Author's response to reviews

Title: The enhanced expression of the matrix metalloproteinase 9 in nasal NK/T-cell lymphoma

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Author's response to reviews: see over
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Dr. Scott Edmunds, PhD,
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Dear Dr. Edmunds,

We send revised manuscript and replies to the reviewer. We have studied the referee's comments carefully and have made corrections which we hope are acceptable.

Yours sincerely,

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[Responses to referee 1’s comments]

“Major Compulsory Revisions”

1. How was “distant involvement-free survival” defined? How often were the patients restaged after treatment? Which procedures were performed (imaging, endoscopy, biopsy)?

   We added the following sentences on page 8, the last para..
   “The distant involvement free survival was calculated from the date when the treatment started to the time of diagnosis of distant metastasis or last follow-up.”
   We also added the following sentences on page 7, the first para..
   “Patients were usually followed every 2 months for the first 2 years and every 3 to 5 months subsequently. Physical and endoscopical examination were performed at every visit and CT were usually performed every 6 months.”

2. As it is a retrospective study, for how many patients were survival data available? For how many was there histological material to perform P-glycoprotein and MMP-9?

   We added the following sentences on page 8, the last para..
   “All but 3 surviving patients had a minimum follow-up of 2 years and the follow-up periods of the other 3 patients were, 1, 1, and 4 months. The median follow-up of surviving patients was 94 months.”
   We also added the following sentences on page 9, the second para..
   “We used biopsy samples of 43 patients and samples of 3 patients were not evaluable because they were too tiny.”

3. Concerning Figures 4 and 5: were B and T-cell lymphomas joined together? This must be clearly stated in the text or legend of the figure. This analysis should be done separately for T and B lymphomas, as each of them could have a different biological behavior.
We added the following sentences on the legend of Fig.4 and Fig.5.
“All classifications of B and T-cell lymphomas were joined together and analyzed.”

We also added the following sentences on page 12, the second para..
“The similar result was obtained, when this analysis was also done separately for T and B cell lymphomas. The 5-year survival rate for P-glycoprotein positive and negative in T cell lymphomas was 68% and 67%. The 5-year survival rate for P-glycoprotein positive and negative in B cell lymphomas was 85% and 71%.”

We also added the following sentences on page 13, the last para..
“The similar result was obtained, when this analysis was also done separately for T and B cell lymphomas. Distant involvement free 5-year survival rates for patients with MMP-9 negative, and MMP-9 positive in T cell lymphomas were 89%, and 57%, respectively. Distant involvement free 5-year survival rates for patients with MMP-9 negative, and MMP-9 positive in B cell lymphomas were 100%, and 62%, respectively.”

4. Even if not statistically significant, the p values should be put in the survival curves. It seems that, for Fig 5 the p value should be significant.

We put the p value in Fig.2, Fig.4, and Fig.5.

5. Which are the known features related to a poor outcome in lymphomas: cell of origin, responsiveness to chemotherapy? Which are the known molecular mechanisms of chemoresistance?

The following sentences were described on page 14, the line 9.
“The reasons for the resistance to chemotherapy are not clear. The overexpression of MDR1 phenotype is considered one of the major determinants for the ineffectiveness of chemotherapy in nasal NK/T-cell lymphoma patients [10, 11]. However, there was no significant difference in overall positivity for P-glycoprotein between NTL and non-NTL in our
study (Table 2). Positive immunoreactivity for P-glycoprotein was not found to be an important prognostic factor for patients’ survival (Fig. 4). Kim et al. reported similar results obtained by using the same antibody, mouse anti-human C-494 antibody on paraffin-embedded sections [14]. These results indicate that the frequent expression of MDR (P-glycoprotein-positive) phenotype may account for a certain proportion [12], but not all, of the failure of chemotherapy [31]. Necrosis is also a constant microscopical feature in nasal NK/T cell lymphoma, usually with a zonal pattern of distribution that suggests a vascular pathogenesis. Poor drug delivery owing to tissue necrosis resulting from angiodestruction by nasal NK/T cell lymphoma cells might be an important contributory factor [8]."


The following sentences were described on page 16, the 3rd para..

“Progress in the treatment for nasal NK/T lymphomas has been slow due to the rarity of the diseases, geographic variation, relative chemoresistance, and lack of randomized trials. There is no consensus about optimal therapy and recommendations are based on anecdotal reports, small series, and phase II trials. There is general agreement that results with CHOP alone are so poor in adults with most peripheral T/NK cell lymphomas [36]. So, the combined radiotherapy and chemotherapy was used [37]. We previously reported 65 patients with mature T/NK-cell lymphomas treated with radiotherapy between 1983 and 2002 to analyze the influence of radiotherapy doses and chemotherapy doses and clinical parameters on
in-field disease control in order to assess the optimal radiation doses for treatment of mature T/NK-cell lymphomas [38]. There were no significant differences in radiosensitivity among subtypes of mature T/NK-cell lymphomas, at least between nasal NK/T cell lymphoma and peripheral T-cell lymphomas, unspecified. Radiation doses of 50 Gy or more may be required to obtain in-field control of mature T/NK-cell lymphomas [38].”

7. In this setting, which is the role of MMP-9? P-glycoprotein may be a factor for chemoresistance, but is MMP-9 also responsible for chemoresistance or only a factor that facilitates dissemination of the tumor? May this be overcome if every patient receives chemotherapy?

The following sentences were described on page 14, the line 21.
“Necrosis is also a constant microscopical feature in nasal NK/T cell lymphoma, usually with a zonal pattern of distribution that suggests a vascular pathogenesis. Poor drug delivery owing to tissue necrosis resulting from angiodestruction by nasal NK/T cell lymphoma cells might be an important contributory factor [8].

We added the following sentences on page 16, the second para..
“Inhibition of the function of MMPs is being pursued for anticancer therapy. TIMPs (tissue inhibitors of metalloprotease) were first compounds to be considered for clinical development. However, the lack of effective methods of systemic gene delivery has limited the clinical utility of this approach, whereas the development of synthetic inhibitors of MMPs has been actively pursued and widely tested in clinical trials [35].”
8. In this sense, the third paragraph of the introduction should also be revised.

We added the following sentences on page 6, the first para..

“MMP-9 can be also responsible for chemoresistance of NK/T-cell lymphoma. Higher expression of MMP-9 could result in tissue necrosis due to more angiodestruction. Poor drug delivery owing to tissue necrosis might be an important contributory factor [8].”

9. Figure 4: the survival plateau is reached with 70% of the patients alive. Are they the same as in Fig. 2?

As we answered to your question 1, we used biopsy samples of 43 patients and samples of 3 patients were not evaluable because they were too tiny. Two of 3 patients who were not evaluated immunohistochemically died of lymphomas. Calculations of Fig.2 included these 3 patients, but calculations of Fig.4 did not.

10. Survival curves: for clearness, the p values should be put in the survival curves even if not statistically significant.

We put the p value in Fig.2, Fig.4, and Fig.5.
[Responses to referee 2’s comments]

“Major Compulsory Revisions”

1. The group on nasal NK/t-cell lymphoma should be compared with a group of NK-T-cell non-nasal lymphoma.

We added the following sentences on page 15, the 3rd para..

“We also reported the expression of MMP9 in 158 patients with non-Hodgkin’s lymphomas [34]. Almost all of the patients with nasal NK/T cell lymphoma and anaplastic large-cell lymphoma expressed MMP9. In this study, 15 of 17 (88%) patients of nasal NK/T cell lymphoma expressed MMP9. Five of 8 (63%) of peripheral T-cell lymphomas, unspecified expressed MMP9 and 4 of 4 (100%) of peripheral T-cell lymphomas, unspecified expressed MMP9. In contrast, only a small fraction of the patients with extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue type and follicular lymphomas expressed MMP9. About 50% of the diffuse large B-cell lymphomas (DLBCL) expressed MMP9. Overall survival rates of patients who expressed MMP9 were significantly lower than those who did not.”

We also added the following sentences on page 16, the last para..

“We previously reported 65 patients with mature T/NK-cell lymphomas treated with radiotherapy between 1983 and 2002 to analyze the influence of radiotherapy doses and chemotherapy doses and clinical parameters on in-field disease control in order to assess the optimal radiation doses for treatment of mature T/NK-cell lymphomas [38]. There were no significant differences in radiosensitivity among subtypes of mature T/NK-cell lymphomas, at least between nasal NK/T cell lymphoma and peripheral T-cell lymphomas, unspecified. Radiation doses of 50 Gy or more may be required to obtain in-field control of mature T/NK-cell lymphomas [38].”

“Minor Essential Revisions”

1. The increased proportion of MMP positive patients could be present in all T-cell lymphoma (all at poor prognosis) and this should be verified.
We added the following sentences on page 15, the 3rd para..

“We also reported the expression of MMP9 in 158 patients with non-Hodgkin’s lymphomas [34]. Almost all of the patients with nasal NK/T cell lymphoma and anaplastic large-cell lymphoma expressed MMP9. In this study, 15 of 17 (88%) patients of nasal NK/T cell lymphoma expressed MMP9. Five of 8 (63%) of peripheral T-cell lymphomas, unspecified expressed MMP9 and 4 of 4 (100%) of peripheral T-cell lymphomas, unspecified expressed MMP9. In contrast, only a small fraction of the patients with extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue type and follicular lymphomas expressed MMP9. About 50% of the diffuse large B-cell lymphomas (DLBCL) expressed MMP9. Overall survival rates of patients who expressed MMP9 were significantly lower than those who did not.”

2. The prognosis of Asian population as well as Central American population is not so poor as European population (cfr the recent paper of Pagano et al Ann Oncol 17-794-2006) and that should be stressed.

We added the following sentences on page 4, line 9.

“The prognosis of Asian population as well as Central American population is not so poor as European population [7].”

Quality of written English: Needs some language corrections before being published

I asked my English colleague to read and criticise my manuscript. He suggested several improvements in the language and in many places the text has been reworded. I do hope that this will help to clarify the text. A ‘WORD’ English spelling corrector has been used to correct irritating errors.

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
We revised our manuscript to describe details of statistical methods that we used.

We added the following sentences on page 7, the first para..
“Patients were usually followed every 2 months for the first 2 years and every 3 to 5 months subsequently. Physical and endoscopical examination were performed at every visit and CT were usually performed every 6 months.”

We added the following sentences on page 8, the last para..
“All but 3 surviving patients had a minimum follow-up of 2 years and the follow-up periods of the other 3 patients were, 1, 1, and 4 months. The median follow-up of surviving patients was 94 months. The distant involvement free survival was calculated from the date when the treatment started to the time of diagnosis of distant metastasis or last follow-up.”