Author's response to reviews

Title: Ki-67 is a Strong Prognostic Marker of Non-Small Cell Lung Cancer When Tissue Heterogeneity is considered.

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Author's response to reviews: see over
Spiral array does not add better information when compared to conventional whole sections neither in terms of costs nor in terms of reproducibility.

Ki67 has been previously studied in NSCLC as prognostic marker, but without any practical role. The study herein does not add news on this topic. The only good new is that highest expression is more prognostic than average or lowest expression. Different studies explored the predictive value of Ki67 to chemotherapy, but none revealed significant influence. Ki67 index has also a lower significance when compared to new predictive biomarkers (e.g., ERCC1, RRM1, EGFR, ALK, others).

Heterogeneity of Ki67 values is a major problem in several tumors, as correctly underlined by the authors. This is true well-known also in NSCLC.

-We agree with this comment. Recently, some new prognostic markers have revealed more specific than Ki-67 expression. But we think that these are controversy, and immunohistochemistry and evaluation of Ki-67 is wide-spread and more useful than that. We made the additional statement to the last of **Discussion** (page 20) as follows.

“Recently, some molecular markers, including the excision repair cross-complementation group 1 (ERCC1), ribonucleotide reductase M1 (RRM1), epidermal growth factor receptor (EGFR), and ROS1, were revealed as a predictive marker for survival benefit and could also predict the effect of medical treatment[20-22]. However, immunohistochemistry (IHC) of these markers are controversial and have a limited use in larger institutions. On the other hand, IHC of Ki-67 was distributed widely, applied to various organs, and established for technique and evaluation of IHC. In this point, IHC of Ki-67 is more common and useful in routine work. Therefore, we
considered that evaluation of Ki-67 expression is still important and the results of our study is significant.”
The study could be strengthened if the recent classification of pulmonary adenocarcinoma could be applied (Travis WD, Brambilla E, Noguchi M, et al. The new IASLC/ATS/ERS international multidisciplinary lung adenocarcinoma classification. J Thorac Oncol 2011;6:244 –285) and a breakdown of Ki67 reading depending on the subtype (lepidic vs acinar vs solid, vs papillary/micropapillary) as well as Ki67 in mucinous vs non mucinous adenocarcinoma would be of great value. Also a comparison of the Spiral Array to a conventional tissue section in displaying various subtypes of adenocarcinoma on the reels would be helpful in a pilot group.

Similarly did Ki67 correlate with differentiation of squamous cell carcinomas?

- We agree with this comment, and also recognize that analyses in new classification by IASLC are important. But we could not review of whole slide corresponding the core of Spiral Array and not reclassify it into new classification, because we formed this cohort anonymized on collecting it. So, we use the histological subtype of adenocarcinoma acquired at collecting of this cohort, and analyzed it with MIB-1 expression (HS and HeS). We added this results as ‘additional file 2’, and made the additional statement to last of Results (page 17) as follows.

“On collecting this cohort, we formed it anonymized. Thus whole slide specimen corresponding the core of Spiral Array was not available and we could not review and reclassified ADC into histological subtypes in new classification of lung adenocarcinoma proposed by International Association for the Study of Lung Cancer (IASLC). In SqCC, we could not evaluate the correlation of Ki-67 expression with the differentiation of SqCC. There is
no significance between histopathological subtype, according to WHO classification in 2004, and HS or HeS [Additional file 2].”

Similarly, in squamous cell carcinoma, we could not analyze correlation of differentiation of squamous cell carcinoma with Ki-67 expression.

p15: It is unclear how Ki67 HS showed 21 score 0- please explain

We defined score 0 of Ki-67 HS as <1% of Ki-67 positive tumor cells, so 21 cases of score 0 have Ki-67 positive tumor cells but percent of positive tumor cells are less than 1%.

p16: HeS is a differential between HS and LS- how was this correlated with poor survival? Did high HeS vs low HeS show any differences?

- We have already presented it by Kaplan-Meier curve in Figure 4.

The small cell carcinomas, albeit of limited stage, should be excluded from this study as tumor heterogenity is rarely an issue in this tumor.

- We agree with this comment. We excluded 3 small cell carcinomas from analyses.

TTF-1 is a marker of lung differentiation- Was there any correlation with Ki67 scores and expression of TTF-1 on tumour? Any association with molecular changes (EGFR/KRAS/ALK)
We did not immunohistochemical staining of TTF-1, EGFR, KRAS, and ALK, so we could not analyze them.
Reviewer 3

1. The term 'non small cell lung cancer' is used in the title and in the text, yet the material includes also 3 small cell carcinomas. I would remove the latter from the analysis or drop the term 'non small cell', with preference for the former solution.

- We agree with this comment. We excluded 4 small cell carcinomas from analyses.

2. Pathological stage in the 'Results' sections is described by stage groups, and I would prefer the full pTNM classes.

- TNM described in the text and additional file 1 were intended to pTNM, originally. We added a postscript to TNM in the text and additional file 1 with 'p' or 'pathological', respectively.

3. 'Correlation' is used in the results, while no correlation coefficient was used; I think it is better to say that eg. there was a significant differences in HS between stages, and not that the there was a correlation between HS and stage.

- We amended the descriptions based on this comment, as follows.
Page 15
Before “Several variables including T factor, pathological stage, and vascular invasion were correlated with HS of Ki-67 scoring”

After “Several variables including pathological T factor, pathological stage, and vascular invasion had significant differences with HS of Ki-67 scoring”

Page 15
Before “And T factor, N factor, pathological stage, pleural invasion, and pulmonary metastasis were correlated HeS”

After “And pathological T factor, pathological N factor, pathological stage, pleural invasion, and pulmonary metastasis had significant differences with HeS”

Page 18

Before “HS was evidently correlated with OS in NSCLC, ADC, and SqCC”

After “there was a significant differences between HS and OS in NSCLC, ADC, and SqCC”