Author's response to reviews

Title: The mannosylated extracellular domain of Her2/neu produced in P. pastoris induces protective antitumor immunity.

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Author's response to reviews: see over
Dear Dr. Bucceri,

We hereby submit for publication a revised version of 1923754115263936. We have carefully considered the constructive criticism of the reviewer and address all his comments. A point-by-point response to all issues raised by the reviewer is provided below. Except from the revised manuscript we provide a marked-up copy of the changes made from the previous article file as a SUPPORTING INFORMATION file. We thank you very much for your attention to this work and hope that you will find the revised manuscript suitable for publication in BMC cancer.

Yours sincerely,

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Response to Reviewer: Ai-Li Shiau:

1. “The authors missed the point regarding the use of mouse tumor cells overexpress "human" Her2/neu protein as the tumor model, rather than using those naturally overexpress "mouse" Her2/neu, to test their ECD of human Her2/neu protein as the tumor vaccine. They stated “xenogenic immunization with recombinant human ECD/Her2 most likely induced cross-reactive CTL against murine HER-2/neu which rejected the transplantable tumors expressing murine HER-2/neu”. In fact, they used human Her2/neu-overexpressing mouse tumors. This issue should be thoroughly re-discussed.

We were asked to comment the use of the recombinant human ECD/Her2 molecule as a vaccine against the growth of mouse tumor cell lines overexpressing “human” Her2/neu protein, rather than using tumors cells naturally overexpressing “mouse” Her2/neu. Given that mouse and human Her2/neu receptors have a homology of approximately 90% [1], vaccination with our human ECD/Her2 would generate two sets of ECD/Her2-specific T cell clones: those recognizing the xeno-human epitopes and those directed against the self-mouse epitopes. The latter would be of low-avidity due to self-tolerance mechanisms against self-Her2/neu. In contrast, ECD/Her2-induced mouse T cell clones recognizing the xeno-human epitopes would be highly activated (as high avidity clones) generating a cytokine milieu which would provide a bystander activation of the low-avidity T cell clones recognizing the self-mouse determinants, thereby rendering them capable of performing cytotoxic activities against a transplantable tumor overexpressing “mouse” Her2/neu. Moreover, the xeno-human determinant specific T cell clones could themselves mediate robust antitumor effects in vivo by cross-recognition of their murine analogs. We think that this satisfactorily addresses the reviewers’ comment, but since we haven’t used in our experiments a mouse Her2/neu overexpressing tumor cell line, we feel that the above text should not be included in “Discussion”. Instead, we have replaced the paragraph in “Discussion” (page 12, lines 1-20 “Negative selection…B cell compartment”) with the one attached below.

In our paper we have used the human ECD/Her2 to vaccinate mice against human Her2/neu overexpressing transplantable tumor cell lines. Our results show that in this particular model the human ECD/Her2 proved to act as a strong vaccine inducing in vivo efficient antitumor responses. Whether this vaccine would have similar results in phase I studies vaccinating Her2/neu positive cancer patients remains to be addressed in the future. Of course, one could think that due to tolerance mechanisms against the self-human Her2/neu protein, this could possibly be less effective in humans. However, the fact that ECD/Her2 is a large molecule including a plethora of immunogenic epitopes increases the chances for detecting vaccine-specific immune responses followed with positive clinical results. Of note, there are now phase I and phase II studies published with single Her2/neu peptide vaccines inducing clinical improvement [2] which are encouraging of using our ECD/Her2 molecule in clinical trials.


The paragraph in “Discussion” (page 12, lines 1-20 “Negative selection…B cell compartment”) was replaced by the following one.

“Negative selection in the thymus is capable of deleting almost all of self-reactive T cells [25, 26]. However, because this process is not complete, several peripheral tolerance mechanisms persist [26]. Because most known tumor antigens, including Her2/neu, come from self-protein, these incompletely removed self-reactive T cells must be activated before being applied to tumor immunotherapy. Recently, several reports have shown that xenogenic vaccination could break self-tolerance and inhibit the progression of established tumor in syngeneic or transgenic mouse models [27-29]. In our study, xenogenic immunization with recombinant human ECD/Her2 induced robust antitumor responses against murine tumor cell lines overexpressing human Her2/neu. Given the high homology between human and murine Her2/neu [30], we propose that our human ECD/Her2 vaccine will also induce cross-reactive CTL against murine Her2/neu, rejecting transplantable tumors expressing murine Her2/neu, thus breaking tolerance against self-Her2/neu, as also proposed in other tumor models based on xeno-vaccinations [8, 31]. In addition, given that ECD/Her2 is a potential source of immunogenic determinants recognized by CD4+ T helper cells [32], then it is obvious that activation of the latter cell population will also lead to generation of B cell clones producing anti-Her2/neu antibodies, thus overcoming any tolerance mechanisms affecting the B cell compartment.


2. “In the data (Fig. 4 and 7) of two animal studies, the tumor volumes of the mice should also be shown.”

In figures 4 and 7, tumor growth analysis showing tumor volumes was added.