Author's response to reviews

Title: HER2 overexpression and amplification is present in a subset of ovarian mucinous carcinomas and can be targeted with trastuzumab therapy

Authors:

   Jessica N McAlpine (mcalpine_j@yahoo.com)
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Version: 2 Date: 11 July 2009

Author's response to reviews: see over
Thank you for the thoughtful comments and suggestions provided on our paper. **HER2 overexpression and amplification is present in a subset of ovarian mucinous carcinomas and can be targeted with trastuzumab therapy.**

The manuscript has been changed with track changes apparent. We are pleased with this revised submission and feel it is a stronger product. In addition to the changes made in the manuscript we would like to address in this letter the reviewers specific comments/concerns. Their original text is included with our comments seen in a contrasting colour.

Please do not hesitate to contact us with questions.

On a separate note: prior email attempts at changing me to corresponding author have been unsuccessful. Can you please tell me how on your automated submission system I am able to do this?

Sincerely,

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Reviewer’s report #1
Title: HER2 overexpression and amplification is present in a subset of ovarian mucinous carcinomas and can be targeted with trastuzumab therapy
Version: 1 Date: 1 May 2009
Reviewer: A Serrano-Olvera
Dear Editor:
Regarding this manuscript: “HER2 overexpression and amplification is present in a subset of ovarian mucinous carcinomas and can be targeted with trastuzumab therapy”, authors: Jessica N McAlpine, Kimberly C Wiegand, Russell Vang, Brigitte M Ronnett, Anna Adamiak, Martin Kobel, Kenneth D Swenerton, David G Huntsman, C Blake Gilks and Dianne M Miller.
I would like to express my comments:
1. Is the question posed by the authors well defined?
a. Yes, it is.
2. Are the methods appropriate and well described?
a. Yes, the methods are described adequately.
3. Are the data sound?
a. The results observed by the authors are similar that those reported by another.
4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
a. Yes, but a previously report informed results from 142 samples’s tissues of mucinous ovarian carcinoma.
5. Are the discussion and conclusions well balanced and adequately supported by the data?.
a. Regarding the role of trastuzumab in mucinous ovarian cancer, I feel that the conclusions do not have a solid base since 2 patients were only dealt with the monoclonal antibody. On the other hand, the utility of the HER2 as prognostic factor was recognized in cases with recurrence, but the finding was observed in a small number of cases.
6. Are limitations of the work clearly stated?
a. Yes, it is.
7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
a. Yes they do.
8. Do the title and abstract accurately convey what has been found?
a. Yes, it do.
9. Is the writing acceptable?
a. Yes, it is.
I recommend to fit the conclusions to a become attached state but with the findings observed in the analysis and not only by the clinical experience that the authors had when trying 2 patients. Nevertheless, it is important to let the door to designed clinical trials open. I consider that the changes that the authors must perform must be considered like essential minor revisions.
I am thankful for the opportunity and the offered confidence to allow to review this interesting study.
Alberto Serrano, MD.

Thank you. I think we have softened our conclusions somewhat and are honest about the limits in interpreting response to therapy in the two clinical patients, limits in number of cases, limits of technique, etc. However, this is a rare tumor (perhaps even more rare than previously recognized, reference 4 of manuscript) with no good treatment options and we have demonstrated a rate of HER2 overexpression and amplification that is greater than in breast cancers where it is routinely tested for and targeted. Ultimately, more cases are needed in order to make more broad conclusions regarding prognostic and predictive potential.

Reviewer's report #2
Title: HER2 overexpression and amplification is present in a subset of ovarian mucinous carcinomas and can be targeted with trastuzumab therapy

Version: 1 Date: 10 May 2009
Reviewer: Karina Dahl Steffensen

Reviewer's report:
Major Compulsory Revisions: Comments number: 2,4,9
Minor Essential Revisions: Comments number:3,5,6
Discretionary Revisions: Comments number:1,7,8

The HER2 overexpression and amplification is present in a subset of ovarian mucinous carcinomas and can be targeted with trastuzumab therapy.
The paper by McAlpine et al investigates the HER2 expression and amplification in mucinous ovarian carcinomas and in mucinous borderline ovarian tumor. Furthermore, they aim at evaluating the potential for trastuzumab therapy for this histological subtype of ovarian cancer.

Development of targeted treatment represents a new option with the hope of improving the outcome for patients with epithelial ovarian cancer. Promising results have been observed in VEGF directed therapy, where rather high response rates have been reported, even in heavily pretreated patients. Nevertheless, results from HER2 directed therapy has been less promising and far from the results in breast cancer. The growing literature on HER2 expression in ovarian cancer also indicates that only a minority (<10%) of ovarian tumors are strongly positive for the HER2 receptor compared to breast cancer where 20-30% of breast tumors show HER2 overexpression.

Epithelial ovarian cancer consists of many subtypes of ovarian cancer and recent data has shown that some subtypes of ovarian cancer show different types of mutations compared to the more common serous type (ref: Kurman RJ and Shih leM, J Int J Gynecol Pathol. 2008 Apr;27(2):151-60). Therefore, it may be of relevant interest whether some subtypes of ovarian cancer show HER2 overexpression rendering this receptor of potential interest for HER2 targeted therapy for some subtypes of ovarian cancer where current available treatment is rather limited.
Overall, this manuscript is interesting and well written. The study design and methods used are appropriate. Although, it would benefit for some improvements and alterations, especially concerning the conclusion that HER2 amplification is not of prognostic significance. Please see the comments below.

I have the following comments and suggestions for the authors:

1. Page 3:
   Line 9: I believe the statement that 15% of breast cancers show HER2 amplification/overexpression is underestimated according to more recent literature (and the latest reviews: Dean-Colomb W et al, Eur J Cancer. 2008 Dec;44(18):2806-12. Epub 2008 Nov 18., Park JW et al, Clin Breast Cancer. 2008 Oct;8(5):392-401) that seems to agree that approx 25% of breast cancer show HER2 overexpression/amplification. We appreciate the range of numbers that have been published on this issue. However, we maintain that in the larger series, published in excellent journals, from population-based cases our cited value of 15% is accurate (see manuscript references, and added Cheang et al, JNCI 2009 reference 10).

2. Page 5:
   I strongly believe that the methods section would benefit from a flow chart or a more detailed explanation on how 34+3+3 ovarian carcinomas and 15+7 borderline tumors add up to the 33 ovarian cancers and 16 borderline tumors as described in the results section. Agreed. We had this in a prior version and had been cut. Thank you for suggesting and we agree it clarifies significantly. What is the reason for the missing samples? Now addressed in text of paper and referenced. The selected cases consist of cases from different sources. Have all the paraffin embedded tumors from the different sources been uniformly treated and preserved under the same circumstances? (same formalin treatment, preservation time, etc.?) All cases were handled, fixed, processed, and stored identically. Although blocks may have been of differing age/time periods, the breast cancer literature encompassing thousands of cases suggest testing of HER2 in these circumstances is valid (i.e., issues of antigen degradation).
   Were all the tumors from the primary debulking surgery prior to any chemotherapy treatment? Now added this information-see Table 1. The authors should address in more detail the characteristics of the included patients, e.g. age, grade, stage, and also parameters like carcinosis and residual tumor after primary debulking surgery since these are classic parameters with influence on prognosis beside the potential influence of HER2 expression. Done. See table and results.

3. Page 7:
   Line 4 from bottom: “were had”? (just a minor detail) Sentence omitted/changed.

4. Page 8:
   Line 5: I might be mistaken, but I am not sure for what reason the statistics described on page 7 were chosen and used for describing the lack of association between HER2 status and recurrence.
   A much simpler association could be provided by showing the data in a 2 x 2 cross tabulation when reporting a significantly or non-significantly association between variables and to show these data as cross tabulations with p-values (using #²statistics or Fisher Exact test) in the tables. In that way the data
compared are presented more clearly to the reader.
Ovarian Cancer HER2 normal HER2 amplified Total
No recurrence 17 6 23
Recurrence 10 0 10
Total 27 6 33
#² = 3.19, P = 0.07 Fishers Exact test: P = 0.09 (one-tailed), 0.14 (two-tailed)

The statistical section of this manuscript has been overhauled and which tests were performed is (we think) quite clear now and in keeping with Dr. Steffensen’s suggestions. A statistician was consulted and added to the list of authors.

5. Page 7:
Line 19: Trastuzamab. Please correct to Trastuzumab. Done

6. Page 10:
Case 2: This is an unusual clinical presentation (brain metastasis) for a patient with ovarian cancer. We used to think that mucinous ovarian tumors were very common, but newer data seems to agree that most mucinous tumors apparently growing in the ovaries are metastatic disease from other primary tumors such as colorectal tumors or cervical tumors. Clinicians should be aware when an answer comes back from a frozen section that says mucinous tumor – they should comprehensively look for a primary occult cancer. Were any pathology revision performed on this patients’ tumor to re-evaluate the diagnosis? Pathology review performed on all and now stated in manuscript. Clinical histories of all cases were also reviewed to rule out the possibility of a GI cancer metastatic to the ovary. These are primary ovarian tumors.

In addition, in this version of the manuscript we have emphasized why a brain met might be very plausible in the individual described as we know her therapy could not cross the blood-brain barrier.

7. Page 11:
Line 7-10: Regarding correlation between IHC and FISH. Other Authors have also reported on correlation between HER2 IHC and HER2 FISH in ovarian cancer. (E.g.: Steffensen KD et al: “The prognostic importance of COX2 and HER2 expression in epithelial ovarian cancer.” Int J Gynecol Cancer 2007, 17, 798-807). I think it would be relevant to add this reference to the discussion since it is in line with and supports the data discussed in this section. This reference is an excellent paper and was read/reviewed in doing this project. The problem in most papers looking at HER2 expression in ovarian cancer, including the above reference, is that specific histologic subtypes were not separately described. The background “noise” in trying to look for HER2 in ovarian serous cancers (with a high frequency of aneupoloidy, etc) is very different than the situation in breast or in ovarian mucinous tumors. In addition, these papers differ on the rate of concordance between IHC and FISH (reference 22). To submit one reference that was in the context of all EOC seemed misleading.

8. Page 11:
Line 11: Have the authors considered that the mismatched results may be due to intratumoral heterogeneity of HER2 status, especially in TMA where only a smaller fraction of the tumor is evaluated for HER2 expression and therefore the
evaluated spot may not always be representative for the tumor? We were delighted to share this part of the story in our manuscript. See additional text and figure 5.

9. Page 8:
The major caveat in the presented paper is the missing data on patient characteristics, survival data and time to recurrence – especially concerning that the authors are making a conclusion of the prognostic impact of HER2 status. (This was also not a part of the presented aims of the study). The authors describes that 10 out of the 33 ovarian cancer cases recurred. I guess none of the borderline tumors recurred due to their usually very good prognosis? How long was the follow up time for recurrence? If the majority of the ovarian cancer cases were FIGO stage I and II then a much larger material is required to make any firm conclusions on the prognostic impact of HER2 expression in mucinous tumors. Since the number of events (recurrence and deaths) in stage I and II tumors are rather low then usually a very large number of included patients are necessary to achieve sufficient statistical power to estimate the prognostic value of a potential marker.

In this case no survival data are reported and I think that the authors should modify (or better leave out) their conclusion that HER2 is not of prognostic significance since I am not convinced that this statement if adequately supported by their data in this small patient material. We have added all clinical information and accompanying survival data we had available and feel this greatly strengthens the paper. We appreciate the suggestion and encouragement to include what had been pared out.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests.

Reviewer's report #3
Title: HER2 overexpression and amplification is present in a subset of ovarian mucinous carcinomas and can be targeted with trastuzumab therapy
Version: 1 Date: 10 May 2009
Reviewer: Adelaida Garcia-Velasco

Reviewer's report #3:
1. Objectives:
   - The question posed by the authors is well defined.

2. Methods:
   - Statistical analysis is not properly described. The tables and numbers of your analysis are not shown. What kind of data base and statistical program did you use? Did you do a cross tab with Chi-square analyses to asses the association between HER2 amplification and prognosis? Did you try a survival analysis using time to recurrence as a target? Did you analyzed the prognostic impact of
established prognostic factors as FIGO stage, type of upfront surgery or performance status at diagnosis? None of these data is described in the article and in my opinion they are essential for the reader in order to fully evaluate the prognostic implication (or not) of HER2 amplification. **Added and described now. See reviewer #2 responses and new version of the manuscript. A statistician was consulted and added to the list of authors. Thank you.**

Minor essential revisions:
- You should describe if you had central pathology revision and if the immunohistochemistry was reviewed by one, two, or three pathologists. **Done.**
- The two cases described, are included in the case series with full HER2 analyses? It is not stated clearly. You should clarify it on the "case selection" paragraph and on Table 1 (results. **Done—see flowchart Figure 1. Cases not included. Flowchart describes retrospective cases. The clinical cases were identified at time of recurrence then tested and treated and followed prospectively.**

3. Results
Major compulsory Revisions:
- Clinical data of the series is not described neither in the text nor at the presented table. This information is essential to evaluate the prognostic analysis. **Done**
- The comparison of the HER2 expression or amplification in order to determine if it has "changed from the time of initial presentation to recurrence" stated as one of the objectives of the study is not described (or not done?) in the two cases treated with trastuzumab prospectively. **Done.**
- The statistical analysis is not shown. **Done/shown**

Are the data sound? Discretionary revisions.
You do have a good number of patients, all of them with a subtype of ovarian cancer that has scarcely been studied in this setting as a different entity from epithelial ovarian cancer. But, I think you can take more interesting results of this series if complete the analysis with data of clinical prognostic factors, and maybe you should wait and do the prognostic analysis with a greater number of events (recurrences). **Of note in terms of power analysis 41 cases would be needed to show prognostic significance.**

4. The manuscript adhere to the relevant standards for reporting and data deposition.
5. The discussion and conclusions well balanced and adequately supported by the data.
6. The limitations of the work are not clearly stated: Discretionary revisions.- You do not describe if the clinical data of the patients are not fully shown because you don't have access to them or for other reasons. **Had access to but had pared down paper and omitted in the originally submitted version. Now have included again.**

7. The writing: the article needs some language corrections before being published. you should carefully revise the description of the cases. **We think/hope these have all been addressed. Thank you.**

**Level of interest:** An article whose findings are important to those with closely related research interests
**Quality of written English:** Needs some language corrections before being published
**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics. *Strengthened and easy to comprehend now. Thanks.*

**Declaration of competing interests:**
I declare that I have no competing interests