Manuscript ID: 28654

Title: Phase IIb trial of in vivo EP mediated dual-plasmid HBV DNA vaccine in CHB patients under lamivudine chemotherapy

Journal: World Journal of Gastroenterology

Response to Reviewers' comments

Dear Dr. Garcia-Olmo,

We thank you for your careful consideration of our manuscript. We appreciate your response and overall positive initial feedback, and made modifications to improve the manuscript. After carefully reviewing the comments made by the Reviewers, we have modified the manuscript to improve the presentation of our results and their discussion, therefore providing a more complete context for the research that may be of interest to your readers.

We hope that you will find the revised paper suitable for publication, and we look forward to contributing to your journal. Please do not hesitate to contact us with other questions or concerns regarding the manuscript.

Best regards,

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Reviewer #1

1- How do you select your vaccine booster intervals? (12, 28 and ....) did you have any differences or scaling system for it? if yes, please stated in the manuscript

Response: Our vaccine administrations were at weeks 12, 16, 24 and 36 on the whole trial course under lamivudine antiviral therapy, representing the booster intervals of 4, 8 and 12 weeks. As a therapeutic HBV DNA vaccine, we chose the vaccination schedule based mainly upon the regimens already evaluated on human subjects [*Hepatology 2004; 40(4):874-82.* and *Gut 2014; 64(1):139-47*], in which the HBV DNA vaccine was given at weeks 0, 8,16, 40 and 44 with the booster intervals of 4, 8 and 24 weeks, in an attempt to induce a strong and durable T cell immune response. We had done dose scaling on this schedule of prime and boosts in our previous phase I trial [*Medical Journal of Chinese Peoples Liberation Army 2013; 38: 204-09*] and as a result, we got the optimal dose of 4 mg dual-plasmid DNA vaccine for phase IIa trial [*J Viral Hepat 2012; 19: 581-593*]. However, we did not have any scaling system for study of the differences of our vaccine booster intervals.

2- You should more discuss about CHB in introduction section, there is no information about CHB

Response: We agree with the Reviewer. We added some background information about CHB in the first paragraph of Introduction section.

"Chronic hepatitis B (CHB) is a chronic infection caused by the hepatitis B virus (HBV). There is no specific symptoms and the diagnosis is based on the clinical description accompanied by laboratory findings (IgM anti-HBc-negative and positive result for HBsAg, HBeAg, or HBV DNA)[1]. The CHB burden is global, but more significant in Asia, Pacific Islands, sub-Saharan Africa, Amazon, and Eastern Europe[2]. Hepatitis B virus (HBV) is endemic in China, with about 110 million HBV carriers and at least 300,000 people dying from HBV-related diseases each year[3]."

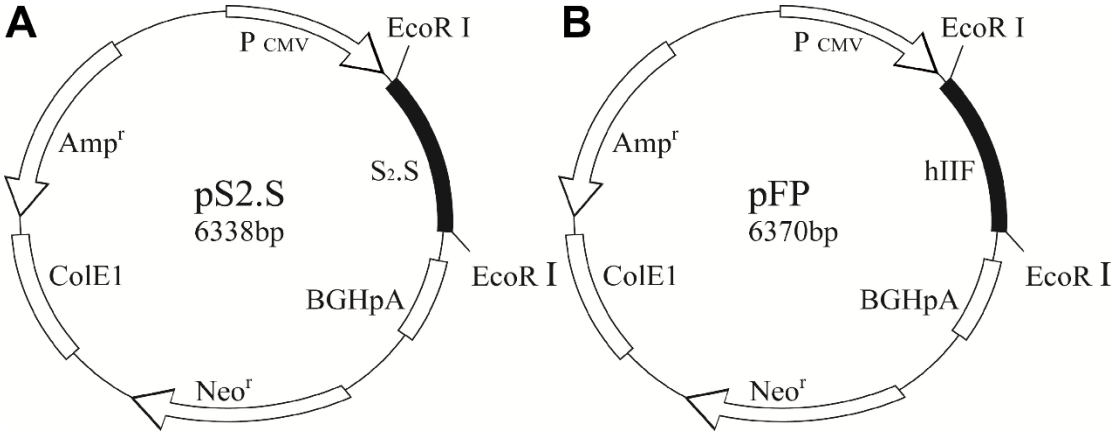
3- "Characteristics of the patients" section should be in materials and methods not

result

Response: We agree with the Reviewer. This section was moved to the Methods.

4- What was your eukaryotic expression vector? please state it with name in methods section

Response: We revised the plasmid construction method in the manuscript, and a new Supplementary Figure 1 was added.



Supplementary Figure 1. Plasmid construction schematic.

In the "EP-mediated dual-plasmid HBV DNA vaccine administration" section, we added "The S2.S gene was amplified from the plasmid pHBVα1 with the whole HBV s gene fragment (type ayw) by PCR, and then inserted in the eukaryotic expression vector pcDNA3.1+ after the use of the EcoR1 enzyme. Supplementary Figure S1A shows a representative map of the vaccine plasmid pS2.S of the HBV DNA vaccine." and the names of vectors.

5- Why do you use this kind of linker? did you investigate its effect on IL-2 and interferon expression? did you evaluate other kind of rigid or flexible linkers?

Response: The pFP plasmid expressed the fusion protein IL2/IFN-γ as adjuvant in dual-plasmid vaccine. There is a linker "-AGSGGGGS-" between the sequences of the two cytokines (IL2 and IFN-γ). The linker is flexible and enhances the bioactivity of the fusion protein.

We analyzed the fusion protein bioactivity using the software Modeller6v2 and

Combinatorial Extension (CE) (Figure A, B, C, below). Results showed that the fusion protein coincided with the natural three-dimensional structure of IL2 or IFN-γ (Figure A, B) and the two bioactive domains were separated and exposed (Figure C).

In addition, our previous experiments proved that the plasmid were correct. The constructed plasmid (pFP) was transfected into COS-7 cell, and the cytokine IL-2 or IFN-γ was detected by ELISA. The double plasmids association (pFP+pS2.S) or pS2.S alone were injected into mice Balb/c by in situ electroporation, and specific humoral and cellular immunity responses were examined [*Wei, Q.K. et al., Int J Clin Exp Med 2015*]. The results showed that this plasmid pFP could enhance the immune response using double plasmids (pFP+pS2.S) group compared with pS2.S alone.

Other sequences such as "-GGGGS GGGGS GGGGS-(G4S)3" were tested as linker, but failed.

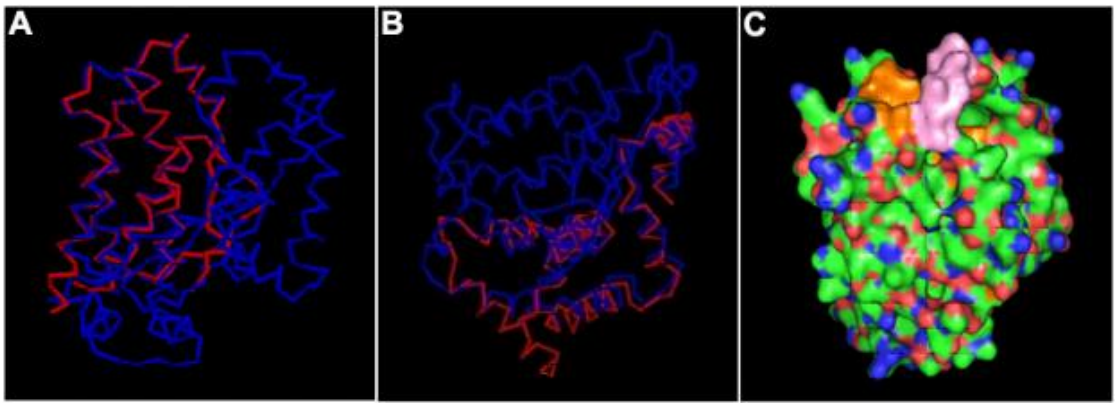


Figure. A. and B. Compression of the fusion protein with natural IL-2 or IFN-γ in three-dimensional structure. (Blue: fusion protein, red: IL-2 or IFN-γ). C. Bioactive domains of IL-2 (orange) or IFN-γ (pink) in the fusion protein.

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Response: We addressed all points raised by the Reviewer in our response above and in the manuscript.

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Response: The manuscript was edited according to the guidelines.

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