Author's response to reviews

Title: The relation between deoxycytidine kinase activity and the radiosensitising effect of gemcitabine in eight different human tumour cell lines.

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

Please find enclosed the revised version of our manuscript entitled: "The relation between deoxycytidine kinase activity and the radiosensitising effect of gemcitabine in eight different human tumour cell lines".

Our modifications and comments of our manuscript, in line with the comments of the referees, in detail:

Referee #1 (Richard L Momparler)
Discretionary revisions
1) The suggestion of the referee to mention the mode of action of gemcitabine of producing a "chain termination" and the role of this for radiosensitisation is included in the discussion session on page 15, para 1.
2) ECV304 cells had a high value of 15.29 nmol/h/mg. The remark of the referee that dCK plays a significant role if it is very low is right. This is published by Kroep et al Mol. Cancer Ther 1, 371-376, 2002. A remark on this has been added to the discussion (p 15/16).

Referee #2 (Mario Del Tacca)
1) The combination index analysis calculated by the Chou-Talalay equation can be used to define the interaction between chemo- and radiotherapy. Leonard et al (Cancer Research 56, 5198-5204, 1996) used this method to determine synergism and the construction of isobolograms after treatment with the combination of chemo- and radiotherapy. We cited to this study, reference 52.
2) In tables 2 and 3, we have substituted the commas with points.
3) We enlarged the figures and included the titles of the x- and y-axis in the figures for CAL-27 and PANC-1 cells. To make these figures more clear, the legends are included into each figure.
4) We moved the sentences describing data on previous studies on dCK activity and sensitivity (references 41, 54 and 55) from the results section on dCK to the introduction (page 4, para 2).

Referee #3 (Ian J Stratford)
1) Abstract, a remark has been included on the relation between gemcitabine phosphorylation and the degree of radiosensitisation (see also below)
2) Page 4, para 1, lines 13/14: the NCI website does not provide information on dCK activity in the NCI-60 cell lines panel. Only a micro-array analysis of dCK (GC32777) is given on the website; this correlated positively with the sensitivity to both ara-C and AC-ara-C. Data for gemcitabine could not be extracted from the website. Because of this we did not add the data of the 60-cell line panel to the paper.

3) Page 4, para 1, lines 17-19: We consider this question as one of the aims of this study. The cytotoxic effect of gemcitabine was correlated by the dCK activity, and we observed an increased radiosensitising effect with a higher gemcitabine dose, this could mean that a higher rate of drug phosphorylation resulted in a greater radiosensitising effect. We rephrased this sentence. In the past a clear relationship between dFdCTP accumulation and drug concentration was observed. A sentence has been added to the introduction and discussion.

4) In the past the accumulation of dFdCTP has been related to the activity of dCK although not all cell lines included in the current panel were evaluated for dFdCTP accumulation. However, since this general pattern was observed in cell lines from a different origin, we feel that a general statement on dFdCTP accumulation and dCK activity can be made.

5) Page 8, para 3, line 1: We deleted “rates”.

6) Page 8, para 3, line 3: The word survival is substituted by “surviving fraction”.

7) Page 9, para 3, line 1: We agree with this comment that true radiosensitisation is observed when the efficacy of radiation is enhanced with drug concentrations that themselves have no effect. We deleted the word “true”, but still consider this finding as an important message.

8) Page 11, para 2, line 10: The dose enhancement factor (DEF) used the calculate the correlation coefficient is the mean DEF, as indicated in the text.

9) We agree with the referees comment that an upwards-bending survival curve is unlikely. Probably, the SRB assay is less suitable in the setting of a radiation with 8 Gy in combination with a cytotoxic agent, because of the large amount of cell kill. Therefore, we deleted this point (8 Gy) from our survival curves, resulting in a continually downward bending curve. As suggested by the referee, we omitted this analysis.

10) Page 13, para 2, line 4: Gemcitabine inhibits DNA repair after treatment with radiotherapy, gemcitabine has already to be incorporated into DNA before irradiation. Therefore, radiosensitisation can occur when gemcitabine is given prior to the radiation treatment (Wachters et al, Int J Radiat Oncol Biol Phys 57, 553-562, 2003) In addition, most of the papers describing the radiosensitising effect of gemcitabine used a 24 h during treatment of gemcitabine immediately before irradiation. Less radiosensitisation was observed when the gemcitabine treatment followed the radiotherapy. In line with the literature, we used this schedule to investigate the cell line and the concentration dependency and the role of dCK.

11) Page 13, para 3, line 1-4: We rephrased our text on this page in line with the comment of the referee.

12) Page 22, table 1: The concentration units for the IC50 and ID50 values are included in the table.

Taken together, the reviewers’ profound evaluation of our manuscript has helped us to change and rephrase part of our experimental work in a more straightforward way. Our manuscript in its revised form has benefited from it and we hope to have convinced you and the reviewers to reconsider our manuscript for publication in BMC Cancer.

Looking forward to a positive response,
Yours sincerely,

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