Author's response to reviews

Title: Chromosomal imbalance in the progression of high-risk non-muscle invasive bladder cancer

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Author's response to reviews: see over
Reviewer's report
Title: Chromosomal imbalance in the progression of high-risk non-muscle invasive bladder cancer: a case control study
Version: 1 Date: 24 December 2008
Reviewer: Marta Sanchez-Carbayo

Reviewer's report:
In the manuscript Zieger et al evaluate chromosomal imbalance in the progression of high-risk non-muscle invasive bladder cancer. It is suggested to incorporate the following minor changes:

- The incorporation of case:control concept in the title is not fully appropriate. It would be better to delete this latter part.

  The mentioning of the study design was a requirement of BMC Cancer acc. “instructions for authors”. It has now been deleted. Indeed, from a statistical viewpoint, it was a case-control study with retrospective selection of the sampled patients based on known outcome. Patients with progression were the cases, those without the controls.

- The main problem in the interpretation of the results is the mix of the two version of the arrays as well as primary and recurrent tumors. It is suggested to exclude the 10K data (since they are very few), and perform the analyses on the primary and the recurrent cases independently to check how they may validate each other or how different they are.

  We partly agree that the mix of two array versions was problematic. However, it was first at the level of interpretation of the results, that the two array types were mixed. The data processing and analyses, although performed in a similar way, were completely independent. This is written more precisely now in the M&M section. As we demonstrated, number of samples is everything because of the huge variation of the observed changes, and the high number of possible confounders (as discussed below). There were 19 10K-arrays out of total 48, this means 40% of samples.

  The mix of primary and secondary tumors was partly intended, because if chromosomal instability were an inherent property of progressing tumors, this should be independent of stage and should also be present in recurrent tumors. Of course we did perform a separate analysis of primary vs. recurrent tumors, showing a similar level of changes in primary and recurrent stage T1 tumors, while stage Ta tumors (which were almost exclusively recurrences) had fewer changes. All this was independent of future progression. However, again, numbers became small and consequently the analyses lost power. The central message was, however, clear: it was the actual tumor stage that impacted the number of chromosomal changes, not the future progression.

- Another critical issue is the potential impact of BCG or other intravesical treatment on the results. Thus similar cases, either primary or recurrent having received previous exposures (maybe different), they should be grouped and compared separately and contrasted with cases being treated with other intravesical chemotherapy (if it is the case).

  Again, we partly agree. But again, the number of samples did not allow for further subgrouping. Of course, we included BCG as a confounder, but found no significant impact of this treatment. This was specified in the manuscript. No other intravesical chemotherapy was given (one exception – see Add. Table 1).
Reviewer's report
Title: Chromosomal imbalance in the progression of high-risk non-muscle invasive bladder cancer: a case control study
Version: 1 Date: 22 October 2008
Reviewer: Per-Uno Malmstrom
Reviewer’s report:
The authors present a negative result regarding the predictive value of chromosomal alterations as a progression marker in non-muscle invasive bladder cancers, this in contrast to several reports from the literature of the opposite. Firstly it is important that negative results are published. As with positive results the result can be true or false (see below).

Major Compulsory Revisions
The manuscript is somewhat unstructured. For example is the aim mentioned in the Methods and not in the Background.
This was corrected
The definition of chromosomal imbalance, the key factor analyzed, is not fully described. Neither is the authors’ interpretation of this discussed.
This is correct. Chromosomal instability (CI) and chromosomal imbalance are used somewhat synonymous in the text, because CI is the more familiar term. In fact, CI is defined as an increased tendency to acquire chromosomal aberrations. This includes aneuploidy, which cannot be assessed with the methods used here. The term “chromosomal imbalance” covers DNA copy number alterations and loss-of-heterozygosity of parts of or whole chromosomes, but not the entire genome. A definition is now given in the Background section and in the Abstract.
The material selection is interesting with primary and unrelated secondary tumors. Even if it is mentioned that primary and related secondaries are usually clonally related a deeper analysis by f.ex. presenting the results of also this later group could be rewarding for the understanding.
We did no analysis of clonal relationship between primary and secondary tumors, since they were unrelated. We did analyses of primary and secondary tumors separately, and this is mentioned in the text. We omitted a presentation of these results, since primary and secondary tumors were not different with regard to chromosomal imbalance, when we controlled for stage (there were more stage Ta tumors among secondary tumors). See also comments to the first reviewer’s considerations.
The tumor material is crucial for these type of analysis. One possible cause of a false negative result could be insufficient tumorfraction in the analyzed material. How did the authors check that not too much non-tumor tissue was contained in the specimen?
We trimmed the specimens under the microscope, as specified in the M&M section. In some cases, we used previously purified DNA, where this quality control was no longer possible. The result of these samples was, however, not different from the rest.
Minor Essential Revisions
No array profile is depicted for the readers to evaluate the quality of the tests. I am not sure what the reviewer means with “array profile” – some lists of Call-rates, numbers of outliers, or a picture of the array scan? The Additional figures show mean and spread of the data to demonstrate the variation, however, these are obtained from processed data and allow no conclusions of the array quality. Moreover, the figures are constructed using software which is not commonly used.
The correlation between qPCR and SNP arrays result is not shown. We have considered this in a previous version, but omitted the figure for better understanding. The correlation coefficient (r=0.67) was added to the Results section.
Table 1 is difficult to read with a mixture of tumor characteristics and methods. Since CIS-information is not essential for the understanding, this information has been omitted and tables 1A and 1B gathered in one table. Further clinical information is still accessible in the additional material.
The syntax is not high quality f.ex the “progression precess” is mentioned in the conclusions. Do the authors mean process?
Yes.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a