September 02, 2014

Dear Editor, Ya-Juan Ma,

Please find enclosed the edited manuscript in Word format (12127-Review).

Title: Prolonged overall survival in gastric cancer patients after adoptive immunotherapy.

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The manuscript has been improved according to the suggestions of the reviewers:

1 The format has been updated according to the guidelines.

2 Revision has been made according to the suggestions of the reviewer

(1) Interesting article. No information about the aprovation of the therapy regimen, which is not common clinical practice by the ethical committee have been given. Only for the retrospective evaluation of data. If author have the approvation of the ethical committee to perform the therapy they should give data about it, otherwise the article cannot be accepted for pubblication. The article cannot be evaluated for pubblication before to have these necessary data.

Response: We have now included details of the ethical approval of the therapy regimen as follows: The treatment regimen and the retrospective case control study were both approved by the Medical Ethics Committee of PLA General Hospital, China. All of the included patients signed written informed consent. (P7)

(2) the novelty of the immunotherapy should be acknowledged, however major drawbacks are evident: - EAAL should be absolutely spelled out at first appearance in the abstract

Response: We have acknowledged the novelty of EAAL immunotherapy (in P6) and explained EAAL at first use in the abstract

-page 5: "...Activated lymphocytes were generated using anti-CD3 monoclonal antibody (OKT3) and IL-2 as described previously [24]....".Please, check whether reference is correct

Response: We have checked this reference and altered the reference list accordingly to cite Tsoukas CD, Landgraf B, Bentin J, Valentine M, Lotz M, Vaughan JH, Carson DA. Activation of resting T lymphocytes by anti-CD3 (T3) antibodies in the absence of monocytes. J Immunol. 1985 Sep;135(3):1719-23.

- page 5: "...human AB serum…", please spell out

Response: We have now explained it fully.

-page 6: "....The eighty-four patients (aged 40-85 years) enrolled in the present study…": these are already results and should be put in the appropriate section

Response: These have been moved to the results.

- page 6 "...(cellular immunotherapy time ranged from 2-24, total immunotherapy time was 242, median immunotherapy time was 5)...". It is not clear what 'cellular immunotherapy time' is: is it the number of cellular immunotherapy administrations per patient (number of treatment cycles)? is it the time elapsed (in months) since study inclusion? anyhow, they are results and should be put in the appropriate section

Response: These have been clarified and moved to the results.

- overall survival is not defined: in particular what is the T0 (study entry)? the time of EAAL treatment? the time of surgery? time of histological diagnosis?

Response: This has now been defined as "from the diagnosis (nearly identical to the start of chemotherapy because the patients underwent the treatment soon after the diagnosis) until death or last follow up". (P8)

- page 7 "....EAAL cell proliferation at different time points is summarized in Fig. 1...": 'summarized' is not correct. It seems actually a 'representative example of T cells proliferation from a patient of EAAL cohort'

Response: We have now altered this as suggested.

- page 7: "....After 13.55±1.25 d of culture, the total cell number went from about 7.65±1.52×106 to 8.76±1.82×109…", please specify in the methods when the cells are counted before and after incubation

Response: This has been included in the Methods section "Cell survival and proliferation assessment" subsection (P 6-7)

- page 7: please specify in the methods what 'Proliferation multiplicity' is and how is calculated, as it is not just the percentage increase in number of cells, which is about +14%

Response: This has now been explained in the methods section as follows: Isolated peripheral mononuclear cells from the patients were appropriately diluted and

cultured. Before and after culture, a cell suspension was counted using a counting chamber under a microscope. The total cell number was calculated by the cell concentration multiplied by the volume, and the cell proliferation multiplicity was calculated as the ratio of cell number before and after culture. (P7)

- page 7: "...The survival rate of effector cells was 97.57±0.94**%** (Table 1)... "please specify in the methods how Survival rate is measured (trypan blue dye?)

Response: This was typan blue dye and has now been added. (P7)

- page 7: "...After in vitro culture and proliferation, the percentages of CD3+, CD3+CD8+, CD8+CD27+, CD8+CD28+, and CD3+CD16+/CD56+ cells increased remarkably (P<0.05), while CD3+CD4+, CD4+CD25+, CD3-CD16+/CD56+ (natural killer cells, NK) were overtly decreased (P<0.05); no significant change was observed in CD4+CD25+CD127- cells (P =0.448, Table 2)....": this is not what is said in the abstract (which is "...the percentages of CD3+, CD3+CD8+, CD3+CD4+,CD8+CD27+, CD8+CD28+, CD4+CD25+, CD4+CD25+CD127-, CD3-CD16+/CD56+ and CD3+CD16+/CD56+ cells increased sharply (P <0.05)...")

Response: This has now been corrected in the abstract. (P9)

- page 7: "...Among the 58 pathologically confirmed stomach cancer patients...", please specify "...among 58 'screened' patients"

Response: We have altered this as you suggest. (P9)

- page 8: "Chemotherapeutic features": timing and setting of chemotherapy delivery is completely incomprehensible: authors should clarify 1) if overall survival is calculated since start of chemotherapy, 2) 2 patients received only EAAL: were they metastatic/locally advanced? or radically resected?, 3) in the EAAL group 33 patients received first or subsequent line chemotherapy meaning they were metastatic or inoperable locally advanced, how does this fit with 10 patients in the EAAL group being stage I or II? is this stage at diagnosis or at study entry? if it is at diagnosis and patients are included in the study later on when they develop inoperable recurrence, then using stage in multivariate analysis is meaningless! 4) it is first said that 7 patients in the EAAL group received adjuvant chemotherapy and then that EAAL was administered with adjuvant chemotherapy in 13 patients! 5)it is methodologically incorrect to perform a unique survival analysis for patients with radically resected cancer on adjuvant treatment and patients with metastatic/inoperable disease

Response: We have tried to address all of these points to clarify this section.

1) overall survival was calculated from the diagnosis (nearly identical to to the start of chemotherapy because the patients underwent the treatment soon after the diagnosis) until death or last follow-up (P8).
2) The 2 patients that received only EAAL were radically resected stage I patients. (P10)
3) Among the EAAL group, 10 patients were diagnosed at stage I or II, and 6 of them were found without disease progress until the last follow-up. Among the 6 patients, 4 received adjunctive chemotherapy but the other 2 did not receive any chemotherapy. The rest 4 patients with stage I or II were found cancer recurrence and metastasis, but only 2 of them received first-line chemotherapy after the disease progress, while the other 2 patients received adjunctive chemotherapy. For stage III patients, 1 just received adjunctive chemotherapy because tumour recurrence and metastasis were not observed until the last follow-up, while all the others received first-line and subsequent chemotherapy. Therefore, among the 42 patients in EAAL group, 2 did not receive chemotherapy, 7 received adjunctive chemotherapy instead of first-line chemotherapy. As a results, a total of 33 patients received first-line and subsequent chemotherapy.
All the stages of cancer were at study entry.
4) 7 patients received EAAL therapy after the adjuvant chemotherapy, but 13 patients received EAAL therapy in combination with chemotherapy. (P10)
5) We agree that there is great difference in survival time between the patients with radically resected cancer and metastatic/inoperable patients. It would be better to separate the two types of patients in the survival analysis. However, as this is a retrospective study, we took these patients as a whole to observe the difference between EAAL therapy and non-EAAL therapy among the cancer patients with different stages with the help of sub-group analysis.

- finally: inclusion criteria for EAAL treatment is missing, how were they selected for EAAL+chemo or for chemo alone?

Response: We have clarified the inclusion criteria in the methods section as follows: The inclusion criteria for the EAAL treatment group were as follows; patients who received EAAL therapy according to the cell therapy records accessed through the China PLA General Hospital electronic medical reviewing system, the patients were histologically or cytologically diagnosed with gastric cancer and had a life expectancy > 12 weeks; with an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2. Patients with an ECOG score > 2, an incomplete medical history, or who were lost to follow-up were excluded.
In parallel, 42 patients were selected for the control group from the China PLA General Hospital electronic medical reviewing system as histologically confirmed gastric cancer patients who were admitted to the same hospital in the same first admission month as the EAAL patients, who had experienced surgery, chemotherapy or radiotherapy. Patients with a history of cell therapy and ECOG > 2 were excluded. The control candidates were grouped and numbered according to their clinical cancer stages, and were randomly selected to match the same number

of the patients as EAAL patients using the Statistical Package for Social Science (SPSS) 17.0 (SPSS Inc., Chicago, IL, USA). Clinical data were collected in the same fashion and those with incomplete medical history or lost to follow-up were substituted. (P7-8)

was this trial registered in a clinical trial registry? what is the registration number? (i.e. clinicaltrial.gov)

Response: **This was a retrospective study and so was not undertaken in the form of a clinical trial but rather as a result of the treatment the patients received after treatment decisions from their clinician and the patients. As such this was not a registered trial.**

(3) The manuscript entitled: "Prolonged survival in gastric cancer patients after adoptive immunotherapy" is an interesting study which is missing the required details for a proper scientific review. For instance: -Patient descriptions, case and control matching, ethical guidelines, patient consent, duration of follow-up, description of "loss to follow-up", specific details of EAAL generation and biosafety measures, time of EAAL therapy in regards to conventional surgery (partial/total gastrectomy), radio and chemotherapy....are missing. -Tables and graphs are vague and lack description of the mentioned indices -Some units of the obtained values are missing. These are only some of the points that do not allow for a proper review of the manuscript findings. Therefore, a major revision (by which all the needed details become available for review) is required at this time.

Response: **The requested information has now been included and we hope have clarified to your satisfaction.**

In detailed response to your points: Patients' basic characteristics: With the cell therapy records, we searched 58 histologically confirmed gastric cancer patients who received EAAL treatment between October 2006 and December 2009. After the exclusion of those with incomplete medical records or ECOG >2 or who were lost to follow-up loss, 42 patients were included in the EAAL group. Patients were classified as 3 groups, which were: 1) group 1 containing 10 stage I and II patients, 2) group 2 containing 12 stage III a and IIIb patients and 3) group 3 containing 20 IIIc and IV patients. As to the selection of control patients, we primarily randomly screened 246 patients who were admitted to the hospital in the same first admission month as EAAL patients, and finally, 10 patients with stage I and II, 12 patients with IIIa and IIIb, and 20 patients with IIIc and IV were selected.

In the EAAL group, 34 patients were male and 8 were female. In control group, 33 were male and 9 were female. The mean age for the EAAL treatment group and control group was 57.71 ± 11.84 and 58.97 ± 11.17 years, respectively, and no significant difference was found between the two groups (P=0.740). The patients were also classified as <60 group and ≥ 60 group according to their age, which can be seen in Table 3.

The EAAL patients received a total of 288 cycles of chemotherapy, and the median cycles were 7. Two patients did not receive chemotherapy, 7 patients received adjunctive chemotherapy after surgery, 13 patients received first-line chemotherapy, and 20 received second and multi-line chemotherapy. Two patients received EAAL therapy alone, 13 received EAAL with the adjunctive chemotherapy, 16 patients received EAAL during the first-line chemotherapy, 7 received EAAL during the second and multi-line chemotherapy, and 4 received EAAL during the first and second-line chemotherapy.
Control patients received 264 cycles of chemotherapy, and the median cycles were 6. Three patients did not receive chemotherapy, 6 received adjunctive chemotherapy after surgery, 16 received first-line chemotherapy, and 17 received second and multi-line chemotherapy. During the treatment period, the chemotherapeutic protocol included mFLOFOX6 (oxaliplatin+ 5-Fu+ Calcium Folinate), mDCF (docetaxel+cisplatin+5-Fu), DF (docetaxel+5-Fu), XELOX (oxaliplatin+ Capecitabine), SOX (oxaliplatin++ Tegafur), FOLFRI (Irinotecan+5-Fu+ calciumfolinate), mECF (epirubicin +cisplatin+5-Fu), Capecitabine and Tegafur. Patients were then classified as ≤ 6 cycles group and > 6 cycles groups according to the chemotherapeutic cycles. In addition, patients were also classified as "yes" and "no " according to whether the patients had received surgery or chemotherapy treatment. The subgrouping can be seen in Table 3.

The preparation of EAAL and assessment of bio-safety of EAAL were detailed in Materials and methods section. (P 6 and 7).

The legends to tables and graphs are revised.

3 References and typesetting have been corrected

Thank you again for considering our manuscript for publication in the *World Journal of Gastroenterology*.

Yours Sincerely,

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