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

**Authors:**

Emiliano Cocco (emiliano.cocco@yale.edu)
Joyce Varughese (joyce.varughese@yale.edu)
Natalia Buza (natalia.buza@yale.edu)
Stefania Bellone (stefania.bellone@yale.edu)
Michelle Glasgow (michelle.glascow@yale.edu)
Marta Bellone (marta.bellone@yale.edu)
Paola Todeschini (paola.todeschini@yale.edu)
Luisa Carrara (luisa.carrara@gmail.com)
Dan-Arin Silasi (dan-arin.silasi@yale.edu)
Masoud Azodi (masoud.azodi@yale.edu)
Peter E Schwartz (peter.schwartz@yale.edu)
Thomas J Rutherford (thomas.rutherford@yale.edu)
Sergio Pecorelli (s.pecorelli@aifa.gov.it)
Charles J Lockwood (charles.lockwood@yale.edu)
Alessandro D Santin (alessandro.santin@yale.edu)

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**Author's response to reviews:** see over
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To whom it may concern:

We enclose our revised 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. We appreciate the comments of the reviewers and have the following responses/revisions:

Reviewer: Sarah Temkin

*The methods section does not include information on the number of normal cervical specimens that were tested for IHC to TF.*

We have added that 8 normal cervical specimens were tested by IHC to the manuscript. (page 5)

*HPV testing of specimens is reported in the results section but not described in the methods.*

We have clarified this information in both the results section and added a section and a new reference describing the methods we used in HPV testing (page 4).

*The data are sound. The responses of cell lines to IDCC, IL-2 enhancement and effect of complement are described in figure 3, 4, and 5. Responses are shown for between 1 and 3 cell lines per experiment in each figure. This reporting of results would be greatly improved by reporting either all of the cell line responses or a composite of the cell line responses in these figures. Reporting only one cell line response in a figure is inadequate.*

As mentioned in the manuscript, the graphs are representative of all the tumor cell lines tested. In Figure 3, we chose to show 3 different cell lines, representing primary cervical tumors of different histologies (squamous cell and adenosquamous) as well as a recurrent cervical cell line to show that despite their differences, they were all responsive to Hi-con dependent cell-mediated cytotoxicity. In Figures 4 & 5, we chose one representative cell line to display the trend we saw with all cell lines, as making a panel with 11 different graphs was a very busy figure and detracted from the point that we were making. Again, we clearly stated in the manuscript that Figures 4 & 5 are representative of the data we have with all cell lines.

*The discussion and conclusions could be stated more clearly. The last paragraph on page 12 does not seem to be relevant to this manuscript. The first paragraph on page 13 which describes the limitation of using IL-2 in conjunction with vaccine therapy also needs to be more clearly tied to the rest of the manuscript.*

We believe that the last paragraph on page 12 is quite important to this manuscript as it describes part of the mechanism by which Hi-con mediates cytotoxicity in vitro and in vivo, namely through natural killer cell activity. It also provides our rationale for performing experiments in the presence of IL-2. The first paragraph on page 13 does not describe the limitation of using IL-2 in conjunction with vaccine therapy, but rather discusses the limitations of using antibodies or antibody-like molecules as therapeutic agents in immunosuppressed cancer patients. In this paragraph, we discuss how IL-2 has been used clinically to augment patients’ immune systems and how this can make Hi-con therapy more efficient and beneficial in vivo.
Reviewer: Haim Werner

*Figure 4: Please insert asterisk above right (black) column to denote statistically significant difference versus no Il-2.*

This change has been made in Figure 4.

We hope that our revised manuscript will be acceptable for publication in BMC Cancer.

Sincerely,

Alessandro D. Santin, M.D.

Please send proofs and correspondence to:
Alessandro D. Santin M.D., Yale University School of Medicine, Department of Obstetrics, Gynecology & Reproductive Sciences Rm. 305 LSOG, 333 Cedar Street; PO Box 208063, New Haven, CT, 06520-8063. Phone: 203-737-4450. Fax: 203-737-4339. E-mail: alessandro.santin@yale.edu