Reviewer's report

Title: Expression of marker genes in the lymph nodes predicts the recurrence of squamous cell vulvar carcinoma.

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Reviewer: Matthias Choschzick

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The study analyzed the gene expression profiles of four vulvar carcinomas with positive and negative lymph nodes. There were seven genes significantly overexpressed in positive lymph nodes in comparison to negative lymph nodes of the same patient. The results were evaluated in 20 patients and a predictive value for groin recurrence was found.

There are number of issues with this manuscript:

The aim of the study is a little unclear: is it the prediction of recurrence as in the title or the evaluation of marker genes for lymph node involvement as claimed in the conclusions of the abstract. In the latter case a detailed comparison between sensitivity and

The authors assume that the detection rate for metastases in routine histological examination is low, but they use this method as negative control in their analysis – this is contradictory

The number of HPV positive cases is too low in this analysis (should be at least 20%) because this may strongly influence the expression results – perhaps the results are only applicable in HPV negative patients – this should be discussed or the tumor set must be changed.

RNA is very instable – this may be a disadvantage of the method proposed.

What kind of staging system used the authors? Most tumors in their study are T2, but in our experience most vulvar carcinomas are pT1b or pT1a according the new WHO classification. There are no information about the histological subtype of tumors. Two patients in tabe 1 have the node stage x. This seems peculiar because the study deals with the lymph nodes of them. Do the authors examine the lymph nodes at first surgery or at time of recurrence? The authors give no overview about the number of examined lymph nodes per patient. What is the reason to examine more than one positive lymph node per tumor?

The authors should give more information about follow-up data of the patients in the manuscript

In my view the results of the study confirmed only the occurrence of tumor cells in lymph nodes, but this information can be achieved more accurately by microscopic examination. This may explain the “prognostic” effect found in the
Kaplan-Meier analysis. Therefore, the results only mirror the known adverse prognostic effect of lymph node status in vulvar cancer as the authors also stated. The used cut-off value seems artificial.

In this study frozen material was used, in the routine most people use fixed tissues. This issue may be hamper application in daily practice – this should at least be discussed.

The results about CA12 are interesting. Have the authors similar results in terms of CA9?

**Declaration of competing interests:**

I declare that I have no competing interests