**Editorial comments:**  
Changes to be made by the author(s) regarding the manuscript:  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

**Thank you. Minor grammatical changes have been made throughout the manuscript.**

2. Please provide an email address for each author.

**This has been provided in the title page.**

3. Keywords: Please provide at least 6 keywords or phrases.

**An additional keyword was provided to total 6. This is located on the title page.**

4. Please remove commercial language and replace it with generic terms: Jim7, Xinapse Systems Ltd, etc.

**Thank you. This language has been removed.**

5. 1.5: How to confirm that tumors have been developed?

**The only way to confirm that tumors have been developed is an imaging study as we describe in this manuscript.**

6. 2.1: Please mention how proper anesthetization is confirmed.

**This has been added to methods section 2.2.**

7. 5.2: How to remove bladder tissue?

**Additional details have been added to section 5.**

For how long and at what temperature is the tissue fixed in 10% formalin? How histologic examination is done? What are the staining agents? Please add more details here or provide relevant references.

**Additional details have been added, now listed as section 6, as requested.**

8. Figure 3: Please explain what the green arrows and red circle represent in the figure legend.

**Additional detail has been added to the Figure 3 legend.**

9. Discussion: Please discuss critical steps within the protocol.

**A paragraph describing critical steps has now been added to the discussion.**

10. References: Please do not abbreviate journal titles.

**References were previously cited using the JoVE EndNote Styling, which does abbreviate journal titles. The style in this manuscript has now been changed to include full journal names as requested.**

11. Please revise the table of the essential supplies, reagents, and equipment to include the name, company, and catalog number of all relevant materials.

**A revised materials table has been provided.**

**Reviewers' comments:**  
Reviewer #1:  
  
Manuscript Summary:  
This is a straightforward manuscript describing MRI to assess tumour burden in a mouse model of carcinogen-induced bladder cancer. The authors present the benefits and limitations of the model well and highlight how the technique may address a major challenge in the field - that BBN-induced tumours are highly heterogeneous. Indeed, the ability to stratify tumours according to stage prior testing experimental therapy should lead to greater consistency in reported results. While the manuscript is well written and organised, there are a few minor concerns, detailed below.  
  
Major Concerns:  
No major concerns  
  
Minor Concerns:  
1. MRI or MR is never defined in the text - it would be convention to define the acronym at first use.

**MRI is now defined in the introduction. Thank you.**

2. In the paragraph describing limitations (lines 67-75), it may be appropriate to discuss availability of MRI for mice. Is this technique/are the machines widely available, for example?

**Thank you for bringing this up. Dedicated small animal MRIs are becoming more common and we have added a sentence regarding use of MRIs as shared resources into the discussion.**

3. In the Protocol:  
1.1 It is indicated that male mice should be used for this protocol, but no explanation for why is provided. This should be mentioned or sex should not be specified.

**Thank you for this comment. Male mice develop bladder cancer more quickly than female mice in the BBN model, and a fair amount of work has been done to investigate why this is, so this should be emphasized to the reader. Additional details and citations have been added to the manuscript.**

2.1 and 2.2 should likely be inverted? Might it make more sense to inject animals with water prior to anesthetising them?

**Yes, thank you, this has been corrected.**  
  
2.4 Monitor body temperature and respiration is mentioned, but no indication of how is mentioned - by instrumentation, by eye?

**Thank you for mentioning this critical point. Monitoring is performed with instrumentation. Additional details have been added to the methods, discussion, and table of materials.**  
  
5.1 As euthanasia protocols can vary widely by institute, it would be better to remove the phrase "of CO2 euthanasia followed by secondary cervical dislocation" (line 135) leaving only "using standard operating procedure in accordance with local IACUC guidelines.

**Thank you, this statement has been adjusted in accordance with your recommendation.**  
  
4. In the representative results, the importance of correlation between bladder weight and bladder wall measurements is over-emphasised with respect to the correlation coefficient. An rs of 0.37 is not a strong correlation, as the strength of correlation decreases as the correlation coefficient approaches 0. The p-value only indicates whether the relationship measured occurred by chance. Thus, this is a relatively weak correlation (rs = 0.37), however, it is likely not due to chance (p=0.009). Additionally, it might be helpful to show the best fit line, as there appears to be one outlier in the graph with a much thinner bladder wall, and it is unclear how much this point drives the relationship.

**Thank you for your careful review. We agree that rs of 0.37 is not a strong correlation, and have changed the wording in the text to reflect this. Removing the outlier as you mention increases the spearman coefficient. We opted not to place a fit line through the graph, as bets fit (i.e., regression) lines are not typically used for correlations.**

5. In figure 5, p-values are presented, however, the tests used to generate them are not stated. The test used and the variables measured should be stated in the legend. Appropriate tests for the data in 5A and 5B would include a nonparametric Kruskal-Wallis test, with a post-test to correct for multiple comparisons, in which the authors compared all tumour stages to the control T0 stage. In 5C and 5D, a nonparametric Mann-Whitney would be an appropriate test to determine statistical significance.

**Thank you. Indeed** **the nonparametric** **Kruskal-Wallis and** **Mann-Whitney tests were used as you describe. This is now included in the figure legend as suggested.**

6. It is stated that "…the MRI derived BLAwall parameter and the BW data demonstrates a direct association with tumour stage…" (line 153-154). This is also likely overstated as the bladder wall values overlap almost entirely in Ta, T1, T2, and T4 tumours (5A). The same can be said for weight and all tumour stages except T3, T4 (5B). Analysis by Kruskal-Wallis testing will reveal statistically significant differences among the different groups. Those differences should be emphasised. Importantly - the potential lack of a statistically significant difference across all tumour stages does not diminish the potential applicability of the technique, however, the limitations of the "fast quantitation" analysis should be clear.

**The word “direct” has been removed from the manuscript as we agree that this may be overstating the association. In addition, as you suggest, the name of the statistical tests performed was added.**

7. In the discussion, it is emphasised that this technique will enable investigators to stratify animals prior to experimentation. It would be of interest if the authors commented on the very diverse range of tumours analysed in the course of this study. Tumours ranging in stage from Ta to T4 are reported - were all of these stages observed after 20 weeks of BBN treatment? How might one stratify such diversity?  
  
**We have added an additional statement and citation regarding tumor stratification into muscle-invasive (T2 or greater) and non-muscle invasive (T1 or less), as is standard management in human bladder cancer.**

Reviewer #2:  
  
Manuscript Summary:  
The manuscript by Glaser et al., descripts a detailed MRI protocol to evaluate tumor burden in situ, particularly the BBN-induced bladder cancer (BCa). Like human, male mice are much more susceptible than females to the BBN-induced BCa (Kaneko and Li, 2018). A recent report from this group also demonstrated that the BBN carcinogen induced BCa recapitulates molecular features of human muscle invasive BCa (Fantini et al., 2018). However, there is a lack of effective tools to accurately monitor bladder tumor burden longitudinally. A reliable MRI based protocol to assess tumor size and stage is timely and invaluable for the field of BCa study. Therefore, this manuscript is suitable for publication. A few minor comments:  
  
Minor Concerns:  
  
1. A better description of MR image analysis is recommended. Specifically, how the representative axial view is selected (4.3), and how the ROI is determined to draw BLAout and BLAin.

**Thank you for your review and comments. Additional details have been added to this section as suggested.**

2. Figure 4 and 5, please indicate the number of mice used for the analysis, and the statistic method used in Figure 5A and B. Please also highlight in Figure 5A and B which two groups are compared.

**Additional details have been added regarding the statistical tests used for Figures 5A-B (Kruskal-Wallis test), and for Figures 5C-D (Mann-Whitney tests). The number of mice is also now included.**

3. Recent advances of using the BBN model should be referenced as well.

**Thank you. Additional citations, including Kaneko and Li 2018 have been added to the discussion as you suggest.**