “even” was dropped according to the reviewer’s suggestion.
   Changes to the manuscript: page 13, line 13.

Minor Points:

1. “statement that the 4-5 days that the DiSC assay takes is too long…”
   The authors accept this objection and adapted the manuscript according to the suggestions of the reviewer.
   Changes to the manuscript: page 3, lines 21 to 25.

2. “lowest concentration of ara-C is not low enough .... the authors may wish to increase that”
   The authors thank for the useful recommendation.
   No changes to the manuscript.

3. “… correlating LC50 values with Km-values”
   Yes, we tried to correlate the Km-values with LC50 values, but for the LC90 values there seemed to be better correlation between the absolute figures.

Discretionary Revisions:

1. “… remarkable that the authors report >10% assay failure…”
   The authors thank for the useful suggestions and will check for adding additional antibiotics to the culture mediums.

2. “… remarkable points for the Km-values at 6-8 hours in figure 2”
   This issue was addressed under major revisions, point 2 (see above).

I hope that I could sufficiently address all points of the reviewers. I am looking forward to hearing from you.

Yours sincerely,

Dr. Peter Staib