Reviewer’s report

Title: Co-expression of nuclear and cytoplasmic HMGB1 is inversely associated with infiltration of CD45RO+ T cells and prognosis in patients with stage IIIB colon cancer

Version: 2 Date: 13 October 2009

Reviewer: Luigi Laghi

Reviewer’s report:

I read with very interest the manuscript entitled “Co-expression of nuclear and cytoplasmic HMGB1 is inversely associated with infiltration of CD45RO+ T cells and prognosis in patients with stage IIIB colon cancer” by Peng et al.

The Authors investigate the relation between the immunolocalization of High-Mobility Group Box 1 (HMGB1) and the intratumoral infiltration of immunological cells in a subset of patients affected by colorectal cancer (stage IIIB, T3N1M0).

Although the field of investigation is interesting, the manuscript is highly confuse and needs to be extensively improved.

MAJOR COMPULSORY REVISIONS (which the Author must respond to before a decision on publication can be reached)

The main criticism is related to the method used by the Authors to estimate the density of immunological cells inside the neoplastic tissue.

The method is extremely subjective. In addition, several data have been omitted and should be added to replicate the described results.

• In particular, how the Authors counted the immune cells? What is the extension/size of the tissue sections that were? I.e., the Authors indicate only 10 different fields but not report what is the extension of each field. How the Authors distinguished and counted the cell clusters in the analyzed tissues? What criteria dictate a cut-off of 16 cells for CD3 density and of 24 cells for CD45RO density?

• How the Authors addressed the problem of the heterogeneous distribution of immunological cells inside the tumour? It would be interesting to know whether the Authors had assessed the issue of intra-sample and inter-sample variability.

• Additionally, the Authors recognized different subset of immunological cells (including CD4, CD8 and CD56) but omit to discuss why these cells have not been compared with HMGB1 expression and its immunolocalization.

• Figure 5 is not sufficiently clear to demonstrate a double immunohistochemistry for HMGB1 and CD45RO. The Authors should improve their staining procedure.

MINOR ESSENTIAL REVISIONS

The Authors used the terms nuclear and nucleolar throughout the manuscript.
However, because these terms indicate two different cellular localization, they should be more consistent and define with a single term the immunolocalization of HMGB1.

The tumoural tissue cannot be easily recognizable in Figure 1D.

In the Legend of Figure 1 and 2, the Authors stated that “all of the antigens were stained on the membrane”. However the term stained should be changed with the most appropriate “immunolocalized”.

DISCRETIONARY REVISIONS
None

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

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

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