Author's response to reviews

Title: Influence of Oral Glutamine Supplementation on Survival Outcomes in Locally Advanced Non-Small Cell Lung Cancer Patients Treated with Concurrent Chemoradiotherapy

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Author's response to reviews: see over
Dear Editor,

We received the comments of all 4 Reviewers and revised the manuscript, final version of which was also uploaded to the system. All Reviewers criticized our manuscript in several aspects, most of which are related with structure of data presentation and our manuscript writing style in the original manuscript. Respecting the suggestions by the reviewers, we revised and reconstructed our manuscript accordingly as recommended. Therefore, we performed some major and minor modifications and additions in order to address all issues suggested, and presented them in a point-by-point manner in the responses to reviewers section below. We omitted the “Figure 1” as recommended by the Reviewer 2, and included 2 additional tables as recommended by the Reviewer 1. We omitted few references and added new ones, therefore total number of the references increased to 52. During the revision process, we got valuable helps from our colleague Savas Topuk, therefore we added his name in the revised manuscript. Finally, since we do not live in a natively English speaking country, and as pointed by the reviewers, the revised manuscripts were evaluated by commercial language editing company namely “Biosciencewriters”. However, we are ready to get help from any language editors you recommend for any linguistic problem the Reviewers might experience in our manuscript. We hope we could satisfy suggestions by all 4 Reviewers, which we believe increased statistical power and relevancy of our current version of manuscript.

Best Regards,

Attachment: Response to reviewers

On behalf of all co-authors,

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Response to Reviewers

Reviewer 1

We appreciate and would like to thank Reviewer 1 for his/her kind recommendations of paramount importance, which we believe will significantly increase the relevance of our current revised version of the manuscript.

Minor Compulsory Revisions

1- Comment: 1. Abstract: Background section in the abstract is not defined very well, it sound more like the Aim section and need more clear explanation of background.

Conclusion section in abstract: Regarding methodology of the study current results cannot suggest, mybe better words: show association.

Response: As recommended by the reviewer, the “Abstract” section of the original manuscript was revised thoroughly, and a clear explanation of the background of the study was made in the “background” subsection of “Abstract” with relevant modifications in the current revised form of the manuscript. Meanwhile, as pointed by the reviewer, related to our manuscript writing style, the “Conclusion” section of the original manuscript was not relevant with the methodology used. Therefore, we modified the “conclusion” subsection of the “Abstract” accordingly as recommended.

2- Comment 2. 2. Background: a) The following sentence should be rewritten more clearly: 

"Although improvements in target definition tools and advent of sophisticated RT techniques, and elimination of elective irradiation of lymph node regions reduced the volume of normal tissue exposed to high dose radiation to a significant degree, because of the need for irradiation of subclinical tumor extension, it appears that normal tissue toxicity
and its consequences will remain as a treatment challenge for RT clinics that is not anticipated to be solved in near future.”

**Response 2.** We would like to apologize for the ambiguous statement related to our manuscript writing style. As pointed, the related sentence was revised, simplified and replaced with “Improvements in target definition and the advent of sophisticated RT techniques, combined with elimination of elective irradiation of clinically uninvolved lymphatics, have significantly reduced the volume of normal tissue exposed to high-dose radiation with a resultant reduction in incidence and severity of treatment-related toxicity [1]. However, because of the need to irradiate subclinical tumor extension, normal tissue toxicity and its consequences likely will remain a challenge for the foreseeable future [2].” in the current revised form of the manuscript as recommended.

**3- Comment 3.** b) Add reference after...marked GLT depletion develops over time which cannot be compensated by increased synthesis.

**Response 3.** This issue was addressed by citation of relevant reference as recommended.

**4- Comment 4.** c) Add reference(s) ... concerns have been raised whether administration of GLT might stimulate tumor growth (TG), and therefore, negatively alter tumor control and survival outcomes following anticancer treatment.

The hypothesis to the study question is not clearly stated (the proposed effect of oral glutamine on survival)! And why they oral dosage of 3x10g/day per day?

**Response 4.** Last paragraph of the “Introduction” section was revised, and modified accordingly to clearly explain the hypothesis of our study with citation of the related references as recommended. Likewise, the concern of the reviewer about the reason why we used high doses of oral glutamine was addressed by the relevant modifications in the methodology section.
5- **Comment 5.** 3. Methods: The methodology of the study is very weak. There is no explanation how they control the ingestion of glutamine in the treatment group. Maybe they have just better food intake if the control group was not able to afford glutamine use because of socioeconomic reasons.

Side effects of treatment should be listed and explained in the Methods section! stages of ARIE should be explained.

**Response 5.** We would like to apologize for our confusing manuscript writing style and for submitting our study with such limited methodology that does not actually reflect our daily practice. Obeying the advices directed by the reviewer, entire methodology of the manuscript was revised thoroughly, and modified with relevant clarifications. The concern by the reviewer about the ingestion status of glutamine in the treatment group was addressed by the necessary explanation in the “Glutamine Supplementation” section of the current revised manuscript.

6- **Comment 6.** 4. Results: a) "The unique acute toxicity that may be attributed to GLT prophylaxis"....What is that, needs explanation.

Did patients in the control group have no nausea?

**Response 6.** We would like to again apologize for the confusion related to our manuscript writing style. As we explained in detail in the “Glutamine Supplementation” section of the current revised form of our manuscript, glutamine supplementation was started 1 week before the initiation of oncological treatment. Therefore, the concerned rate of nausea and vomiting presented in the first paragraph of the “Results” section represented the adverse events experienced in the absence chemoradiotherapy. Moreover, since it would not be easy to discriminate nausea and vomiting caused by glutamine alone from that experienced during the delivery of glutamine in conjunction to chemoradiotherapy, which also causes
nausea and vomiting and controlled with antiemetic therapy, the adverse events related with the administration of glutamine alone was chosen to be evaluated and presented.

7- **Comment 7.** b) ARIE, needs explanation in Methods

**Response 7.** ARIE was graded according to RTOG-ARIE scoring criteria and the grade of ARIE reflected the worst grade observed in each patient. A new table with the name of “Table 1” depicting the RTOG-ARIE grading criteria was created as recommended.

8- **Comment 8.** c) "Weight change (WC), which is the absolute difference between pre- and posttreatment weight measures, is a parameter that is independent of pre-treatment weight with the potential to underestimate the value of pre-treatment body mass." ...this goes in Methods and needs reference

**Response 8.** The related statement in the 3rd paragraph of the “Results” section of the original manuscript was carried to the “Method” section under the new subsection with the name “Patient evaluation and scoring of ARIE” in the current revised form of the manuscript with citation of a relevant reference as recommended.

9- **Comment 9.** d) What are RECIST criteria? Needs explanation in Methods.

**Response 9.** At our institution, since we use PET-CT for staging, radiotherapy treatment planning and response evaluation purposes in nearly all cases requiring radiotherapy, we currently use PERCIST response evaluation criteria as standard approach. However, at the time the patients included in the study was being treated, EORTC 1999 recommendations were obeyed for the response evaluation. Therefore, we would like to apologize for that mistake done during the writing process of our study. The response evaluation criteria used in the study should have been EORTC 1999 guidelines. This issue was clarified in the “Methods” section under a new subtitle “Response Assessment and Follow-up”. The
EORTC criteria were summarized in a new table with the name of “Table 2” as recommended.

10- Comment 10. Discussion: a) See the comment 1, the use of words "suggest no tumor protection.." cannot be justified according to weak methodology.

Response 10. As concerned previously by the reviewer in his/her 1st comment, and responded by us accordingly, weak methodology related to our manuscript writing style was overcome by thorough revision of the entire “Methods” section in the light of the recommendations given by the reviewer. Likewise, conclusion was re-designed accordingly by paying attention to not use the word “suggestion”.

11- Comment 11. b) Limitations of the study must be exposed! It seems that not just glutamine but also factors as better socioeconomic status and just better doesn’t change the treatment outcome.

Response 11. Although it seems reasonable to drive such assumptions at first look to our previous methodology presented in the original manuscript, as we tried to clarify the follow up and nutritional issues in the current revised manuscript, the principle concern should be related with the reimbursement policy of social security system in our country and presence or absence of patients’ health insurance rather than the socioeconomic status of the patients. As an example, while some patients with no health insurance coverage could not receive glutamine, other patients from the same socioeconomic level could reach by their health insurance. On the other hand, patients from higher socioeconomic levels could pay for their glutamine although they do not have health insurance. Likewise, the total amount of glutamine to be paid and given by the national health insurance is predetermined by government policies. However, as we aforementioned, this is totally related to troubles of our national social security system, and we believe that such kind of
comparison by taking socioeconomic status in account may confuse the readers, and not be reasonable to be generalized. Nevertheless, such comparisons in another trial may yield impressive results.
Reviewer 2

We appreciate and would like to thank Reviewer 2 for his/her worthwhile review and kind comments which we believe will significantly increase the value of the current revised version of the manuscript.

Major compulsory revision

1. **Comment 1.** This MS summarized results of a monocentre retrospective data analysis with the aim to evaluate whether (high dose) oral glutamine supplementation can cause harm in patients with LA-NSCLC. The rationale to perform this analysis remains unclear: the authors themselves mentioned in the Background chapter that glutamine supplements can contribute to reduce the extent of tissue damage caused by CRT. Suddenly in the last paragraph of this chapter, the authors mentioned some “concerns” which have been raised whether glutamine can stimulate tumor growth etc. – unfortunately, no references are cited to support this “hypothesis”! Is that only a “rumour”? As recently outlined in ref. 39, glutamine can be seen as conditionally indispensable in oncology without the risk to support tumour growth or to decrease survival rates. What is, thus, the reason to present these retrospective data? If the safety (long-term or acute or both?) of glutamine application is the primary goal, survival can not be the unique evaluation variable (as the Title suggest). Moreover, several positive aspects of glutamine treatment (see Table 2) are not adequately honoured. A more “objective” data presentation (eg, Effects of oral glutamine on ... in..) may be more adequate.

2. **Title.** See general comments above.

**Response 1.** This study takes its root from the hypothesis whether many agents exerting strong radioprotective effects on normal tissues, have possible protective actions against the effect of radiotherapy on the tumor tissues as well. There were conflicting results on the
issue. For example, amifostine, which is a strong radioprotector, was found to have no detrimental effects on survival outcome in a recent meta-analysis by Bourhis et al (Bourhis J et al. J Clin Oncol. 2011), suggesting no tumor protection or growth stimulating action. On the contrary, erythropoietin, which has been used successfully for stimulation of erythropoiesis in various cancers, negatively impacts survival outcomes for most tumor types (Bohlius J et al. Lancet 2009). Therefore, based on the strong radioprotective effects by the Gln as previously shown by two studies (Topkan E et al, Lung Cancer 2009, and Algara M et al, Int J Tadiat Oncol Biol Phys, 2007), and considering the conflicting results of different radioprotectors on potential tumor growth promoting actions, we carried out this study. We would like to apologize for the misunderstanding and consideration of our starting point as “rumour” by the reviewer, which is totally related to our manuscript writing style. Related paragraph was revised and required modifications were made to clarify our starting point and logic behind this study with citation of relevant references.

Moreover, as we aforementioned, in our previous study, we revealed a beneficial role of oral Gln in the reduction of ARIE incidence and severity, as well as maintenance of body weight, in LA-NSCLC patients treated with C-CRT (Topkan E et al, Lung Cancer 2009), and our primary aim in the current retrospective analysis was to evaluate protective or growth stimulating action of Gln on tumor tissue, if any, in clinical terms by comparative survival analyses according to the status of Gln supplementation. Therefore, the pointed data were not presented in detail in the original manuscript. However, as recommended by the reviewer, the related positive aspects of glutamine treatment noted in our study population has also been presented in this current revised version of the manuscript to increase the relevance of our study. Therefore, we would like to give our special thanks to the reviewer for his/her kind comment, which we believe added much to the impact of our manuscript.
2. **Comment 2.** 3. Background. 1st paragraph: It is mandatory to support the comments with actual references. If not existing, this chapter should be deleted.

**Response 2.** As recommended by the reviewer, the related statements are cited with relevant references.

3. **Comment 3.** 3. Methods - Inclusion criteria: What about nutritive support of the patients? Is there any information available?

**Response 3.** This issue has been clarified in the “Glutamine Supplementation” subsection of “Methods” section by necessary additional information as recommended.

4. **Comment 4.** 5. Results. See general comments above.

**Response 4.** Please refer to responses 1 to 4.

5. **Comment 5.** 6. Keeping the primary aim of the analysis in mind, data presented in Figure 1 are not motivated.

**Response 5.** As pointed by the reviewer, the “Figure 1” is omitted and removed from the manuscript as recommended.

6. **Comment 6.** 7. Discussion. Generally, it is difficult to understand that there is an “absence of data on TG stimulating potential .... of glutamine”. In all intervention studies published yet, side effects of oral glutamine would have been reported. Obviously, the study presented was also not planned to evaluate glutamine “toxicity” but only summarizes effects with or without glutamine supplementation.

Consequently, most of the discussion is focused on the beneficial effects of glutamine compared to no glutamine (see also general comments).

**Response 6.** We would like to again apologize for the confusion related to our manuscript writing style. The only few adverse events related with the administration of glutamine is
nausea and vomiting, and as pointed by the reviewer, we were aware of the fact that the toxicity of any intervention study should be presented besides its advantageous effects on the subject. Therefore, we had planned to present those findings if noted in our patient records. However, as we explained in detail in the “Glutamine Supplementation” section of the current revised form of our manuscript, glutamine supplementation was started 1 week before the initiation of oncological treatment. Therefore, the concerned rate of nausea and vomiting presented in the first paragraph of the “Results” section represented the adverse events experienced in the absence chemoradiotherapy. Moreover, since it would not be easy to discriminate nausea and vomiting caused by glutamine alone from that experienced during the delivery of glutamine in conjunction to chemoradiotherapy, which also causes nausea and vomiting and similarly responses to appropriate anti-emetic therapy; therefore, the adverse events related with the administration of glutamine alone was chosen to be evaluated and presented.

7. **Comment 7.** 8. Conclusions: prospective randomized clinical trials cannot be requested to evaluate glutamine toxicity!

**Response 7.** As recommended by the reviewer, the related statement in the original manuscript has been omitted and removed from the “Conclusion” section of the current revised form of the manuscript. Being aware of the fact that Gln is a safe agent even at high doses except for easily manageable nausea and vomiting, we, in the original manuscript, did not mean to invite large randomized clinical trials to evaluate the potential tumor cell protecting and/or growth promoting actions of Gln rather than evaluation of its toxic effects by Gln. Therefore, we would like to apologize for that misunderstanding that is totally related to our manuscript writing style. However, in the last sentence of the “Conclusion” we invited studies with larger cohorts and/or comprehensive meta-analyses
to enlighten the widely discussed tumor growth stimulating potential of Gln this hot topic in oncologic practice, which has also been emphasized by the reviewer 4.

**Minor essential revisions**

8. **Comment 8.** 9. 2nd paragraph and throughout the MS: the correct standard abbreviation for glutamine is either Gln or Q.

**Response 8.** The abbreviation “Glt” previously used for “Glutamine” has been replaced with “Gln” throughout the text in the current revised form of the manuscript as recommended.

9. **Comment 9.** 10. Ref. 1 to 4: Rather old – there are several actual reviews available describing clinical effects of glutamine.

**Response 9.** The related references have been revised and replaced with the updated new references as recommended.

10. **Comment 10.** 11. Table 1: Please, check the p-values given (038? 042?).

**Response 10.** We would like to apologize for the mistakes done during the writing process of the manuscript. The pointed Table was revised and necessary corrections by using necessary punctuations have been done as recommended.
We appreciate and would like to thank Reviewer 3 for his/her nice feelings about our efforts on the subject, and for the comments of paramount importance which we believe will significantly increase the impact and relevance of results presented in our current revised version of the manuscript.

**Major Revisions**

1. **Comment 1.** Re BMC Cancer manuscript: “Influence of Oral Glutamine Supplementation on Survival Outcomes in Locally Advanced Non-Small Cell Lung Cancer Patients Treated with Concurrent Chemoradiotherapy” by Topkan, Parlak and Pehlivan.

   This is a retrospective investigation on the impact of prophylactic oral glutamine supplementation on survival outcomes of patients with Stage IIIB non-small cell lung carcinoma (NSCLC) treated with concurrent chemoradiotherapy (C-CRT).

   Overall the results from the study indicated that glutamine did not promote tumor growth. Glutamine had no significant effect on the disease progression at the follow-up of 24.2 months, but resulted in reduced incidences of the late esophageal toxicity, reduced treatment delays with shorter treatment course and no significant body weight.

   1. The question posed by the authors is well defined.

   2. The methods used are appropriate and well described.

   3. The data are sound.

   4. The manuscript adheres to the relevant standards for reporting and data deposition.

   **Response 1.** We would like to thank to the reviewer for his/her valuable evaluation and kind comments on our work.

2. **Comment 2.** 5. The discussion and conclusions are adequately supported by the data. To my opinion, the Discussion is too long and needs to be reduced.
Response 2. The manuscript has been revised and “Discussion” section was shortened significantly as recommended with efforts to not lose general style and aims of the study. However, respecting to the recommendations of other three reviewers we had to discuss some additional issues including the limitations of the study. As a result because of additional paragraphs manuscript could only be shortened to a little extent. We wish it will satisfy your valuable recommendation.

3. Comment 3. 6. The limitations of the work are clearly stated.

7. The published work is acknowledged.

8. The title and abstract accurately convey the results.

Response 3. We would like to thank to the reviewer for his/her kind comments on our work. However, to enlighten the readers of the journal and emphasizing the fact that retrospective series can only be useful in generating hypothesis rather than guiding the literature the limitations of present study has been extensively discussed in the last paragraph of the “Discussion” section.

4. Comment 4. 9. Overall, the manuscript is well written. Some sentences, such as on p. 10 (Discussion): “In this setting, results of recent.....” and on same page “Confirming these results, our....” need to be revised.

Response 4. The recommended linguistic revision and modifications have been made in the current revised form of the manuscript, by the help of a professional editing company (Bioscience Writer).

Minor Essential Reviewers

5. Comment 5. The symbol of glutamine is Gln, not GLT.
**Response 5.** The abbreviation “Glt” previously used for “Glutamine” has been replaced with “Gln” throughout the text in the current revised form of the manuscript as recommended.

6. **Comment 3.** The data presented could be of interest for the clinical practice, as there is a concern over glutamine providing to a host with cancer since it may stimulate tumor growth. I would recommend publication.

**Response 3.** We would like to thank to the reviewer for his/her kind feelings on our work.

**Reviewer 4**

1. **Comment 1.** Is the question posed by the authors well defined? The question is good defined. It should be told that it is a glutamine therapy and no supplementation only. The
resulting question is then –> Is my supportive therapy limiting the effectivity of radiochemotherapy in lung cancer patients?

Response 1. We would like to thank to the reviewer for his/her kind review and comments on our work.

2. Comment 2. Are the methods appropriate and well described? The authors are discussing the limitations of retrospective analysis. This method is appropriate but we have missed statistical plans regarding the number of patients who are necessary to give sufficient answers.

Response 2. We totally agree the comment of reviewer on the importance of number needed to signify a moderate difference for any study comparing two or more factors. However, our current study represents an unplanned analysis of our previously reported study (Topkan et al, Lung Cancer 2008) in a larger study population, and all available patients were included. Therefore, we may have missed the chance to conclude more relevantly. But we are happy to mention that we are planning to address the same question in a multi-institutional study in a group of 550 patients to realize 5% survival difference at 3 years. We will be grateful if our present effort be accepted as only hypothesis generating which will guide our planned study.

3. Comment 3. Are the data sound? Probably the groups are very small. The supportive effects were primary end points and are impressive. The secondary endpoint survival is calculated. A correct information needs larger patient groups or the method of metaanalysis (example: Bourhis J et al: MAART project – J Clin Oncol 2011).

Response 3. Methodology section has been restructured with new sub-sections, and re-written by adding information about the nutritional management of patients, toxicity scoring and response assessment and patients’ follow-up. Furthermore, the cohort size
limitation has been discussed as a limitation in the last paragraph of the “Discussion” section. Please also refer to our response the 2nd comment. Additionally, in the last sentence of the “Conclusion” we invited studies with larger cohorts and/or comprehensive meta-analyses to enlighten this hot topic in oncologic practice.

4. **Comment 4.** Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes, despite the missed statistical plan.

   **Response 4.** We would like to thank to the reviewer for his/her kind opinion on our work. For the statistical plan used in our manuscript, please refer to our response to the 2nd comment by the reviewer.

5. **Comment 5.** Are the discussion and conclusions well balanced and adequately supported by the data? The discussion reflects the benefits of glutamine supplementation / administration. It does not explain the critical points of cytoprotection. Do we really know all effects of glutamine on the tumour and normal cells? Do we have enough information regarding the interactions between irradiation / chemotherapy and glutamine?

   **Response 5.** We are in line with the reviewer that all possible mechanisms of action of Gln on normal healthy and tumor cells are not determined yet. However, possible mechanisms of selective radio-/chemoprotection exerted by Gln are extensively discussed in this revised manuscript (Both in “Introduction” and “Discussion” sections).

6. **Comment 6.** Are limitations of the work clearly stated? No.

   **Response 6.** As pointed by the reviewer, a new paragraph about the limitations of our study has been prepared and integrated to the “Discussion” section as the last paragraph as recommended.
7. **Comment 7.** Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes, but it would be useful to look for other studies with cytoprotective agents as amifostine or selenium.

**Response 7.** As the reviewer recommended, we searched the literature for other studies evaluating the cytoprotective effects of other agents. There were 2 most striking results on the issue. One with the strong radioprotector amifostine, which was found to have no detrimental effects on survival outcome in a recent meta-analysis (Bourhis J et al. J Clin Oncol. 2011). On the contrary, the other one was erythropoietin, which has been used successfully for stimulation of erythropoiesis in various cancers, negatively impacted survival outcomes for most tumor types (Bohlius J et al. Lancet 2009). As recommended by the reviewer, we could also integrate information regarding the use of selenium, but we decided to add only these two most commonly practiced radioprotectors (amifostin and erythropoetin) with two conflicting tumor growth stimulating potential by the citation of related references in the last paragraph of the “Introduction” section, and would like to ask the reviewer if it is suitable not to mention about selenium. However, if insisted, we are ready to integrate information about selenium.

8. **Comment 8.** Do the title and abstract accurately convey what has been found? With limitation.

**Response 8.** Abstract section has been revised appropriately to give additional information about our observations on glutamine’s beneficial effects on weight retention, reduction of acute and late radiation induced esophagitis, and prevention of treatment delays.

9. **Comment 9.** Is the writing acceptable? Yes.
Response 9. We would like to thank the reviewer for his/her kind comments on our work. However, in an effort to further improve linguistic style we revised the whole manuscript by the help of a professional editing company (Bioscience Writers).

10. Comment 10. Summary: The real interesting manuscript should be revised and should be stated that survival is a secondary end point of each supportive care. The statistical analysis has to be discussed in extenso. The limitations must be clear and further steps should be defined well, e.g. prospective randomized studies or meta-analysis.

Response 10. The effects of Gln on acute and late radiation-induced esophageal toxicity, BMI change, WC, PWC during treatment, and needs for hospitalization and/or treatment breaks were comparatively analyzed, and related information has been added to “Statistics” section of methodology and also honored in abstract of this current revised manuscript. But, as these issues were previously addressed in our previous study (Topkan et al, Lung Cancer 2008), for this current study, the primary endpoints were determined as overall- (OS), locoregional regression-free- (LRPFS), and progression-free survival (PFS) difference between two cohorts. We are in line with the reviewer that survival outcomes must be accepted as secondary end points for any supportive care study, however, we believe that considering our previous study together with this current one we believe that this issue will significantly be solved. Additionally, in the last sentence of the “Conclusion” we invited studies with larger cohorts and/or comprehensive meta-analyses to enlighten this hot topic in oncologic practice.