Reviewer's report

Title: Polymorphisms and plasma levels of IL-27: impact on genetic susceptibility and clinical outcome of bladder cancer

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Reviewer: Silvia Selinski

Reviewer's report:

A very interesting and well-described and conducted study though the statistics and figures have to be improved and results have to be discussed more critically. The manuscript is clearly structured, the objectives are also clear. Most of the statistical analyses are well documented.

The main problems are the application of the statistical methods and the discussion of the results considering the few relevant observations. Furthermore a table with the study group characteristics and especially the distribution of IL-27 plasma levels is missing and the figures have to be revised.

In particular I have the following remarks:

Major Compulsory Revisions

1. Statistical analysis – Cox regression/number of events:
   a. In most Cox regression analyses the critical number of events is missing. From the data and figures 8 and 9 it is clear that for the rs17855750 GT/TT genotypes there are only 2 events in MIBC cases and 1 event in NMIBC cases. The remaining GT/TT cases are censored. Thus, it is critical to derive the "protective" effect from so few observations. This must be discussed and handled with care. Furthermore, I'd expect in such a situation a chi-squared test checking the frequency of events (total, relapses only, death only) in rs17855750 GT/TT vs. GG. This usually helps to interpret the data.
   b. Please revise the Kaplan-Meier plots. All curves must be plotted using a solid line.

2. Study group characteristics, IL-27 levels, Figures 1-7:
   a. A table of the study group characteristics is missing: distribution of all variables, age and IL-27 levels e.g. with minimum, maximum, IQR, median, mean, standard deviation.
   b. The IL-27 level distribution in subgroups (Figures 1-8) and the respective p values could be presented in a separate table. Thus most figures can be omitted.
   c. Please use Box plots. In case of few observations please indicate them.

3. Statistical analysis - Table 1/Distribution of genotypes in cases and controls:
   Please use logistic regression models adjusted for age, gender, smoking habits and tumor grade to estimate adjusted ORs and CIs.

4. Stratified analysis of the genotype distribution (Table 2): The authors
calculated if genotype distributions differ between subgroups of cases (old vs.
young cases, for instance). For smoking habits, gender and age this is only
useful to check the data for the Cox regression analysis (confounding). For tumor
grade the results can just be reported. For tumor stage (NMIBC and MIBC cases)
the analysis should include the same genetic models used in table 1, results
must to be handled with care due to the small number of GG genotypes. More
useful is a subgroup analysis repeating the analysis of table 1 in the subgroups
of young and older persons (cases and controls), males/females,
smokers/non-smokers unadjusted and adjusted for all but the subgroup variable.

5. Statistical analysis – comparison of the IL-27 levels in 3 groups: A
Kruskal-Wallis test (KWT) was applied for pairwise testing.
a) The KWT is the extension of the Wilcoxon test for a c sample problem. So an
overall p value is missing. Furthermore the assumptions of the test (equal
variances) are not checked/reported.
b) For pairwise tests the Wilcoxon test can be applied but to be honest the level
alpha of significance has to be corrected for multiple testing (Bonferroni,
Bonferroni-Holm, for instance). This is what parametric pair-wise post hoc tests
(Tukey test, for instance) do. Was there a specific reason not to use these tests?
c) GLM models seem to be useful here as plasma levels varied between cases
and controls as well as between rs17855750 genotypes.

6. The conclusions are based on sparse data, please indicate this clearly
throughout the paper and discuss it.

Minor Essential Revisions
1. Abstract: the term "correlated": No correlation coefficient was calculated. So,
please substitute this term.
2. Materials and Methods: Please define "age", in particular, please indicate if
age was recorded as age at first diagnosis.
3. Materials and Methods - Statistical Analyses: Please define low and high risk
tumors as well as the binary age variable. Why 64 years were chosen as
threshold (mean, median?). Thresholds for the Cox analysis of the IL-27 plasma
levels have to be added.
4. Materials and Methods - Statistical Analyses: The authors also assumed an
intermediate mode of inheritance using a multiplicative model (alleles instead of
genotypes). This has to be added.
5. Materials and Methods - Statistical Analyses: The subgroup analysis (age,
gender etc. ) has to be added.
6. Statistical analysis: Fitting separate Cox regression models for NMIBC and
MIBC patient was adjusted also for tumor grade. What is missing is the
distribution of grade in both patient groups separately. But I'll expect that almost
all NMIBC cases have low grade tumors whereas MIBC cases have high grade
tumors. So adjustment for tumor grade can (and should) be omitted.
7. Statistical analysis - Cox analysis of the IL-27 plasma levels: Thresholds for
the Cox analysis of the IL-27 plasma levels (and probably age) are based on the means. Medians are more robust in such a situation. Furthermore, I recommend strongly the use of quartiles for the analysis. Often differences can be observed only between the upper and lower quartile. Kaplan-Meier plots, p values and data are missing.

8. Figure 3: rs17855750 instead of rs153109 (Figure 2).

9. Author contributions: It is unclear who did the statistics.

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

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.