Reviewer's report

Title: Treatment of Lung Cancer Cell Lines with EGFR Inhibitors Generates a Population of Cells with Cancer Stem Cell Properties

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Reviewer: Brigitte Gomperts

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While the authors have responded to the minor revisions and especially improved the statistics and the discussion, they have not addressed the most important point about whether the resistant cell line represents a stem cell population or not. They now no longer claim that the resistant cell line is a cancer stem cell, but that it has stem cell like properties.

One of the claims for "stem cell like properties" is on the basis of the expression of pluripotency genes such as Oct3/4, Nanog and Sox2. The Jaenisch lab has shown that Oct4 expression is shut off in adult cells and claims that Oct 4 is expressed in adult tissues may be due to the fact that levels of Oct4 expression found in such studies were roughly one thousandth of the levels in ES cells, and so could represent random reactivation of Oct4 or activation of a pseudogene. So the levels detected by QPCR are likely so low, that they are of unclear significance. It would be good to show the delta Ct values for this. Sox2 is known to be expressed in the basal and supra basal cells of the adult airway and in some lung tumors, so in this context is not a stem cell marker. The presence of other markers of embryonic pluripotent stem cells is likewise of unclear significance in the adult. The expression of these pluripotency genes also could be artifacts of culture.

Top of page 10 should be changed: Taken together, these experiments indicate that the H1650-ER1 resistant subline expresses markers associated with [embryonic stem cells]. [The significance of this is therefore not clear in adult tissues].

I am not convinced from the data, that the cells from the cultured resistant cell line are truly tumor-initiating cells, and really feel that the in vivo xenografting is needed to answer this question. The title should therefore read: "Properties of resistant cells generated from lung cancer cell lines treated with EGFR inhibitors."The discussion should include the facts that it is unclear what expression of embryonic stem cell markers mean in the context of adult tissues.

My enthusiasm for the paper is therefore diminished. But I think it is important for the results to be published as there are likely other researchers examining EGFR inhibitor resistant cells who would be interested in the data.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.