Format for ANSWERING REVIEWERS

August 17, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: Manuscript revision NO: 4161).

Title: METASTATIC TYPE 1 GASTRIC CARCINOID - A REAL THREAT OR JUST A MYTH?

Author: Simona Grozinsky-Glasberg, Dimitrios Thomas, Jonathan R. Strosberg, Ulrich-Frank Pape, Stephan Felder, Apostolos V. Tsolakis, Krystallenia I. Alexandraki, Merav Fraenkel, Leonard Saiegh, Petachia Reissman, Gregory Kaltsas and David J. Gross

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

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

(1) Reviewer 00033061

General observations This manuscript is generally well written and organized. It is quite unexpected the gender distribution in the population studied. Being the population under study composed of type 1 gastric carcinoid, which develop in the context of chronic atrophic gastritis, I would have expected a high prevalence of female gender. If possible, enter a comment about this.

We thank the reviewer for this suggestion; a comment addressing the almost equal prevalence of GCA1 in both male and female patients in our cohort was included (Revised manuscript, "Results", page 6, paragraph 2, lines 7-9).

Only minor imprecisions should be corrected:
- In the ”results” section (page 6, Basal Evaluation, second paragraph) the authors said that EUS is done to everyone while in the “methods” section it is written that EUS has been made in 6 out of 20 patients.

We thank the reviewer for this observation and we apologize for the misunderstanding; EUS was intended to be performed in all patients; however, it was actually performed in only 6/20 patients. We addressed this point and correct the sentence as required (Revised manuscript, "Results-Basal evaluation", page 6, paragraph 4, line 24).

- Speaking about signs of aggressiveness, the authors write that these one were available in 12 patients .... but they do not explain why these are missing in the other 8 patients (i.e. 40% of the population) ... perhaps because there are no EUS data (but they’ve just said that it was made in all the patients).

Regarding this specific comment, we were referring to signs of aggressiveness found at ***the initial biopsy*** *(as mentioned in the text) taken during the first gastroscopy/EUS. We highlighted this comment in the text. (Revised*

manuscript, "Results-Basal evaluation", page 6, paragraph 4, line 25).

- Again in the “results”, “Treatment” section, paragraph 2 the authors categorize 11 patients in the group of grade 1 and 9 patients in the group of grade 2 : this does not match Table 2.

We thank the reviewer for this important observation; we reviewed our data and made the appropriate corrections in the text as well as in the Table 2: Ki-67 was available in 17 out of the 20 patients included; eleven tumors were defined as ENETS grade 1 (Ki-67 ≤ 2%) and six tumors as grade 2 (Ki-67 between 2%-20%) (Revised manuscript, "Results-Treatment", page 7, paragraph 3, lines 17-19& Table 2).

(2) Reviewer 01164511

Grozinsky-Glasberg et al. reported a multicenter, retrospective analysis describing characteristics and treatment procedures in a wide group of patients with metastatic gastric carcinoids type 1 (GCA1). The topic is interesting and the paper is well designed and written. The authors should add a short paragraph in the discussion section on the future perspectives in the therapy of advanced and aggressive GCA1. Please, briefly describe the potential applications of interferon-beta (Vitale G. et al. Cancer Res 2006; 66(1):554-62. Vitale G. et al. Am J Physiol Endocrinol Metab 2009; 296(3):E599-66. Caraglia M. et al. Curr Cancer Drug Targets 2009; 9(5): 690-704); pasireotide (Wolin EM, et al. Cancer Chemother Pharmacol. 2013 Jun 14); dopamine agonists and dopamine/somatostatin chimera (Kidd M et al. Regul Pept. 2007 Oct 4;143(1-3):109-17.).

We added a paragraph regarding these future perspectives in the treatment of aggressive type 1 GCA, as requested by the reviewer; we also used the suggested references (Revised manuscript, "Discussion", page 10, paragraph 3, lines 26-29, and page 11, paragraph 1, lines 1-8).

(3) Reviewer 00004159

This is a useful retrospective study of a rare disease and provides helpful information on risk factors and prognosis in a cohort of 20 patients.

Major comments:

1. The description of patient characteristics is confusing, specifically in relation to baseline imaging:

a. The paragraph on page 5 entitled “Imaging Assessment” reads: “Seventeen patients underwent 111In-pentetreotide scintigraphy (Octreoscan) or Gallium68-DOTA-TATE/-TOC/-NOC PET at diagnosis. Thirteen patients also underwent computerized tomography (CT) of the abdomen.” …which appears to suggest that of the 17 patients who underwent functional imaging, 13 also had a CT. I assume that this cannot be the case but the manuscript doesn’t specify who had functional imaging, who had CT and who had both (or neither). It might be helpful to include these details in the table. It is surprising that not all patients had a CT scan – can the authors explain.

We thank the reviewer for this comment, and clarify the imaging issue by introducing 2 columns in the Table 3 and describing in details each method used in each patient and its results. All patients underwent imaging assessment (CT, SRS/Ga^{68}-PET-CT, or both), as one may see in Table 3. A short paragraph was introduced to clarify this point (Revised manuscript, Materials and methods, “Imaging assessment”, page 5, paragraph 2, lines 6-9).

b. The following paragraph entitled “Endoscopic and histopathological assessment” (page 5) states that 6/20 patients underwent EUS at baseline but the paragraph on page 6 entitled “Basal evaluation (at diagnosis)”

states that EUS was performed in all patients. Can the authors please explain this discrepancy.
*This point was already addressed and revised at the request of reviewer **00033061**. As mentioned, EUS was intended to be performed in all patients; however, it was actually performed in only 6/20 patients. We address this point and correct the sentence as required (Revised manuscript, "Results-Basal evaluation", page 6, paragraph 4, line 24).*

2. The same paragraph (page 6) refers to “Signs of aggressiveness or invasiveness at first biopsy” but includes in these the presence of lymph node metastasis. Is this intentional? If so, it needs to be made clear that the “first biopsy” includes surgical/endoscopic resection specimens.

We thank the reviewer for this important comment. It was unintentional, as the data on the lymph node involvement by metastatic disease at diagnosis came from the imaging results and not from the histo-pathology. We made the appropriate correction (Revised manuscript, Results - "Basal evaluation-at diagnosis", page 7, paragraph 1, lines 1-5).

3. Page 6 (methods) – please describe statistical tests used in this study. I am concerned that simple t tests have been used which would not be appropriate as I suspect the datasets were not normally distributed.

We thank the reviewer for this comment and apologize for the misunderstanding. Nonparametric ANOVA (Kruskal–Wallis one-way ANOVA) was used to assess and compare different parameters (such as the mean age at diagnosis, the size of the largest tumor, the KI67 etc.) at diagnosis (Table 4), and for the levels of gastrin at diagnosis and following surgical treatment/at last visit (Table 3). Post hoc comparisons were made using Mann–Whitney U test. A p value < 0.05 was considered statistically significant. We address this point and clarified it in the revised manuscript, Statistical analysis, page 17.

4. The observation that serum gastrin levels decreased in all patients (page 9) is interesting and in some cases confusing.

a. Presumably, the gastrin levels given in Table 2 are those from baseline assessment. Could post treatment levels also please be included for individual patients?
The gastrin levels given in Table2 & 3 were indeed from baseline assessment. We included now, as requested, the last gastrin levels, when available, for individual patients (Revised manuscript, Results - "Laboratory and imaging assessment at diagnosis", page 8, paragraph 2, lines 7-10; and Table 3)

b. There is no value given here for 6 of the 20 patients – this needs to be clarified when the mean concentration is quoted elsewhere in the manuscript.
In 6 patients there was no available data on gastrin levels; this point is now addressed in the manuscript as required (Revised manuscript, Results - "Laboratory and imaging assessment at diagnosis", page 8, paragraph 2, lines 7-10).

c. If the value is unknown in these patients, how was the decrease in gastrin levels determined?
The levels of gastrin were available, as mentioned, in 14 out of the 20 patients included, and decreased at last evaluation in the same patients. In the remaining six patients, there was no available data on gastrin, whatsoever.

d. In those patients whose treatment included neither antrectomy nor the use of SSAs, (patients 6, 13 & 14), what mechanism is responsible for a decrease in serum gastrin concentration?. More detail on this would be informative.
As mentioned in the manuscript, the patients who underwent wedge resection also had antrectomy (Manuscript, Results – “Follow-up assessment and treatment outcome”, page 9, paragraph 2, lines 9-10);

therefore, the source of hypergastrinemia was excised, and the gastrin levels went down also in these patients.

5. Please include follow up in each individual patient in the outcome column. It would not be appropriate to consider the patient to be cured if the follow up period was short.
A follow up column for each individual patient has been included now in Table 3, as requested.

6. Page 10 and table 3: are the subgroup of patients with metastatic GCA1 (n=20) included in the total (n=254)? If so maybe they should be excluded and the comparison made between metastatic and non-metastatic cases.
We thank the reviewer for this comment. However, the collection of data on 254 patients coming from 5 international referral centers was extremely difficult and time consuming, and therefore it is practically impossible at this stage to go back to the datasheets of all patients, exclude the metastatic one, and perform again the whole analysis. Moreover, looking at the data presented in Table 4 (previously Table 3), it is clear that even if we would be able to take out the data coming from the metastatic patients from the whole group, the difference between the 2 groups (the non-metastatic GCA1 and the metastatic GCA1) will be even more dramatic.

7. The importance attached to the use of SSAs seems overstated. The authors go so far in discussion as to recommend this as first-line treatment in patients with large (>1cm) tumours. Given the evidence in the literature that, once started, treatment with SSA should be continued indefinitely, it seems that the observed response in this study is insufficient to make such a recommendation and I would suggest a more guarded statement be used instead.
We thank the reviewer for this observation and completely agree that there is still insufficient experience regarding treatment with SSAs in patients with GCA1. We have changed the paragraph and addressed this point, as suggested. (Revised manuscript, Discussion, page 12, paragraph 4, lines 30-31, and page 13, paragraph 1, lines 1-8).

Minor comments
1. Page 6 – It is arguable whether Crohn's disease should be called an autoimmune condition
We agree with the reviewer, and deleted Crohn's disease (Revised manuscript, Results, page 6, paragraph 2, and lines 10-12).

2. Page 11, 3rd paragraph – change GAC1 to GCA1 for consistency
3. Table 3, column 3 – change GA1 to GCA1 for consistency
We performed the corrections as requested.

(1) <u>00504704</u>

This is an interesting report and is well written. My only concern if whether or not all the patient they describe as having type I gastric carcinoid actually had type I gastric carcinoid. There should be strong documentation that these patients had atrophic gastritis and not a gastrin producing NET, or a type III carcinoid. comments This paper would be stronger if the authors show a table with all the features which allowed them to designate their patients as type I carcinoids - when available - B12 levels, gastrin levels, histology showing atrophy, acid secretion , prior use of PPI medicines, etc

We thank the reviewer for his important suggestions.
We added a table (Revised manuscript, Table 2) regarding the features associated with the diagnosis of CGA1 in our patients. It is important to mention that none of the patients presented with ZES and the associated MEN1

syndrome or with characteristics of type 3 gastric carcinoids (Revised manuscript, Results, "Basal evaluation (at diagnosis)", page 6, paragraph 3 and lines 21-23).

The authors stated that gastrin levels fell in all patients on therapy. They should explain how that happens in subjects with a wedge resection of the primary lesion and no other therapy.

As mentioned in the manuscript, the patients who underwent wedge resection also had antrectomy (Manuscript, Results – "Follow-up assessment and treatment outcome", page 9, paragraph 2, lines 9-10); therefore, the source of hypergastrinaemia was excised, and the gastrin levels went down also in these patients.

3 References and typesetting were corrected

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

Sincerely yours,

Simona Glasberg, MD
Neuroendocrine Tumor Unit,
Endocrinology & Metabolism Service,
Department of Medicine,
Hadassah-Hebrew University Medical Center,
P.O.B. 12000, Jerusalem
Israel 91120
Tel. 972-2-6776788
Fax 972-2-6437940