Reviewer's report

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

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Reviewer: Ai-Li Shiau

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This manuscript described the efficacy of the mannosylated extracellular domain (ECD) of the Her2/neu protein produced in yeast for induction of antitumor immune responses in syngeneic tumor models. As Her2/neu is overexpressed in various human cancers and is associated with increased metastatic potential and poor prognosis, Her2/neu has been an attractive target for developing immunotherapy against Her2/neu-overexpressing cancers. The rationale of this study is feasible, and the development of cancer vaccine based on Her2/neu is important. However, the authors provided limited data and left several important questions unanswered.

1. In this study, the ECD of human Her2/neu protein was used as the immunogen to induce antitumor immune responses in mice against syngeneic tumors artificially expressing exogenous human Her2/neu to mimic Her2/neu-overexpressing human tumors. As human Her2/neu protein is a foreign antigen to the mice, it is much easier to elicit potent immune responses in mice against the human Her2/neu antigen expressed on mouse tumor cells. This issue should be thoroughly discussed.

2. The authors described the enhanced immunogenicity of mannosylated Her2/neu protein in the induction of immune responses. Although this may be true in the Her2/neu vaccine, the non-mannosylated Her2/neu produced from E. coli should be tested side-by-side with the mannosylated protein for their immunogenicity and protective immunity against Her2/neu-overexpressing tumors.

3. In Figure 2, mice immunized with ECD/Her2 for three times at days 0, 21, and 42 developed anti-ECD/Her2 antibodies. However, similar antibody levels were induced regardless of the numbers of immunization. Furthermore, at days 62 and 82, the background level of non-specific binding in the ELISA of the samples from the control mice was high. These points should be discussed.

4. The authors claimed that the immunized mice did not elicit any self-toxicity compared to PBS-vaccinated mice without showing any data. As anti-Her2/neu antibody has been shown to exert some side effects, this issue should be addressed.

5. In the animal studies, tumor-free survival of BALB/c mice bearing D2F2/E2 (n=9) was shown (Figure 4), whereas tumor volume of HHD mice bearing ALC/neu tumors (n=4) was shown (Figure 7). For better presentation of the
efficacy of ECD/Her2 vaccine and the consistency of the data, both tumor volume and survival in the two tumor models should be shown. Furthermore, in Figure 7, the numbers of the mice per group should be increased.

6. Figure 5 showed the immune serum from ECD/Her2-vaccinated mice could reduce the proliferation of SK-BR-3 cells. However, the degree of cell growth inhibition was not 13%. Therefore, whether the antibodies contained in the sera from ECD/Her2-immunized mice have significant anti-proliferative effects in SK-BR-3 cells cannot be confirmed.

Level of interest: An article of limited interest

Quality of written English: Acceptable

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.