Author's response to reviews

Title: Prognostic impact of Skp2, ER and PGR in male and female patients with soft tissue sarcomas

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Author's response to reviews: see over
Our response: In univariate analyses, expression of ER had no impact on survival for men (p=0.089), but high expression of ER was associated with improved survival in women (p=0.041). High expression of PGR was associated with reduced survival in men (p=0.010), but had no impact in women (p=0.832), Figure 2. In co-expression analyses men with the ER-/PGR- phenotype (N=39, p=0.013) had improved survival whereas women with the ER+/PGR+ phenotype (N=25, p=0.001) had improved survival, Figure 3. In the multivariate analysis expression of SKP2 had no significant impact on survival for men (p=0.088), while high expression was significantly associated with worse survival in women (p=0.006), Table 7.

In patients with low expression of ER (N=112), men had improved 5-year survival (69%) compared to women (47%, p=0.002), while there were no differences (p=0.376) between men and women in patients with high expression of ER (N=72). In patients with low expression of PGR (N=132), men had improved 5-year survival (69%) compared to women (55%, p=0.013), while there were no differences (p=0.271) between men and women in patients with high expression of PGR (N=57). There were no differences in survival between men and women in univariate analyses of patients with low (N=109, p=0.529) or high (N=67, p=0.233) expression of SKP2 (data not shown).

Reviewer 1: The nationality of the patients also significantly affects patient outcome, the authors should discuss it more detail.

Our response: In univariate analyses the 5-year survival were 62% in the Norwegian and 44% in the Russian patients (p=0.005), table 1. While the Norwegian patient cohort is unselected (recruited among the general population of North Norway), the Russian cohort is subjected to an unfavourable prognostic bias as a considerable part of this material is from the Arkhangelsk Regional Oncology Centre (more poor prognosis patients). Among the Norwegian patients, 64% (84/131) of the soft tissue sarcomas (STS) were histological high grade tumors, while this was the case in 89% (55/62) of the Russian patients (P<0.001). In the multivariate analysis, controlling for other important prognostic factors, there was no differences in survival between Norwegian and Russian patients (p=0.143), Table 7.

Reviewer 1: As a study lumping various sarcoma types together for survival analysis, the authors should further mentioned the limitation caused by the heterogeneity of tumor types.

Our response: The data collection introduced problems in identifying adequate numbers of similar patients with similar tumors and with the same treatment traditions. These are well known problems when conducting STS studies. Our findings are in large hypothesis generating, and to be more conclusive future STS studies must be based on large, multi-institutional and multinational studies with possibilities to establish adequately sized STS patient cohorts of homogenous tumor groups. However, all tumors investigated herein had mesenchymal derivation and belong to the same generic group.
Reviewer 2: Sarcomas are very heterogeneous group so I find it a little difficult to assume that all subtypes adhere to this relationship. It would be interesting to know if the relationship is stronger in certain subtypes than others or at least present data showing ER, PR and Skp2 by histological subtype. Or if there are not enough subtypes for analysis, a comment made in the discussion section about this.

Our response: There are small numbers of patients in the different histological subtypes of STS and dividing each subgroup in men and women makes no sense. In univariate analysis, if we combine men and women in one group, high expression of ER were associated with improved survival in patients with rhabdomyosarcoma (N=9, p=0.040). High expression of PGR were associated with poor survival in patients with synovial sarcoma (N=12, p=0.010), data not shown. There were no significant differences in survival according to high or low expression of SKP2 in any of the histological subtypes. We have included a table of percentages of high expression of ER, PGR and SKP2 according to the different histological subtypes, Table 2. Using chi-square test, there are no differences in overall in overall expression of ER, PGR and SKP2 with respect the different histological subtypes.

Reviewer 3: Pictures showing low/high PGR expression in soft tissue sarcomas are missing in Figure 1. Moreover, it is not clear which portion of the cores at x100 is shown at x400 magnification.

Our response: We have now updated Figure 1 with pictures showing low/high PGR expression. We considered to mark the portion of the cores x100 shown at x400 magnification, but the areas of high magnification selected were from different places in the different cores and sometimes hidden behind the x400 magnification. We decided to keep the original layout.

Reviewer 3: Results section, pag. 8: "and 42% of patients (N=193) were male" It should be reported n=81 for clarity.

Our response: It is now corrected “42% of patients (81/193) were male”

Reviewer 4: Several of the authors' primary conclusions involve differences in prognostic associations of markers by gender. However, the authors do not formally test for this interaction but rely on whether the individual p values are "significant" in men and/or in women. They should instead directly test the statistical significance of the interaction.

Our response: We have now updated the “result” section. In univariate analyses of patients with low expression of ER (N=112), men had improved 5-year (69%) compared to women (47%, p=0.002), while there were no differences (p=0.376) between men and women in patients with high expression of ER (N=72). In patients with low expression of PGR (N=132), men had improved 5-year survival (69%) compared to women (55%, p=0.013), while there were no differences (p=0.271) between men and women in patients with high expression of PGR (N=57). There were no differences in survival between men
and women in univariate analyses of patients with low (N=109, p=0.529) and high (N=67, p=0.233) expression of SKP2 (data not shown).

Reviewer 4: Table 7 (multivariate analysis) should explain in more detail the differences between the individual factor-level p values vs. the overall significance p value.

Our response: The difference between the individual p-value and total p-value in the multivariate analysis is relevant in cases where there are more than two categories for a given variable. Overall p-value is calculated based on a general assessment of all categories for the given variable, but the individual p-value only calculates the significance of a given category versus the reference category.

Reviewer 4: The study includes ~30 reported (raw) P-Values from a variety of sub-analyses. Many of these p-values are near the threshold of 0.05 and deemed significant if below this cut-off. The authors should consider correcting for multiple hypotheses (by Bonferroni, for example), in which case only a few of their most significant associations would remain significant at the 0.05 level.

Our response: Type I errors occur when inappropriate significance levels are used. In biological studies it has become a norm to use P < 0.05 as the cut-off point where a difference is considered significant. This gives that one in twenty tests for the same difference will be a type I error. When conducting a large number of tests the chance of an erroneous positive result thus increases. There have been developed several approaches for reducing the chance of type I error in the setting of multiple testing. The drawbacks of these are the increased chance of type II error. There is no consensus whether such methods should be used in prognostic studies. We chose not to conduct a correction of multiple testing as we see our studies as hypothesis generating. This results in an increased risk of type I errors, but decreases the chance of type II errors.

Reviewer 4: It would also help the readability of the paper if the authors stated their primary hypotheses and focused presentation of results and interpretation on these, prior to also reporting all of the sub-analyses.

Our response: Our major hypothesis is that a different prognostic significance of Skp2 in men and women exists and is related to diverse gender expressions of ER and PGR. Our hypothesis was confirmed. We found diverse prognostic DSS impacts from gender related expression of Skp2, ER, PGR and DSS in STS. In men, but not women, an ER positive/PGR negative co-expression profile was an independent negative prognostic factor for DSS. In women, but not men, high expression of Skp2 was associated with reduced DSS.