Reviewer's report

Title: Systematic analysis of 18F-FDG PET and metabolism, proliferation and hypoxia markers for distinction of head and neck tumors

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Reviewer: Tove Gronroos

Reviewer's report:

This manuscript is from a well-known research group at the Radboud University, Nijmegen. The group is especially recognized for optimizing local therapy by means of advanced imaging techniques and biomarkers in the field of clinical oncology.

This is a thoroughly conducted study on 14 primary head and neck cancer xenografts, which were imaged with 18F-FDG small animal PET and evaluated for immunohistochemical (IHC) markers related to metabolism, hypoxia, proliferation and blood perfusion. The aim of the study was to systematically analyze if IHC assessment of molecular markers and PET parameters could distinguish separate head and neck cancer tumors. The main finding of this study was that out of nine analyzed IHC parameters a cluster of 6 random parameters could classify 70% correctly, i.e. differentiate between the tumor lines, whereas 18F-FDG/PET could not.

FDG-PET/CT is a powerful molecular imaging method, which is increasingly being used in radiation planning today. Hence, it is important to acknowledge the advantages and disadvantages that this methodology can supply to a physician in planning the therapy. The current study brings new perspectives to the field.

The aim of the study is important, well defined and the paper is written in good language. In general, the research was conducted meticulously and thoughtfully and presented in a logical order. However, texture analysis and statistical methods are very advanced and from a readers point of view, quite difficult to follow/interpret in both text and figures.

Minor essential revisions

1. On page 6 authors state that animals were injected with FDG 45 min before the PET-scan, but there is no comment on whether the animals were kept anaesthetized during this time period? From Supplementary Figure S1 and the low FDG uptake into the muscle tissue one could interpret that so was done, but for clarity this should also be stated in the text.

The same figure shows a surprisingly low uptake of FDG into the brown fat, any reason/explanation to this?

2. In total, animals (mice) were injected i.v. with 300 ul of liquid. This is a lot for a mouse. Could this amount have any effect on the physiology and/or FDG
biodistribution in the mice?

3. Page 7: Tumors were divided into two pieces; one for IHC and one for radioactivity counting. Please, discuss the possibility that these pieces were not identical/homologous in their expression of markers or for FDG uptake?

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

**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