Reviewer's report

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

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Reviewer: Gertjan JL Kaspers

Reviewer's report:

General
The concise manuscript describes the results of an important preclinical study, searching for the optimal in vitro assay to predict chemosensitivity in vivo. Since the DiSC-assay has major disadvantages, the authors studied the usefulness of another assay on caspase-3 activation. In AML, the predictive value of the DiSC assay regarding clinical outcome has not been extremely well established. Therefore, a major disadvantage of this study is that clinical outcome was not included. The current conclusion is that caspase-3 activation is not a good surrogate marker of chemosensitivity, but it can not be excluded that caspase-3 activation correlates better with clinical outcome than the DiSC-assay results.

An advantage of the DiSC assay is that it measures maximum total cell-kill, as established on cell lines and patient samples. The caspase-3 activation assay conditions were examined on proliferating cell lines, and it is possible that stable Km values are not reached after 18 hours in patient samples.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
- include clinical outcome data, preferably both achievement of CR and relapses, as well as overall outcome; and correlate this with the in vitro assays
- include experiments on patient samples for the validation of the caspase-3 activation assay, especially concerning the stability of Km values
- data on page 10 concerning comparison between both assays should also show correlation coefficients, not only p-values
- in the conclusion on page 13, the word "even" seems disputable, and certainly is no conclusion of this paper

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
- the statement that the 4-5 days that the DiSC assay takes is too long is highly debatable. Clinicians would start with an ara-C/anthracycline regimen anyway, and even a few days postponement of treatment would be possible in many patients
- the lowest concentration of ara-C in the caspase-3 activation assay is not low enough, and in the future the authors may wish to increase that
- did the authors try correlating LC50 values with Km-values

Discretionary Revisions (which the author can choose to ignore)
- It is remarkable that the authors report >10% assay failure rate because of bacterial contamination. In thousands of samples now being tested in our laboratory at the VU university medical center in Amsterdam, this certainly is less frequent. Perhaps the authors should refer to several of our papers to see if our culture medium contains additional antibiotics.
- is there any explanation for the remarkable points for the Km-values at 6-8 hours in figure 2?
What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
'I declare that I have no competing interests'