Author's response to reviews

Title: Eradication of intractable malignant ascites by abdominocentesis, reinfusion of concentrated ascites, and adoptive immunotherapy with dendritic cells and activated killer cells in a recurrent lung cancer patient: a case report

Authors:

- Hideki Kimura (hkimura@chiba-cc.jp)
- Toshihiko Iizasa (tiizasa@chiba-cc.jp)
- Aki Ishikawa (aishikawa@chiba-cc.jp)
- Mitsuru Yoshino (myoshino@chiba-cc.jp)
- Masato Shingyouji (mshingyoji@chiba-cc.jp)
- Masaki Kimura (mkimura@chiba-cc.jp)
- Tetushi Hirata (thirata@chiba-cc.jp)
- Akiko Odaka (aodaka@chiba-cc.jp)
- Keiko Matsubayasi (kmatsuba@chiba-cc.jp)

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Author's response to reviews: see over
To reviewer Dr. Masato Okamoto

Thank you for your comments.

First of all, the purpose of this paper is to report a case of intractable malignant ascites successfully treated by abdominocentesis, reinfusion of concentrated ascites combined with adoptive immunotherapy. A case with severe malignant ascites as reported in this paper (every week we had to drain 3000-6000ml ascites) can not survive more than one month. The patient survived more than 10 months with no symptoms after the initiation of the therapy.

Reviewer Okamoto insisted to analyze the phenotype of transferred cells more detail but the purpose of this report is not to analyze precisely the cell surface markers of the infused cells but to report the fact that a patient got a remission of ascites by the treatment. Furthermore cell surface markers like HLA class I, CD1a, CD11c, CD14, CD40, CD80, CD86 were not studied in this case. CD83 is one of the most reliable cell surface makers of activated dendritic cells. If you want to know about the characteristics of the cells obtained from tumor draining lymph nodes of the lung cancer patients after IL2 culture, please refer to the paper “Kimura H, Dobrenkov K, Iida T, et al. Tumor-draining lymph nodes of primary lung cancer patients: A potent source of tumor-specific killer cells and dendritic cells Anticancer Res 25:85-94 2005.” (Ref. No 7)

You required also to test cytokine production of administered cells but we had already reported the cytokine concentration of INF-gamma, before, 8h, 24h, 48h and 5 days after the first intra-peritoneal immunotherapy. The concentration of TGF-beta, and IL12 were also examined but did not changed so that the data was not shown due to space limitations.

2) You insisted to clarify whether the cells called DCs present tumor antigens and induced antigen-specific CTL or not. The precise analysis about the mechanisms of decrease of tumor cells in the ascites is difficult because this is a clinical study and not the experimental animal study in which tumor specific antigens are already analyzed. In the clinical case, tumor antigens are different case by case and may be different in each tumor cell. To analyze the specificity of the case, the experimental study using many ascites patients will be necessary and those studies deserve original articles. Those subjects are too large to handle in a case report.

In the discussion we mentioned about the mechanisms of eradication of tumor cells, but those were our speculations with no proof of specificity. So we changed the sentences as follows:
indicated that these effects were mediated by... to
suggested the possibility that these effects.

This eradication of tumor cells was mediated mainly by the specific immune responses with cytotoxic killer T cells since

To
The precise mechanisms of eradication of tumor cells by the adoptive transfer of activated killer T cells and DCs are not clear, but it may be mediated mainly by the immune responses of cytotoxic killer T cells since... Your opinion that allogeneic tumor cells should be used as target cells in this analysis to prove the specificity of the cell killing is reasonable, but it is impossible now to test allogeneic cells as targets of the cytotoxicity test because the case can't be repeated any more.

3) We did not tested the NK markers in this study because the cells obtained from regional lymph nodes were mainly T cells and DCs that was already reported precisely in the previous paper Anticancer Res 25:85-94 2005.”

4) As mentioned in this paper, cells from ascites were obtained after treatment of adoptive transfer of activated killer T cells and DCs. Those cells (TIL) were cultured in vitro for 2 weeks in KBM-400 lymphocyte medium containing IL-2. The original cell population obtained from ascites consists mainly of CD4 positive T cells (52.6%) and CD8 positive T cells (22.0%; data not shown) containing a small number of tumor cells. In vitro culture of TIL in the presence of IL2 and tumor cells for 2 weeks might have changed cell population to CD8 dominant.

5) In ascites tumor cells were dominant before the treatment and lymphocytes prevailed tumor cells after the adoptive immunotherapy. Cells infiltrating into the abdominal cavity with carcinomatous peritonitis are called tumor infiltrating lymphocytes.

6) We corrected INF to IFN (interferon).

Hideki Kimura MD,PhD
To reviewer Dr Robert Thurer

Thank you for your comments.

We added a sentence in the last of case presentation as follows
P8 line 19
The patient survived more than 10 months after the initiation of the therapy.

Hideki Kimura MD PhD
Chiba Cancer Center