Reviewer's report

Title: Centrosome clustering and cyclin D1 gene amplification in double minutes are common events in chromosomal unstable bladder tumors

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Reviewer: Anne Kiltie

Reviewer's report:

This small study of only 22 patients did not have a clearly stated hypothesis and as such appeared to be an exercise in taking a panel of tests applying them to see if anything interesting came out. The English was generally good. However, reading of the text was greatly hampered by the persistent use of percentages rather than crude numbers. It was not always apparent to which samples these percentages referred.

Major compulsory revisions:

Abstract
The use of percentages rather than crude numbers made this difficult to follow in parts. The Conclusions section appeared to be an extension of the Results section and no actual conclusion was presented.

Background
The first statement is rather odd. Do the authors imply that only one cell is involved. The first paragraph requires references to back these statements up.

In paragraph 2 it is not clear whether the authors are referring to bladder cancer in general or to non-muscle invasive or muscle invasive subgroups.

Is the overarching hypothesis that missegregation appears to lead to CIN but there is currently a lack of evidence for this? If son, the authors should state this here and in the abstract.

Although this represents the first study using primary tumours that provides insights on how HSR and/or DMs might co-exist in tumour cells, this only relates to three cases. Could this have occurred by chance?

The last two paragraphs are rather unclear and disorganised.

Methods
Paragraph 1: How were cases selected for this study? Why were so few samples studied? The pT2G3 tumour is neither superficial nor minimally invasive, the patient had a cystectomy and was only followed up for 4 months, and so should be omitted from this work.

Paragraph 4: The information in paragraph 4 would be useful in Table 1.
Results
The first two paragraphs are very clear; the three most confusing paragraphs are paragraphs 4, 6 and 11.

Paragraph 2 last sentence: remove “almost”. It might be easier to say that T1G3 tumours all had CIN.

Table I is a key element of the results and does not always clearly correlated with what is stated in the text. It requires a more detailed legend, e.g. definition of subpopulation, definition of abnormal centrosome, etc, as it was not easy to follow. A first column of negative, moderate and high CIN would be helpful.

Paragraph 4: all high-CIN samples (n=6) are mentioned but only five results were obtained as there is a question mark against the 6th. Again, crude numbers should be used as well as percentages. What is the definition of an abnormal centrosome? Does “overall” refer to all or only high-CIN samples? When referring to ‘two samples’, etc, it would be very helpful to include the names of the actual samples, as has actually been done later in paragraph 4. Is centrosome size being used as a surrogate for centrosome clustering? If not it would be helpful to add centrosome clustering as a column in Table I.

Paragraph 5: are there not 11 samples with supernumerary chromosomes, rather than seven?

Paragraph 6: This was very difficult to follow as I could not understand the CCND1 column in Table 1. To what does “gain” refer? Is this gain of chromosome 11 by FISH? The CGH data needs to be presented in the table also, to make this paragraph comprehensible, otherwise we are relying on ‘data not shown’. The last sentence says that amplification was detected using CGH in only three cases but we do not know which three.

In paragraph 11 it is not clear to what status U443 has been assigned and the text does not tally with Table I. The U-443 tumour did not show DMs although the lymph node metastasis did, so the first sentence is not a true statement in its current form, and it is impossible to guess which side of the divide the authors have placed this sample for the Kaplan-Meier analysis. The authors need to present the Kaplan Meier survival data. There appears to have been no account taken of the influence on stage and grade in this analysis.

Discussion
Paragraph 1: why look at CCND1 copy number as a surrogate for CIN when the CIN index can be investigated directly?

Paragraph 2: why is it remarkable that on classification of tumours according to their CIN index that all T1G3 samples were in the CIN positive group?

Paragraph 5: ‘lagging’ requires a definition.

Conclusion
This requires some comment regarding the small numbers involved and the need to repeat this study in a larger tumour set to confirm the findings so far observed in only three patients.

In the Background section the authors state that the study will contribute to the understanding of genetic complexity of bladder tumour cells. How has this contributed?

Minor Essential revisions

Page 12 line 13: remove second ‘samples’ after DM.

The fonts in Figure 1 need enlarged.

Figure 3: what is ‘hypothetical’ sequence?

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**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.