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 and the valuable remarks. We agree with most of the points of the reviewers. At the requested passages, we have revised the manuscript accordingly and/or will respond to the comments point by point:

**Reviewer 1 Lucia Altucci:**

**Major Compulsory Revisions:**

The authors may increase the patients number to improve quality of the data and have more statistical relevance. This might be necessary in view of the fact that for solid tumours the published data report a decrease of DNA nucleosomal fragments in responders as pointed out from the authors. An increased number of data would strengthen the value of the manuscript.

1. We agree with the reviewer that a higher patient number would increase the statistical relevance of the data. However, we have to admit that it has already been a big challenge to include these 25 patients with AML in our study as this pathology is far less frequent than solid tumors and as we aimed mainly at patients with newly diagnosed AML who were treated in our institution and who were willing to participate in the additional intensive blood follow-up investigations. As we already have conducted this study for 18 months to collect the presented patient samples it would at least take further 18-24 months to increase the patient number substantially. Because clear (and statistically significant) trends were already seen in this limited patient number, we decided to publish these data – even if they can not provide definite conclusions – and continue with the study to collect more data.

Although this objection is very reasonable and correct, we can not improve the data within a short time frame. Thus, it would be helpful for us to know, whether the manuscript is generally acceptable with the data presented here. All other comments and changes can be done in short time.
Moreover data on the karyotype and immunophenotype of the included patients should be added to table 1.

2. We agree that karyotype and immunophenotype is valuable information for the prognostic stratification of the patients. We thought that – due to the variability of these parameters and because of the limited number of patients – it would not improve the paper very much if we correlated marker levels with these entities. However we can add this information quickly.

The authors should add some data obtained by putting in culture the patient samples thereby showing that blasts of the responders to therapy have an increased apoptotic rate. This would also help also in the understanding the differential response among blasts and normal cells which is a major problem given that normal cells may contaminate the biological value of the increased free nucleosomal DNA.

3. Unfortunately we have only serum and plasma samples of the patients, so we can not perform culture experiments with blasts. However, it can be speculated that also normal cells die during chemotherapy and contribute to the release of nucleosomes. Similar effects were shown in a recent in vitro study of normal and malignant lung cancer cells which were irradiated with various doses (Holdenrieder et al, Tumor Biol 2004;25:321-6). Particularly at low radiation doses, malignant cells were more susceptible to undergo cell death, however also normal cells were killed to some extent. Despite this probable “contamination” of blast and normal cell deaths, it remains interesting, that the nucleosome levels differed significantly between the response and no-response groups. As nucleosome concentration is not only affected by the extent of release but also by the removal capacity and velocity, this second point might also play a considerable role in the differing kinetics.

Indeed, the data reported in Figure 1 seem weak and the increase of free nucleosomal DNA is not really major.

4. We have replaced that figure (Fig 1) by another one showing the values of all patients of the responsive and the non-responsive group as well as the medians for all days. We hope that the differences are more visible.

Further we joined a figure (Fig 2) showing the area under the curve of day 2 – 4 (AUC 2-4) of nucleosomes for the various response groups. This parameter integrates the information given at days 2, 3 and 4 and shows a sensitivity of 56% for therapy response at a specificity of 100%.

The addition of more molecular data would certainly increase the manuscript level.

5. Which kind of molecular data would you request?

Minor Essential Revisions:
English should be revised and orthography verified.

6. If the paper is generally acceptable with the data presented we will have it revised by a native speaker.

Reviewer 1 Nejat Dalay:

Major Compulsory Revisions:
The study comprises 25 patients including two with relapsing disease. The main result of the study is relies on the observation that significantly higher levels of nucleosomal DNA are measured in patients in whom complete response is achieved. Thus, the sample size is limited. I would have preferred to observe the same data in a considerably larger group of AML patients.
7. Please, see point 1 of reviewer 1.

Furthermore, from the data given in Table 2 we can see that the nucleosomal DNA levels are higher in responders. Statistical significance between groups is achieved only for the levels on days 2 and 4. This difference is mainly due to the higher upper range in both cases. However, it is not clear in how many patients higher levels are detected. e.g. whether this is the result of one or two patients displaying very high levels or whether significant increases were detected in most of the patients.

8. In order to avoid random significances at specific days, we performed an overall analysis of variance to investigate the effect of therapy response on marker levels. In this test, all marker values were included to show, whether a marker can distinguish between the various response groups. Only if this test was significant (this was only the case for nucleosomes; p=0.017), the various days were evaluated separately by the non-parametric Wilcoxon test to see which days were the most meaningful ones. Finally, the information of the days 2-4, which turned out to show the most notable differences between the response groups, was integrated in the area under the curve of days 2-4. By proceeding in this way, we avoided to overinterpret very high values in single patients. Further the general effect of days during therapy on marker levels and the general effect of interaction was tested.

In Figure 1 the courses for two representative patients are depicted. It should be indicated in how many patients exactly the same type of course or deviations from it are obtained.

9. Figure 1 was replaced by a more informative one showing the values of all responsive and non-responsive patients (including medians) for all days. Concerning kinetics, 9 of 18 patients with complete remission showed the characteristic course of an initial increase at day 2 followed by a decrease, but only 1 of 7 patients with no remission. All other patients decreased immediately (described on page 8).

Higher levels of nucleosomes in responders and immediate decrease in non-responders are in contrast to several earlier studies which have shown higher and persisting levels in non-responders. The authors explain this by different pathophysiological backgrounds of the tumors which is not convincing and requires further discussion.

10. We are aware of this discrepancy between this study and investigations on solid tumors, but we have no definite explanations.

Nevertheless we think that it is quite reasonable that chemotherapy in AML patients is effective on susceptible blood cells and the effective removal of blasts leads to the initial increase of cell death products like nucleosomes followed by considerable decreases. In contrast, non-effective therapy obviously results in lower cell death rates and less release of products.

The main difference to solid tumors might be due to the fact that these tumor entities are cured by surgery in early stages. Chemo- and radiotherapy is only applied in late, inoperable stages. Then, various effects have to be considered in addition to therapy response such as the aggressiveness of the tumor (corresponding with the spontaneous cellular turnover rate), the access to the blood circulation (better in the metastasized situation) and probably a less functionable immune system which plays an essential role in the removal of circulating nucleosomes. These factors might be responsible for the higher values in non-responsive patients with advanced solid tumors.

The blast numbers are an important factor in AML and are correlated with the disease outcome. Blast counts in the patients are not given. Association between the initial blast counts, response and nucleosomal levels should be indicated.
11. If the paper is generally acceptable with the patient data presented we will do the requested changes/calculation.

Minor Essential Revisions:

Table 1 does not provide useful information. The histology and mode of therapy are not evaluated as variables in the study and can be easily omitted. Alternatively, more relevant information (blast nr, etc) should be added. The number of patients under “Mode of therapy” adds up to 24.

12. The data can be added quickly.

Quality of written English: Acceptable

With exception of the increase of the patient number and the culture experiments we have performed or are able to perform all requested changes quickly. However, before doing so, it would be very helpful, if you could indicate, whether the manuscript is generally acceptable with the data presented, then. Thank you very much.

We would be very pleased if our manuscript is considered for publication in BMC Cancer.

Yours sincerely,

Petra Stieber, MD