Author's response to reviews

Title: Epidermal Growth Factor Receptor (EGFR) gene copy number (GCN) correlates with clinical activity of irinotecan-cetuximab in K-RAS wild-type colorectal cancer: a fluorescence in situ (FISH) and chromogenic in situ hybridization (CISH) analysis.

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Version: 3 Date: 22 July 2009

Author's response to reviews: see over
Title: Epidermal Growth Factor Receptor (EGFR) gene copy number (GCN) correlates with clinical activity of irinotecan-cetuximab in K-RAS wild-type colorectal cancer: a fluorescence in situ (FISH) and chromogenic in situ hybridization (CISH) analysis.

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Ancona, 22 July, 2009
Dr. Sabina Alam
Scientific Editor
BMC Cancer

BMC Cancer MS: 1277489010276854

Title: Epidermal Growth Factor Receptor (EGFR) gene copy number (GCN) correlates with clinical activity of irinotecan-cetuximab in K-RAS wild-type colorectal cancer: a fluorescence in situ (FISH) and chromogenic in situ hybridization (CISH) analysis.

Corresponding Author: Mario Scartozzi (marioscartozzi@libero.it)

Dear Dr. Alam,

please find enclosed the revised version of the above mentioned manuscript. We found the reviewer’s comments very interesting and we therefore modified our paper accordingly. All changes have been also summarized in an accompanying letter, which contains a point by point reply to the reviewers’ remarks.

We hope that our paper can be now considered for publication in BMC Cancer

Sincerely

Mario Scartozzi, MD

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Point by point reply to reviewer’s comments.

Reviewer’s report 1.

Title: Epidermal Growth Factor Receptor (EGFR) gene copy number (GCN) correlates with clinical activity of irinotecan-cetuximab in K-RAS wild-type colorectal cancer: a fluorescence in situ (FISH) and chromogenic in situ hybridization (CISH) analysis.

Version: 1 Date: 6 July 2009

Reviewer: Esther P Black

Reviewer’s report:

Discretionary:

1. Did administration of irinotecan in combination with 5-FU/leucovorin v. alone change the predictive power of FISH or CISH?
   We do agree with the reviewer’s comment. Unfortunately the number of patients who received irinotecan in combination with 5-FU/Leucovorin was too low to draw any suggestion (please also see Results section, page 8).

2. There are several typographical errors in the Patients and Methods section. Misspells and typos have been corrected throughout the text.

Minor:

1. The ROC plots are mislabeled on the x-axis. The label should read (100%-specificity %).
   We do agree with the reviewer’s comment and therefore we modified the paper accordingly (please also see page 21 & 27)

2. Of the 44 evaluable samples, not all were assayed by FISH although all were assayed by CISH. Do the responders as determined by CISH analysis, with the established cutoffs, encompass the responders as determined by FISH? There is no mention of the overlap or correlation between the two methods. Please describe.
   We do agree with the reviewer’s comment and therefore we modified the paper accordingly (please also see Results section, page 10, Discussion section page 11 and table 2 page 19

Major: none

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

Yes, I have competing interests. We published an article in BMCC describing the use of a gene expression predictor of response to anti-EGFR therapy in colorectal cancer. I am co-founder and president of a company which seeks to pursue clinical use of this predictor.
Reviewer’s report 2

Title: Epidermal Growth Factor Receptor (EGFR) gene copy number (GCN) correlates with clinical activity of irinotecan-cetuximab in K-RAS wild-type colorectal cancer: a fluorescence in situ (FISH) and chromogenic in situ hybridization (CISH) analysis.

Version: 1 Date: 8 July 2009

Reviewer: Pierre Laurent-Puig

Reviewer’s report:

This paper deals with the role of EGFR copy number variation and response to irinotecan cetuximab in patients demonstrating a resistance to irinotecan chemotherapy regimen in a series of 44 patients KRAS wild-type patients. They compared two different methods: the FISH and the CISH. The results seem indicate that an increased copy number of EGFR is prognostic of response and time to progression.

Majors remarks

Although this series is small and probably retrospective the results presented here are of interest. Nevertheless, the authors should investigate the others markers of resistance to cetuximab and among them at least the BRAF mutation status.....

We do agree with the reviewer’s comment: data about B-RAF status may be an interesting asset in this setting. Unfortunately recently presented data from far larger series (J Clin Oncol 27:15s, 2009, suppl; abstr 4068) demonstrated that B-RAF mutation is usually present in 5-6% of K-RAS wild type patients. Therefore given our sample size (44 patients) we can assume that approximately 2-3 patients would present such genetic abnormality making this information not useful to our study purposes.

......and they also need to add a table given the individual data concerning the EGFR copy number by the two technics used (CISH and FISH). They should also try to determine if there is any interest to combine the FISH and the CISH methods.

We do agree with the reviewer’s comment and therefore we modified the paper accordingly (please also see Results section, page 10, Discussion section page 11 and table 2 page 19

Minor remarks

The nature prospective of retrospective should be mentioned in the patients and methods section

We do agree with the reviewer’s comment and therefore we modified the paper accordingly (please also see page 4, Patients and Methods section)

In the result section a typographical error mixed CISH and FISH in the same sentences line 7 page 9

We do agree with the reviewer’s comment and therefore we modified the paper accordingly

The legend of the roc curves is often presented a sensitivity and 1-specificity It seems for me that there is a mistake in the x

We do agree with the reviewer’s comment and therefore we modified the paper accordingly (please also see page 21 & 27)

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a
Title: Epidermal Growth Factor Receptor (EGFR) gene copy number (GCN) correlates with clinical activity of irinotecan-cetuximab in K-RAS wild-type colorectal cancer: a fluorescence in situ (FISH) and chromogenic in situ hybridization (CISH) analysis.

Corresponding Author: Mario Scartozzi (marioscartozzi@libero.it)

Declaration of competing interests: I have received reimbursements, fees, funding from Merck.

Sincerely

Mario Scartozzi, MD

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