Reviewer's report

Title: Analysis of and prognostic information from disseminated tumour cells in bone marrow in primary breast cancer. Report of a prospective observational study

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Reviewer: Sabine Kasimir-Bauer

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The paper by Falck et al., summarizes the results of a prospective observational study on DTCs in the bone marrow of primary breast cancer patients who presented with first diagnosis between 1999-2003. It was the purpose of the study to evaluate the presence and the prognostic value of DTCs in these patients. The results were achieved using two different methods.

Although the study is designed correctly and the experimental work has been exposed concisely, some considerations have to be made:

1. The authors should only focus on those patients who finally were included in the study (n=401 patients). In this regard, the flow chart can be deleted.

2. Conclusions drawn, e.g. Abstract: It has to be considered that the BM aspirations were performed several years ago with no standard procedure clearly defined. Nowadays, standards are clearly defined and it is not correct to conclude that “it is too early to implement bone marrow...as a routine clinical method”. To date, BM aspirations during surgery of the primary tumor are part of clinical routine in many University Hospitals, especially in Germany. In case of DTC-positivity, the therapeutic strategy is the intake of bisphosphonates which have been shown to be successful in elimination of these cells. The effect of zoledronic acid or clodronate on DTCs recently has been studied (see Rack et al., Anticancer Res., 2010; Hoffmann et al., Anticancer Res. 2011).

3. In the Introduction, the authors state that BM aspiration is associated with pain and discomfort. In the context of this paper and other studies this is not quite true since all the aspirations had been performed during surgery of the primary tumor under general anesthesia. I agree, for follow-up studies, this procedure is less accepted by patients.

4. The methods: A standard for DTC detection clearly has been defined. Staining for CK+ cells is performed using the murine monoclonal antibody Mab A45-B/B3 (Micromet, Germany), directed against a common epitope of CK polypeptides including the CK heterodimers 8/18 and 8/19. Briefly, the method includes a) permeabilization of the cells with a detergent, b) fixation with a formaldehyde based solution, c) binding of the conjugate Mab A45-B/B3-alkaline phosphatase to cytoskeletal CKs and d) formation of an insoluble red reaction product at the site of binding of the specific conjugate using an APAAP detection. A control antibody (conjugate of Fab-fragment) serves as negative control. Evaluation of
CK+ cells is carried out according to the ISHAGE evaluation criteria and the DTC consensus. The applied antibody A45-B/B3 for the detection of CK-positive cells is directed against a common epitope of CK polypeptides and, in many studies, has been complexed with alkaline phosphatase anti-alkaline phosphatase (APAAP) molecules so that the missing Fc part prevents unspecific binding of the antibody to Fc receptors on mononuclear cells. A BM analysis of 165 non-carcinoma control patients resulted in only 2 false positive results indicating that the A45B/B-3 gives reliable results for the detection of single disseminated tumor cells. This has been published in 1998 by Braun et al. All these criteria have to be included in a revised version.

5. In my opinion, a positivity rate of 25% in normal donors is much too high and might be the main reason for obtaining different results as compared to already published studies.

6. The images for DTCs can be deleted, they are well known to everybody in the field.

7. In the discussion, the clear focus should be discussing the differences of the methods used between 1999-2003 and the methods used nowadays.

8. The chapter about CTCs should be excluded. This is a separate, very complex topic which cannot be enrolled in two sentences citing one method.

In general, the paper should emphasize that the study had been performed 10 years ago with no standard method available. The most critical point is the high false-positivity rate which has to be discussed concisely, especially with regard to methods used during the time period the study had been performed. Furthermore, the authors should then address the improvement of methods used for detection and discuss the current literature, also with regard to therapeutic consequences.

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

**Quality of written English:** Needs some language corrections before being published

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

**Declaration of competing interests:**

I have no competing interests.