Reviewer's report

**Title:** Polysialic acid is associated with better prognosis and IDH1-mutation in diffusely infiltrating astrocytomas.

**Version:** 1  **Date:** 7 May 2014

**Reviewer:** Jennifer Chan

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In this brief manuscript, the authors aimed to characterize the expression of NCAM, polySia, and polySia-NCAM in a range of gliomas and to determine whether polySia-NCAM could be of additional prognostic utility in grade II-IV astrocytomas. They found that polySia and NCAM are frequently expressed, and that PolySia is associated with IDH mutation and better prognosis.

**Major compulsory revisions:**

1. Please describe how samples were scored for positivity or negativity for polySia and NCAM. (For example, if only one cell in the core is positive, was this tumor scored as positive? 10% of cells? Majority?)

2. Among grade II-IV gliomas, the reviewers found that polySia was not associated with WHO grade, yet they found that polySia-NCAM was associated with IDH1 mutation. Because it is known that IDH1 mutation characterizes grade II and III diffuse gliomas and secondary GBM, but is not a feature of grade I gliomas or primary GBM, how do the authors explain the lack of association with grade?

3. Similarly, the authors found positive polySia expression to be associated with increasing proliferation as determined by Ki-67 staining, yet they also found that polySia expression was associated with better prognosis. The authors touch on this point briefly, but should expand on these seemingly discordant findings. (The latter association with better prognosis would be consistent with their finding of polySia association with IDH mutation, but the Ki-67 finding is unexpected.)

4. Please specifically state whether polySia-NCAM is still prognostically meaningful after controlling for IDH1 mutation and patient age. Is the survival effect of polysia-NCAM purely a function of its association with IDH1 mutation? Is the claimed utility of polySia-NCAM expression as a new additional prognostic factor for gliomas additional to the prognostic information that can already be inferred from IDH1 status? The authors did make the survival curves looking at “both positive, “one positive” and “both negative”, however that analysis does not clearly address the question because one cannot discern in the ‘one positive’ group what the effect of is of the individual PSA or IDH positives.

5. The antibodies for NCAM, PSA, and polySia-binding fusion protein is not
adequately described in the methods section. Please explicitly describe the source and conditions, at least briefly.

Minor Essential Revisions:
The scale bar and lettering on Figure 1 is very difficult to read.

Discretionary revisions:
In their characterization of staining, the authors classify tumors as positive or negative, and show only images from one positive and one negative tumor. However, as the authors emphasize that polySia-NCAM is a marker of stem cells, it would be informative and would elevate the paper to know whether polySia and NCAM colocalizes with specific cellular subpopulations within the tumors (e.g. in cells positive for stem cell markers or proliferative markers).

**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 statistician.

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