Reviewer's report

Title: Circulating endothelial cells and other angiogenesis factors in pancreatic carcinoma patients receiving gemcitabine chemotherapy

Version: 2 Date: 6 April 2012

Reviewer: Christine Brostjan

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Review of revised manuscript

“Circulating endothelial cells and other angiogenesis factors in pancreatic carcinoma patients receiving gemcitabine chemotherapy” by Shunsuke Kondo et al., Feb. 2012
Reviewer: Christine Brostjan, Medical University of Vienna, Austria

With respect to the previously listed points of concern the authors have sufficiently responded to and conducted the majority of suggested revisions with the exception of:

Major Compulsory Revisions

The fact that a correlation between blood parameters is no proof for a causal relationship has been acknowledged in the discussion section. However, the abstract still reads “Several chemokines and proangiogenic factors promote the release of CECs…” which should be changed to “Several chemokines and proangiogenic factors correlate with the release of CECs…”.

Minor Essential Revisions

* What was the intraassay variation (standard deviation in % of mean) with respect to the duplicate measurements of samples?

The answer does not match the question. The authors have measured each sample in duplicate and have used mean values for statistical calculation. The standard deviations (SD) of these mean values, when expressed as percentage of the mean, illustrate the divergence of duplicate measurements. Thus, the “intraassay variation” is defined as the mean of standard deviations expressed in percent and reflects the reproducibility of analyses. The authors did not calculate or mention the intraassay variation of their CEC measurements.

* How was blood processed for plasma preparation? The mode of plasma preparation greatly impacts the reliability of VEGF measurements (Starlinger et al. Dis Markers 2011;31:55-65).

The choice of anticoagulant (EDTA, citrate or heparin), the temperature of
plasma processing (at room temperature or 4 degrees Celsius) and especially the elimination of remaining platelets by a two-step centrifugation process (10 min 1000 x g, then 10 min 10,000 x g) is essential to avoid interference with VEGF measurements. These plasma processing details should be specified.

* The abstract mentions cytokine evaluation by ELISA; the methods section does not explain which cytokines were evaluated by ELISA.

The abstract still mentions the ELISA analyses. Since the authors have now stated that they only included data of bioplex assays, the ELISA analyses should be removed from the abstract.

* The authors report that CEC levels measured 28 days after start of gemcitabine therapy were not significantly different from baseline CEC values. In our own study (Starlinger et al. Neoplasia 2011;13:980-90) we observed that CEC values substantially increase when measured within 1 week of the last gemcitabine administration. However, after a treatment gap of 2 weeks, CEC levels have recovered. It would therefore be important to know, whether the authors measured CECs within one week of gemcitabine administration.

Based on the information now given by the authors it can be concluded that CEC measurements on day 28 of gemcitabine therapy were conducted following a 2-week treatment break (i.e. two weeks after the last gemcitabine dose). This is the likely reason why the authors did not detect a significant change in CEC numbers during gemcitabine therapy. While the authors acknowledge this notion in their reply to the reviewers, it has not been clearly stated in the discussion section.

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Based on the manuscript changes that have been introduced into the revised version, the following additional questions have been raised:

Major Compulsory Revisions

The authors have now corrected the distribution of patients with stage III, stage IV and recurrent disease among the collectives with high and low CEC count:

high CECs (# 166 cells/4ml):
- 12 patients of which 10 present with stage III, 1 with stage IV, 1 with recurrence

low CECs (<166 cells/4ml):
- 25 patients of which 5 present with stage III, 18 with stage IV, 2 with recurrence

The difference in stage distribution is statistically significant (P<0.001).

Surprisingly, it is the CEC low patient group (with predominantly stage IV disease) as opposed to the CEC high patient group (with predominantly stage III disease) which shows significantly better PFS and OS. This rather unexpected finding has not been addressed by the authors. Could this be explained by a
difference in performance status and CRP elevation within the CEC high (predominantly stage III) collective?

In table 2, the authors claim that stage IV and recurrent disease as opposed to stage III is associated with an increased hazard ratio of 2.21. While this finding seems plausible, it does not fit the data for CEC low (predominantly stage IV disease) as opposed to the CEC high patients (predominantly stage III disease)!

It may be worthwhile for the authors to compare OS and PFS between patients with stage IV and recurrent disease as opposed to stage III patients to shed light on this discrepancy.

Furthermore, the positive correlation of CEC counts with cytokines (IL-8, HGF) is surprising: While IL-8 and HGF are significantly higher for advanced disease (stage IV + recurrence), high CEC levels are associated with stage III rather than stage IV disease according to table 1. This observation suggests that IL-8 and HGF levels should be negatively rather than positively correlated with CEC numbers!

I strongly believe that a professional statistician should assess the analyses who may be better trained to evaluate these apparent discrepancies.

Minor Essential Revisions

Table 2: The cut-off level for SDF-1 alpha should read 110.6 pg/mL (not 471.3 pg/mL) according to the results text.

Discussion: According to table 3, it is the VEGF (not IL-6) blood levels which correlate with CEC counts. The discussion mentions IL-6 instead of VEGF !

Figure 2: When comparing figures 1 and 2, there is a discrepancy in the number of VEGFhigh patients. While figure 1 indicates that only 3 patients classified as VEGFhigh, 17 patients seem to contribute to the survival curve of the VEGFhigh collective in figure 2 !

Figure 3 (formerly figure 2): The in-text citation of figure 3 in the results section still reads figure 2 and should be corrected to figure 3.

Multivariate analysis: The HR for CRPhigh versus CRPlow reads 2.04 in table 2, but 1.39 in the results section. Which value is correct?

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

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