Author's response to reviews

Title: Microvessel density as a new prognostic marker after radiotherapy in rectal cancer

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Version: 2 Date: 23 December 2008

Author's response to reviews: see over
Dear editorial board,
Thank you for reviewing our manuscript. We have made corrections according to the advices presented by reviewers. We would like to answer and comment the remarks.

Reviewer: Adam AD Dziki.

Operation time after radiation.

I did not find in this paper, when the patients after short course rtg-therapy were operated. The time of radiation and operation was described in materials methods (page 5): “For short RTX group patients, irradiation was given in a dose of 5 Gy five fractions within five days. Surgical resection was performed within a week after RTX. For long RTX group patients, irradiation was given in a dose of 50 Gy/5 weeks with 2 Gy fraction treating 5 days/week. Surgical resection was performed within six – eight weeks after RTX.”

Reviewer: Gaetan Des Guetz.

Criteria for RTX.

Why patients are treated with short course, long course, which criteria?
The criteria for short and long RTX courses are as follows:
Indication for long RTX is unresectable tumor or T4 or N1 status according endorectal ultrasound examination.
Indication for short RTX is a clearly resectable tumor T3 or T4 and N0 status according to endorectal ultrasound examination, younger age patients and those with higher risk of disease recurrence (tumor differentiation grade etc.). These criteria are presented in a section Materials and Methods (Patients and tumor specimens, page 4).

Data for short course RTX.

We have no data about prognostic for short course RTH
There were no statistically significant different of MVD between dead and alive patients in short RTX group.

Survival of patients with lower MVD.

Patients with low MVD seems to have better survival: why MVD was significantly lower in the group of long course RTH.
In Discussion we extended the possible explanation of these phenomena (page 8). It is of note that tumor samples from the short RTX group were taken one week after the radiation treatment. “This suggests that the changes in tumor tissue may be subsequent to the cytotoxic effects of RTX that lead to inflammation that begins in the tumor straight after RTX [14]……….. The tumor samples from the patients that received a long course of RTX were taken 6 – 8 weeks after the RTX. During this period, cytotoxic effects and the inflammatory reaction induced by radiation treatment in the tumor tissue is diminishing, radiation induced necrotic lesions of the tumor are repaired by fibrous connective tissue [16].” (Page 8)

Data for local or metastatic relapse.

We have no data for local and /or metastatic relapse. Could we have relapse rate and mortality rates?
The aim of our study was to investigate the changes in tumor angiogenesis under the influence of different types of radiotherapy to assess the prognostic value of angiogenesis (MVD) in the overall survival after radiotherapy. The date and cause of death of those who died during the follow-up period were obtained from The Lithuanian Cancer Register. So analysis of the data about local and metastatic relapse were out of the scope this study.

A table.
A Table could be useful to resume characteristics of patients.
The demographic and clinical characteristics of the patients are presented in Table 1.

Minor revision.
The first reference must be corrected from G Des Guetz et al not GG.
The first reference was corrected to Des Guetz G

Reviewer: Arjan W Griffioen.

Number of patients.
The patient numbers are small. When the group of 101 patients is divided in 3 groups (no RTX, short and Long RTX) and the latter one even in dead or alive patients, there is not much room for statistics anymore.
During this retrospective study we were able to analyze data from 101 patient. The mean of MVD were examined by Mann-Whitney test for non parametric criteria of small groups. It affirmed our results.

MVD.
Measuring only MVD is not enough nowadays, and it even might be considered a weak parameter due to (inter and intra observer variations).
MVD is one of most parameters which might be prognostic marker in cancer generally and after radiotherapy in rectal cancer specifically. The purpose of our study was to determine prognostic value of MVD in rectal cancer after radiotherapy.

Different numbers of MVD in abstract and results.
Why are the numbers of MVD in the abstract not the same as in the results section?
We thank for the error correction. It was a mistake in an abstract. It must be: “The mean MVD for the long RTX group was 134.8; for the short RTX group – 192.5; and for those not treated with RTX – 193.0.” Page 2.

Figure 2.
Figure 2 does not show anything about RTX.
Figure 2 represents differences of mean ± SE of MVD (microvessel/mm2) in untreated (no RTX), short term (short RTX) and long term RTX groups.
Figure 2. Differences of mean ± SE of MVD (microvessel/mm2) in untreated (no RTX), short term (short RTX) and long term RTX (long RTX) groups.

Figure 3.
Figure 3 does not show anything on dead or alive patients, as stated in results section.
Figure 3 represents distribution between MVD and survival in untreated, short term and long term RTX groups. A black line indicates mean ± SE of MVD for alive patients, a grey one indicates the same parameters of dead patients.
Figure 3. Distribution between mean ± SE of MVD (microvessel/mm²) and survival in untreated (no RTX), short term (short RTX) and long term RTX (long RTX) groups.

Figure 6.
Figure 6 is not called out at all.
There are only five figures in the manuscript.

CD31.
CD31 would be a much better antigen for such studies, since it stains a lot more blood vessels in tumor tissues.
Both CD31 and CD34 are used for endothelial cells staining in the studies of angiogenesis in malignancies. We chose CD34 as it was more commonly used in the studies of angiogenesis in colorectal cancer.

The sentence “the only feature……..”
The sentence 'the only feature....... as compared to latter' is not clear. It should be explained.

The statement (page 7) “Treatment using short RTX did not change the mean MVD as compared to tumor samples taken from those receiving no RTX; the only feature noticeable was that the dispersion of MVD was greater in a former group as compared to latter”. The statement indicates that the mean of MVD in both groups does not differ significantly, but the dispersion of MVD is greater in short RTX group in comparison to no RTX group. It can be edited: the only feature noticeable was that the dispersion of MVD was greater in a short RTX group as compared to no RTX group (Page 7).

Ethics
The information about approval of Kaunas Region Ethics Committee For Biomedical Research was added (Page 6).