Reviewer's report

Title: Circulating cell death products predict clinical outcome of colorectal cancer patients

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Reviewer: Stig Linder

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This is a well conducted study addressing the possible use of serum caspase-cleaved cytokeratin/total cytokeratin as a prognostic biomarker for colon carcinoma. A weakness of the study is the relatively small size of the patient material (49 patients).

Major Compulsory Revisions

1. The paper describes the usefulness of calculating CK18-Asp396/CK18 ratios. This is potentially a very interesting concept, but the underlying biology is complex. On page 8 it is stated that a ratio of 0.20 means "20% apoptosis" and "80% necrosis". This is not correct. CK18 is not always cleaved to completion during apoptosis, and ratios of 0.20 can be observed after treatment of a cancer cell line with an apoptotic agent (differs between cell lines). The CK18-Asp396/CK18 ratio can be used to determine whether a particular compound induces apoptosis/necrosis in vitro using standard compounds as a reference, but it is not possible to say whether a 0.20 ratio means pure apoptosis or a mix between apoptosis and necrosis. However, and importantly, the decrease in the ratio observed in Fig. 2 is very interesting (and does indicate an increasing necrotic component during progression), and the possible prognostic information is also very interesting. The writing of the paper needs to be modified to clarify this issue.

Minor Essential Revisions

1. On page 11 it is written that "the CK18 ELISA also recognizes the M30 antigen". I understand what the authors mean, but the statement is not correct. The term "M30 antigen" is usually used to describe the CK18-Asp396 neo-epitope created by caspase cleavage. This is not recognized by the M65 ELISA. However, the M65 ELISA recognizes the soluble CK18 fragments that are detected in the M30-Apoptosense ELISA (as well as other soluble CK18 C-terminal fragments that are not caspase-cleaved).

2. Different authors have used different nomenclatures to described the CK18 protein complexes detected in the M30 and M65 ELISAs. One possibility is to use "CK18-Asp396" and "total CK18", or "M30 antigen". These designations are not completely correct (the molecules detected are complexes that need to expose to distinct epitopes, for the M30 Apoptosense both the caspase-cleaved epitope and an epitope in the 300-330 region). In this manuscript the term "M30
levels" is often used, which is not good. "M30" is an antibody; this should be changed through-out.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I have a financial interest in Peviva AB that manufactures and markets the ELISA kits used in this study.

I have a scientific competing interest (my group is interested in using the CK18 markers for similar purposes that are addressed here).