Title: Prognostic significance of multidrug-resistance protein (MDR-1) in renal clear cell carcinomas: a five years follow-up analysis.

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Version: 4 Date: 23 November 2006

Author's response to reviews: see over
Author's response to reviews

Definitive Title: 2306172411155395 Prognostic significance of multidrug-resistance protein (MDR-1) in renal clear cell carcinomas: a five years follow-up analysis.

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Version: 2
Date: 5 November 2006

Author's response to reviews:
Thank you for consideration of our manuscript for publication in your journal. We are very grateful for your precise and punctual revisions and for your lots of useful advice. By this cover letter, we want to provide point-by-point responses to your comments. Our paper has been written by observing and respecting the journal style. We have tried to perform major compulsory revisions, minor essential and discretionary ones, so obtaining a new revised and correctly formatted manuscript. In light of the reviewers’ comments, below, you can read a detailed description of all changes we have made since the previous version.

Reviewer's report 1

Previous Title: 2306172411155395 Multidrug-resistance protein (MDR-1)'s prognostic significance in renal clear cell carcinomas: a five year follow-up analysis.

Version: 1
Date: 2 October 2006
Reviewer: Yoichi Mizutani

Reviewer's report:
General
This manuscript has demonstrated that high expression of MDR-1 in RCC may predict worse prognosis. While the studies conducted are of interest, the number of examined was too small to allow valid interpretations of the results.

Pages 3-4. Major clearness has been made about study tumoral population. A previous survey was performed on an initial renal tumour population, represented by 30 RCCs (clear cell type), 3 RCCs (sarcomatoid type), 2 RCCs (cromophobe type), 1 RCC (papillary type) and 1 oncocytoma. This first research was directed to specify the most important prognostic factors in renal neoplastic pathology. By preliminary univariate analyses of the different histopathological, immune-histochemical and clinical parameters, we could identify MDR-1 as the only immune-histochemical factor and tumour stage as
the sole histopathological parameter that were characterized by values that were close to statistical significance. To standardize our study population, we have selected only RCCs (clear cell type) for the further investigations and we have removed the other histotypes because, in the initial sample, they represented too small numerical fractions to be studied by statistical analysis. Successively, Cox multivariate regression analysis (MVA) has been used to confirm independent predictors of outcome among histopathological, immune-histochemical and clinical variables. Therefore, 30 RCCs (clear cell type) were employed in this following study only when a complete and long-term clinical follow-up was available.

Pages 7-8-9. In the Discussion, we have added critical statement (new role of MDR-1 as prognostic factor in renal clear cell carcinoma) about our study and its possible limitations (small cohort of RCC patients, no variety of types of RCCs but only clear cell histotype). Further investigations, maybe on larger and more heterogeneous population, should address to the relationship between MDR-1 expression, renal carcinogenesis, neoplastic progression and degree of differentiation in RCC. Anyhow, our results stimulate promising hypothesis and, as for us, they may be useful not only to predict disease evolution, but also to aid oncologists in the selection of adjuvant post-surgical treatments.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
There are no comments

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
There are no comments

Discretionary Revisions (which the author can choose to ignore)
There are no comments

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions
Level of interest: An article of limited interest
Quality of written English: Acceptable
Statistical review: No
Declaration of competing interests:
I declare that I have no competing interests.

Reviewer's report

Previous Title: 2306172411155395 Multidrug-resistance protein (MDR-1)'s prognostic significance in renal clear cell carcinomas: a five year follow-up analysis.
Version: 1
Date: 8 October 2006
Reviewer: Axel Merseburger
Reviewer's report
General
In this study the authors examine the MDR-1 expression in 30 renal carcinomas by immunostaining. They conclude that high levels of MDR-1 were associated with poorer prognosis in patients with RCC. The authors conclude that MDR-1 is likely to be a prognostic marker for renal cell cancer. I think it is a moderately written, relatively unstructured structured paper with a positive finding in a fairly small patient cohort. However, investigating MDR-1 expression renal cell cancer tissue is an
interesting research approach. In my opinion this manuscript might only be valuable for publication in the BMC Cancer if major revisions were performed. 

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**Major Compulsory Revisions** (that the author must respond to before a decision on publication can be reached)

1. In the discussion there is no critical statement about the study and the possible limitations.

   Pages 7-8-9. In the Discussion, we have added critical statement (new role of MDR-1 as prognostic factor in renal clear cell carcinoma) about our study and its possible limitations (small cohort of RCC patients, no variety of types of RCCs but only clear cell histotype).

2. For the IHC investigations: What tissue was used as negative controls?

   Page 5. For the IHC investigations, serial sections, conducted on our RCC samples, also included non-lesional areas, 5 cm distant from tumoral mass. Negative controls were performed on these sections (the non-lesional ones) and on other sections that comprised normal areas of removed kidneys for surgical non-neoplastic renal diseases; while positive control was executed on sections obtained from a case of infiltrating breast cancer.

3. What adjuvant therapy was used?

   Page 6. 8 patients in stage I have been submitted to adjuvant chemotherapy (Vinblastine 0.2-0.3 mg/Kg i.v.), 1 patient in stage II, 6 in stage III and nobody in stage IV.

4. Which TNM system was used? 2002?

   Page 4. Tumour extent, that, in the course of our previous surveys, was defined by Robson system, in this study, has been revised and classified according to the 2002 TNM system (reported in references 15-16-17, too), for the statistical analyses.

5. Was the surgery open or laparoscopic?

   Page 4. RCC patients underwent open-surgery at the Department of Urology of the University “Federico II”, Naples, Italy, from January 1993 to December 1996. All patients have been treated with radical open-nephrectomy, including resection of peri-nephric fat, Gerota’s fascia, adrenal gland and regional lymph nodes.

6. In Table 1 it says that T2 there is one M+ and 9 M0 with a total of 9 patients, something is incorrect here with the addition!!

   Page 15. Table 1 has been revised, integrated and correctly formatted, in the content, in the label and in the caption, too.

7. Table 2: What means anni??

   Page 16. In Table 2, moreover, years term substitutes for anni and the threshold tumour size has been fixed to 7 cm. Table 2 has been revised, integrated and correctly formatted, in the content, in the label and in the caption, too.

8. Figure 4 is questionable because of the small number of cases and events for a KM-Curve.
Previous Figure 4 has been eliminated because of the small number of cases and events.

9. The English grammar must be revised!

As regards language revisions and the English grammar, we have called for help a native English speaking colleague.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Reference 3: “Stenzel” is correctly spelled “Stenzl”.

In reference 3, we have corrected the name of the first author (Stenzl and no more Stenzel).

2. Reference 11+12+15: the title of the publication is missing.

Pages 11-12-13-14. We have revised the literature, then we have added the titles of the publications that were missing. Other references (n.15-16-17-18-21-34-35) have enriched the bibliography.

3. Table 1: in the size column the 6.4 is incorrectly formatted.

In Table 1, the threshold tumour size has been changed and fixed to 7 cm (no more to 6.4).

Discretionary Revisions (which the author can choose to ignore)

1. Tumor stages should be stated in Roman numbers.

Tumour stages have been stated in Roman ordinal numbers.

2. Result and Discussion section should be separated.

Results and Discussion sections have been separated.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field
Quality of written English: Not suitable for publication unless extensively edited
Statistical review: No
Declaration of competing interests: I declare that I have no competing interests

Reviewer's report 3

Previous Title: 2306172411155395 Multidrug-resistance protein (MDR-1)'s prognostic significance in renal clear cell carcinomas: a five year follow-up analysis.
Version: 1
Date: 17 October 2006
Reviewer: Rodolfo Montironi  
Reviewer's report:  
General  
This is a potentially interesting study. However, I certain number of comments need to be made.  

抗衡 Compulsory Revisions (that the author must respond to before a decision on publication can be reached)  
1. Type of tumour. The authors affirm that they used 30 consecutive carcinomas. According to them the carcinomas were all of clear type. Since there is a variety of types of carcinomas of the kidney, it is hard to believe that they were all of the same type. 

Pages 3-4. Major clearness has been made about study tumoral population. A previous survey was performed on an initial renal tumour population, represented by 30 RCCs (clear cell type), 3 RCCs (sarcomatoid type), 2 RCCs (cromophobe type), 1 RCC (papillary type) and 1 oncocytoma. This first research was directed to specify the most important prognostic factors in renal neoplastic pathology. By preliminary univariate analyses of the different histopathological, immune-histochemical and clinical parameters, we could identify MDR-1 as the only immune-histochemical factor and tumour stage as the sole histopathological parameter that were characterized by values that were close to statistical significance. To standardize our study population, we have selected only RCCs (clear cell type) for the further investigations and we have removed the other histotypes because, in the initial sample, they represented too small numerical fractions to be studied by statistical analysis. Successively, Cox multivariate regression analysis (MVA) has been used to confirm independent predictors of outcome among histopathological, immune-histochemical and clinical variables. Therefore, 30 RCCs (clear cell type) were employed in this following study only when a complete and long-term clinical follow-up was available. 

2. Page 4. There are clinic-pathological data that should go to the results section and should become part of statistical analysis. 

Clinic-pathological data, that were present at page 4 of the previous work, have been inserted in Results at page 6 of this next version. 

3. Page 5. The authors say that inter- and intra-observer variations were evaluated. However, there is no such an information in the text. 

Page 5. Inter-rate reliability between the two investigators examining the immune-stained sections was assessed by the Cohen's K test, yielding K values higher than 0.70 in almost all instances. 

4. Results and Discussion section. This section contains only Results and there is no discussion of the data. 

Page 7. We have created a Discussion section, that has been divided from Results. 

5. Conclusions. This section is quite long and should be designated as Discussion. 

Page 9. We have created a new, more brief, Conclusions section. 

6. Page 7, Second paragraph. The meaning of “inner surface of proximal tubule” is not clear. Do the authors refer to luminal surface? A reference for this paragraph is also needed.
At page 7 of the previous version (and at the same page in the revised manuscript), *luminal surface* term substitutes for *inner surface of proximal tubule*. We have also added references for this paragraph and concept (n. 34-35).

7. Page 14, Table 2. Tumour size. How was the threshold defined?

Page 16. In Table 2 the threshold tumour size has been fixed to 7 cm.

8. Multivariate analysis is missing. This should be done and added.

Page 17. Cox multivariate regression analysis has been done and added. We have inserted other two tables in which results of Cox multivariate regression analysis have been summarized (Tables 3-4).

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**Minor Essential Revisions** (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Page 10. The title of references 11 and 12 is missing.

Pages 11-12-13-14. We have revised the literature, then we have added the titles of the publications that were missing. Other references (n.15-16-17-18-21-34-35) have enriched the bibliography.

2. English needs to be improved.

As regards language revisions and the English grammar, we have called for help a native English speaking colleague.

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**Discretionary Revisions** (which the author can choose to ignore)

There are no comments.

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**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article of limited interest

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

**Statistical review:** No

Finally, we have modified paper title and we have added this sentence in the abstract (Results section):

Page 2. Cox multivariate regression analysis has confirmed that, in our cohort of RCC (clear cell type) patients, the strong association between MDR-1 and worse outcome is independent not only of the adjuvant therapy, but also of the other prognostic parameters (*p* < 0.05).

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In hopes that our new revised research article can be accepted and published and with our best regards,

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