**Author's response to reviews**

**Title:** Early prediction of therapy response in patients with acute myeloid leukaemia by nucleosomal DNA fragments

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**Author's response to reviews:** see over
Manuscript “Early prediction of therapy response in patient with acute myeloid leukemia by nucleosomal DNA fragments”

Dear editors,

thank you very much for reviewing our manuscript again. We have revised the manuscript completely and integrated all requested points of the reviewers – as far as possible. We have marked all changes by red letters in the manuscript and will comment on them point by point:

**Reviewer 1 Lucia Altucci:**

**Major Compulsory Revisions**

The authors should justify what they cannot include (increase the patient's number and blasts studies) and include all data they instead can provide as they say in response to this reviewer and to the other. Indeed, conceptually, a decision has to be taken after reading the manuscript in its full contents, never before.

1. Though the compliance of the AML patients, who received first-line chemotherapy in our hospital, to participate at this observational intensive follow up-study was very good, it took us 18 months to collect the patient samples. We know that the results presented here are preliminary and a higher number of patients would be desirable. However, because clear (and statistically significant) trends were already seen in this limited patient setting, we decided to submit these data for publication. Based on these results, we continue our AML study and hope to present more extended data in the next years.

This reviewer was asking to get in the manuscript some 'more molecular' aspects. This was referred to the possibility to correlate the value in early prediction of nucleosomal DNA fragments with molecular parameters of the effects of the therapy.

2. The data on cytogenetics and immunophenotype are added in table 1 (page 22). Due to the heterogeneity of the data, a correlation with therapy response or marker levels was not performed.
The authors state that it is difficult to get blasts sample; they may nevertheless see if it is possible to include some experiments with blasts cells even if taken from blood. Otherwise they may include just the data that they can.

3. Unfortunately, we had only the cell-free serum and plasma samples of the patients, therefore it was not possible to perform culture experiments with blasts. However, we added the information on the pretherapeutic blast number in the bone marrow and the blast number at day 16 after start of chemotherapy which is known to be prognostically relevant. We correlated the blast numbers at both time points as well as the kinetics of blast numbers with therapy response and nucleosomal DNA levels. Though blast numbers were not associated with therapy response, there was a clear correlation between pretherapeutic nucleosomal DNA levels with bone marrow blast number after 16 days and with the relative reduction of blast number from day 1 to 16, and also between the area under the curve of days 2-4 (AUC 2-4) of circulating nucleosomal DNA reflecting the immediate effect of therapy with the reduction of blast number from day 1 to 16 (page 10; lines 5-15). These results strengthen our hypothesis, that one essential reason for the elevated release of circulating nucleosomal DNA into blood is the effective blast reduction in AML patients (page 16; lines 2-8). As the data of blast numbers was provided by Dr. Braess, we added him to the author’s list.

Minor Essential Revisions:
It will be good to get an improvement in English.

4. The manuscript was completely revised by a native speaker experienced in revising scientific papers in the biomedical field. All changes made by this person are marked in blue.

Reviewer 2 Nejat Dalay:

Major Compulsory Revisions
The initial serum DNA values in the patients with remission is considerably higher than those without response. This leads to the differences between the “areas under the curves”. Why are higher levels of nucleosomal fragments present in the patients who are going to respond before therapy? This issue has not been addressed at all and needs further discussion.

5. It is true that already the pretherapeutic level of nucleosomal DNA was higher in patients with good response than in those with poor response, however, this difference was not statistically significant (table 2). The area under the curve of days 2-4 after start of chemotherapy (AUC 2-4) of nucleosomal DNA includes only the values at days 2, 3, and 4 but not the pretherapeutic value. As the pretherapeutic concentration of nucleosomal DNA (as a result of spontaneously occurring apoptosis) might potentially be associated with the levels during therapy, the level of the area under the curve might also be influenced indirectly by the spontaneous release of DNA. However, as massive apoptosis was induced by therapy (see also the rapid blast reduction) and the AUC is restricted only on days 2-4, we think that the difference between both groups is more likely to be related to the therapy effect.

As I have indicated earlier the blast numbers and other relevant data should be given. Then the assumption “that the higher circulating DNA levels are the result of effective blast reduction” would make sense.

6. We are grateful for your suggestion and have added the requested data (see points 2 and 3).

The response to the reviewer’s comments (Point 10) should be added to the discussion as possible explanations to account for the discrepancy between solid tumors and AML.
7. We have added this discussion point at page 13, lines 5-17.

In Figure 2 a value of 150 (AUC) has been indicated. How did the authors find this value?

8. With respect to the low patient number, we know that this cutoff level might be preliminary. For defining the cutoff, we took the 100% specificity for having no response (all patients with no response should be below the cutoff). Then we calculated the sensitivity for detecting good response. For receiving the same numbers of sensitivity at 100% specificity, the cutoff range could be between 133 and 152 ng/mL.*d.

In view of the small number of patients the appropriate methods and results should also be evaluated by an expert statistician.

9. All calculations in this work were done by a professional statistician who has been working in the field of biomedical statistics for more than 20 years.

The new version of figure 1 is now better and more visible. However, the legends to the figures are not indicated. Only the first two lines in the legend for Figure 1 refer to Figure 1. The rest is the legend for the (new) Figure 2. The authors have added some requested information into the results section (Page 8, lines 7-9). Then the last two lines at the end the page can be omitted. I could not locate the p-values (p=0.259)(page 9, line 2) and (p=0.059)(page 9, line 23) in Table 2.

10. We apologize for his formatting errors and have corrected them accordingly.

With exception of the increase of the patient number and the culture experiments we have performed all changes requested. We would be very pleased if our manuscript is considered for publication in BMC Cancer.

Yours sincerely,

Petra Stieber, MD