Author’s response to reviews

Title: Expression of Tissue factor in Adenocarcinoma and Squamous Cell Carcinoma of the Uterine Cervix: Implications for immunotherapy with hI-con1, a factor VII-IgGFC chimeric protein targeting tissue factor

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To whom it may concern:

We enclose our manuscript entitled “Expression of tissue factor in adenocarcinoma and squamous cell carcinoma of the uterine cervix: Implications for immunotherapy with hI-con1, a factor VII-IgGFc chimeric protein targeting tissue factor” for submission to BMC Cancer as an original research article. There are no conflicts of interest for any of the Authors of this work.

**SUMMARY.**
We evaluated the expression of Tissue Factor (TF) by immunohistochemistry (IHC), real time-PCR and flow cytometry in cervical cancer and the potential of hI-con1, an antibody-like-molecule targeted against TF, as a novel form of immunotherapy against multiple primary cervical carcinoma cell lines with squamous and adenocarcinoma histology derived from primary or metastatic/recurrent site of disease. Sensitivity to hI-con1-dependent-cell-mediated-cytotoxicity (IDCC) was evaluated in 5-hrs-^{51}chromium-release-assays against cervical cancer cell lines *in vitro*. To investigate the effect of interleukin-2 (IL-2) and physiological level of human IgG on IDCC, 5-hrs ^{51}Cr-release-assays were also conducted in the presence of low doses of IL-2 (50 IU/ml) and human serum. Cytoplasmic and/or membrane TF expression was observed in 8 out of 8 (100%) of the tumor tissues tested by IHC and in 100% (11 out of 11) of the cervical carcinoma tested by real-time-PCR and flow cytometry but not in normal cervical keratinocytes (*p*<0.01). All primary cervical cancer cell lines tested overexpressing TF, regardless of their histology, were highly sensitive to IDCC (mean killing ± SD, 56.2% ± 15.9%, range, 32.4%-76.9%, *p*<0.001), while negligible cytotoxicity was seen in the absence of hI-con1 or in the presence of rituximab-control-antibody. Low doses of interleukin-2 further increased the cytotoxic effect induced by hI-con1 (*p*=0.025) while human serum did not significantly decrease IDCC against cervical cancer cell lines (*p*=0.597).

TF is highly expressed in squamous and adenocarcinoma of the uterine cervix. hI-con1 induces strong cytotoxicity against primary cervical cancer cell lines overexpressing TF and may represent a novel therapeutic agent for the treatment of cervical cancer refractory to standard treatment modalities.

Sincerely,

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