Reviewer's report

Title: pO2 Polarography, Contrast Enhanced Color Duplex Sonography (CDS), [18F] Fluoromisonidazole and [18F] Fluorodeoxyglucose Positron Emission Tomography: Validated Methods for the Evaluation of Therapy-Relevant Tumor Oxygenation or only Bricks in the Puzzle of Tumor Hypoxia?

Version: Date: 4 December 2006

Reviewer: Jan Bussink

Reviewer's report:

General
In the paper by Gagel et al. a thorough study is presented where the results of several well and less well established methods for hypoxia assessment are used. To my opinion it is important that results are published were multiple different tumors are assessed even if the correlation is limited. There is an abundance of ‘one tumor model’ studies with good correlation rates between different methods. This study shows that if human tumors are studied correlations may vary substantially. Their conclusion that the methods should be tested in prospective studies is, although not attractive, justified.

It is unclear how the patients were selected. Initial number of patients is 38, 36 had Eppendorf measurements; where are the other two? (are those the lymphoepithelioma and lymphoma patients (top of page 4). How did you go to 22 Fmiso, 31 CPD and 18 TP assessments? Please clarify in the text. There are too many abbreviations in the text.

Extensive discussion is presented with respect to outliers. In the end, was it correct to exclude these patients? If the assays would be used in clinical practice would you recognize these 4 patients and reject their test results if the test would be used to select these patients for hypoxia modifying treatment?

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

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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)

Abstract (page 2):
Last sentence of Methods not clear: from CDs…, was it CDS or CPD? Inconsistent when taking into account information on page 5
There is a ‘open door’ in the abstract:
‘ After exclusion of four outliers the absolute values of the Pearson correlation coefficients increased clearly’.

Methods (page 4):
CDS: was this performed in all patients? Or in 32?
Quantification of perfusion (page 5): Unclear, see also comment concerning Abstract. The definition of TP is unclear.
PET (page 5):
Were both scans (FMISO and FDG) performed in all patients?
Were patients immobilized in a head and neck mask to reduce setup variation?
What was the time interval between these two scans?
How many bed positions were scanned?
Which signal-to-background ratio was used?
Two static scans of FMISO were obtained after 120 min, transmission and emission or two bed-positions?
Why did you choose the time interval of 120 min? Dynamic scanning and scanning after 240 min might have improved the results.
Blood was taken – at which time points? Three samples after 120 min or at different times during the “incubation period”? How were FMISO SUVmean and SUVmax calculated?

Results
CDS (page 7): why was the TP only calculated for 18 patients?
PET (page 7): What was the FMISO SUVmean and SUVmax used for? Only mentioned in the results part, no correlation performed.

Correlations (page 7): Why was no correlation of FMISO SUVmean and max performed? Please clarify CDS parameters and replace 0.056 by -0.056 as stated in Table 1 (page 8).

Discussion
Page 10: Discussion on exclusion of patients unclear especially with respect to patient number 1 and 2. Excluding 3 and 4 because of the observation that the tumors were mainly necrotic is OK. However, a part of the discrepancies may be due to differences in setup and variation.

Page 11: Paragraph on p53 is not relevant for the present study, instead discussion of limitations of the study are more appropriate. For instance, FMISO dynamic scanning, tumor cell turn over due to time factor, results on microregional level and immunohisctochemistry.

Conclusions
Page 12: hetereogeneity of the results and limitations of individual assays could be emphasized.

Tables and Figures
Table 1 (page 21): Please clarify different number of patients data are based on: 18, 20, 22... in legend.

Figure 1 (page 22): depict linear best fit and r2?

Figure 2 (page 23): Definition of normoxic and hypoxic tumor based on needle electrode measurements, MISO T/B lower in hypoxic patient! Maybe comment on this (page 19)?

Figure 3 (page 24): Why different layout compared to figure 2?

Discretionary Revisions (which the author can choose to ignore)

What next?: Accept after minor essential revisions

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

Quality of written English: Acceptable

Statistical review: No

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