Reviewer’s report

Title: Expression and biological-clinical significance of hTR, hTERT and CKS2 in washing fluids of patients with bladder cancer.

Version: 2 Date: 10 August 2010

Reviewer: Susanne Fuessel

Reviewer’s report:

Mezzasoma et al. determined transcript levels of three bladder cancer-associated factors in bladder washing fluids originating from 36 patients with bladder cancer and from 58 control subjects. The distribution of the transcript levels were evaluated with regard to the discrimination between tumor and non-tumor patients and to a dependence on tumor stage.

The objectives of the study are introduced in a comprehensive manner and procedures are well described. Statistical analyses revealed that the single markers seem to be insufficient for a reliable stratification between tumor and non-tumor patients as well as between patients with non muscle-invasive and muscle-invasive tumors. The combination of the transcript markers apparently improved the diagnostic power of the assay possibly reflecting complex alterations of molecular processes in tumor development and progression.

Results are discussed in detail and limitations of the study – the relatively low numbers of subjects particularly in the tumor patient group – are stated.

Major Compulsory Revisions:

1. The authors measured transcript levels of the housekeeping gene beta-actin for normalization purposes. But all results presented in the tables and figures seem to represent the pure transcript levels of hTR, hTERT and CKS2. Furthermore, it remains unclear what the unit “ng/µl” in tables 1 and 2 does represent. If the authors had used normalized transcript levels the variables would be dimensionless. The use of non-normalized data for diagnostic purposes appears doubtful since the total volume of bladder washings and the numbers of exfoliated cells are varying between the different patients. This must be clarified.

2. The use of non-normalized data can also be the reason for the conflicting result that hTR transcript levels decrease in tumor patients in comparison to the controls. Other studies have shown increased transcript levels of hTR in urine specimens from bladder cancer patients and a correlation between hTR expression and telomerase activity which is specific for tumor cells.

3. In this context, correct citation of reference 25 in the discussion chapter should be verified.

4. The authors should also check the specificity of the primers and probe used for hTR detection.

5. Additionally, the authors should explain the logistic regression model in more
detail to make the differences between the ROC analyses of the single markers presented in the third and the fourth paragraph clear.

Minor Essential Revisions:
6. Did the authors use a LightCycler instrument from Roche or the MX3005P System from Stratagene? This should be revised.

Discretionary Revisions:
7. The numbers of subjects in each group could be already mentioned in the abstract.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests.