Reviewer's report

**Title:** Upregulation of Claudin-4, CAIX and GLUT-1 in distant breast cancer metastases

**Version:** 3  **Date:** 18 September 2014

**Reviewer:** Ben Davidson

**Reviewer's report:**

This paper analyzed the expression by immunohistochemistry of Claudin-4, EGFR, CAIX, GLUT-1 and IGF1R in 97 paired primary breast tumors and their distant metastases.

Claudin-4 was the most frequently expressed protein in both primary and metastatic tumors, followed by GLUT-1, CAIX and EGFR. Positive to negative conversion from primary carcinoma to metastasis was observed in 37.5%, 19%, 12%, 6% and 0% of tumors for Claudin-4, GLUT-1, CAIX, EGFR and IGF1R, respectively, while the opposite occurred in 65%, 47%, 43%, 9% and 0% of cases for IGF1R, EGFR, GLUT-1, CAIX and Claudin-4, respectively.

Upregulation of Claudin-4 in the metastases was significantly associated with positive lymph node status, negative to positive conversion of EGFR with negative PR status, high MAI, CAIX upregulation in metastases with younger age and with adjuvant chemotherapy, and negative to positive conversion of GLUT-1 with older age.

This is an interesting paper addressing an important clinical issue.

**Minor Essential Revisions**

**Abstract, Results:** It is unclear whether the p-values represent the total sum of upregulated and downregulated cases for each protein or merely the negative to positive changes. Based on Table 1 these values appear to represent both changes, but this may be stated more clearly in the abstract. In addition, it would have been more informative to perform a comparative case-matched analysis of the entire expression sum at each site using the Wilcoxon Signed Ranks test and provide this value for each protein.

**Abstract, Conclusion:** The authors may consider deleting the word ‘probably’ in the final sentence.

**Materials and methods:** What was the IHC score cut-off? Was any expression considered positive?

**Reference # 20** appears to be in non-English language and should therefore be removed from the manuscript.

**Figure 1:** Staining examples for all 5 antibodies should be shown.
Level of interest: An article of importance in its field

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