**Reviewer’s report**

**Title:** HPV positive, wild type TP53, and p16 overexpression correlate with the absence of residual tumors after chemoradiotherapy in anal squamous cell carcinoma.

**Version:** 0 **Date:** 17 Nov 2017

**Reviewer:** DC Gilbert

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This is an interesting study looking at HPV involvement, p53 mutation and HIV status in Anal SCC treated with chemoRT that adds further data to the emerging literature on this topic. This is of clinical relevance as at present treatment is determined using tumour stage alone in a one size fits all approach - personalisation using biomarkers should improve outcomes.

The study is well presented and well performed - the scientific analyses are appropriately carried out and described.

My main criticism is that this report only has 6 month data to correlate with the biomarkers analysed - 6 month complete response is a meaningful endpoint but is by no means definitive - 3 year locoregional control rate would be considered the more relevant endpoint with recurrences (local and distant) occurring predominately in the first year, but significant numbers in year 2 and a few in year 3 (ACT 2 data - James et al Lancet Onc 2013).

**Suggestions:**

Title: should make this explicit (and care with 'predict' as this is prognostic not predictive data) i.e. something like 'HPV...... and correlations with complete response to chemo-radiotherapy in anal squamous cell carcinoma. HIV doesn't correlate so care with this in the title.

Background - para 2, more could be said here (or alternatively in the discussion) with respect to the clinical rationale for a better understanding of biomarkers in ASCC (for a review see Jones et al BJC Jan 2017). A discussion around the improved response to HPV+ SCC across tumour sites (HNSCC, vulval etc) and the reasons for this would be relevent too - including p53 status in HPV+/- tumours

Methods - FISH for TP53 - why was this done and why only in the samples with mutations detected? please explain.

Results para 3 - insert para break after 'and 50% more than control' so 6 month CR is its own paragraph. Move the p53 mutation data from this paragraph to the next subheaded paragraph ('mutational status......).
Discussion - the inverse correlation between p53 mutation and HPV status has also been described in HNSCC (Westra Clinical Cancer Research 2008) and Vulval and Penile Cancers again supporting a crucial role in HPV- oncogenesis and potentially resistance to treatment

The importance of Immune recognition in ASCC, whether measured systemically or in tumour samples could also be included in the discussion (Balermpas et al. Oncoimmunology. 2017 Feb 6;6(3):e1288331. Martin D et al. Front Immunol. 2017 Sep 29;8:1225; Gilbert et al Br J Cancer. 2016 Jan 19;114(2):134-7) as this links many of the other observations.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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I am able to assess the statistics

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