Author’s response to reviews

Title: A segregation index combining phenotypic (clinical characteristics) and genotypic (gene expression) biomarkers from a urine sample to triage out patients presenting with hematuria who have a low probability of urothelial carcinoma

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Author’s response to reviews: see over
A segregation index combining phenotypic (clinical characteristics) and genotypic (gene expression) biomarkers from a urine sample to triage out patients presenting with hematuria who have a low probability of urothelial carcinoma

Laimonis Kavalieris, Paul J O'Sullivan, James M Suttie, Brent K Pownall, Peter J Gilling, Christophe Chemasle and David G Darling

Reviewer 1 Kian Chong

Major Revisions
1. The statement has been reworded to provide more information on study participation criteria. Please see page 8, lines 3-5.
2. Table 2 has been altered to include the information that the reviewer has requested.
3. The text has been reworded to address the reviewer’s concern (page 8, lines 3-5). The study population is not skewed as the Cxbladder test was conducted before the conclusive cystoscopy. The patients were consented, recruited and prospectively sampled for Cxbladder prior to retrospective cystoscopy.
4. We agree with the reviewer that the statements in the Methods Section, and Table 1 are confusing. We have reworded the Methods Section (page 9, lines 7-9) and Table 1 to make this clearer.
5. We have reworded the Methods Section (page 9, lines 7-9; page 12, lines 15-16) of the results to explain better the fact that hematuria incidence is a zero term for microscopic hematuria.
6. There is no change in ‘severity’: this is simply the trigger point for diagnosis of microscopic hematuria. Therefore, we do not believe that amendments to the manuscript are necessary.
7. We do not imply that we will be interpreting the G+P Index differently. This is a derivative of the analysis. Therefore, we do not believe that amendments to the manuscript are necessary.

Minor Revisions
1. We have changed Table 3 and added ‘Non-UC’ to the legend to define Controls.
Reviewer 2 See-Tong Pang

Major Revisions
1. We did not present False Negative rate data for microhematuria for the reasons correctly stated by the reviewer. We have amended the legend to Table 4 to make this clearer. Whether it is safe to ‘let it go’ based on the G+P Index is up to how each individual clinician uses the test, and it would be improper for the authors to make a recommendation. The method cannot differentiate urothelial from renal cell carcinoma, however Cxbladder Detect correctly identified 100% of upper tract carcinomas (O’Sullivan et al. J Urol 2012;188:741–747)
2. Cxbladder Triage will be used as a ‘rule-out test’, by clinicians, to identify those patients who do not require a full clinical urological examination. It will be used at initial presentation with either micro- or macrohematuria.
3. The negative and positive values for the genes are a result of model fitting of the markers and do not represent any underlying biology. We thank the reviewer for pointing out inconsistencies in the nomenclature of the genes, which we have corrected to the new nomenclature. Please see page 11, line 16.
4. This question is fully covered in O’Sullivan et al (2012).

Reviewer 3 Ioannis Leotsakos

Major Revisions
1. We have added a comment that evaluation of upper urinary tract cancer was covered in the O’Sullivan et al (2012) paper (page 7, line 16). Thank you to the referee for requesting this clarification.
2. The distribution of cancers, as requested by the reviewer, is already presented in Table 2. We fully agree with the statement that “it cannot be stated that low-grade tumours have no consequences”, as stated by the reviewer.

Minor Revisions
1. We have compared Cxbladder with the clinical truth as determined by cystoscopy and appropriate follow up. Hence our data are comparable with clinical practice.
2. We have added this information on page 8, lines 10-12.
3. We fully agree with the reviewer. We are seeking increased detail in subsequent clinical trials to improve our data.
4. We agree with the reviewer. Further studies are progressing.

We recognize the valuable comments made by all of the reviewers.

Please also note the following additional minor amendments that have been made upon subsequent review of the manuscript:
- The Conclusions (marked; page 17, lines 19-21) have been amended to ensure that they are consistent with the corresponding text in the Abstract
- The authors listed in reference 36 have been corrected (marked; page 23, line 6).

James M Sutte PhD, Laimonis Kavalieris, PhD and Paul O’Sullivan PhD.

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