Title: Genetic polymorphisms in the endothelial nitric oxide synthase gene correlate with overall survival in advanced non-small-cell lung cancer patients treated with platinum-based doublet chemotherapy.

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Reviewer: Elisa Giovannetti

Reviewer's report:

The manuscript by Fujita et al. describes an investigation to determine the association between endothelial nitric oxide synthase (eNOS) polymorphisms and clinical outcomes in 108 non-small-cell lung cancer (NSCLC) patients receiving standard platinum-containing doublet regimens. The authors assessed the 2 recently described eNOS polymorphisms (G894T in exon 7 and a VNTR polymorphism in intron 4). The results suggest a correlation between the VNTR polymorphism and overall survival.

Overall, the manuscript deals with an interesting topic because NSCLC is a leading cause of cancer death and biomarkers of NSCLC clinical outcome should improve its clinical management and outcome. Additionally, the role of eNOS in carcinogenesis, if further evaluated/developed, might lead to clinically relevant applications, both for prognostic and therapeutic purposes.

However, some conclusions drawn from the data are overstated and there are several comments listed below that the Authors should take into consideration:

- The abstract and text of the manuscript should clearly identify which was the primary endpoint (i.e. correlation with overall survival, OS) of this study.
- It is not clear, from the methods and the results section, why the Authors included in the study 6 patients with stage IIIA.
- The Authors should further clarify the schedule of the chemotherapy regimens.
- The authors started from 108 patients, and focussed on OS of all these patients, but then they presented in response data referring only to 66 evaluable patients out of the 89 patients who received carboplatin-paclitaxel regimen. The Author should be encouraged to provide also the response data of the patients treated with the other 2 regimens. However, in order to assist the reader, the Authors should add a flowchart showing the number of the clinical/pharmacogenetic data available from all the subjects enrolled in the study.
- Considering the small number of cases, the comparison was performed between bb and ba/aa VNRT genotypes. However, the author should provide information whether the heterozygous genotype was associated to eNOS mRNA levels similar to those reported for the a/a genotype.
- The Authors reported in the text that they “investigate whether eNOS intyron 4..."
VNTR was associated with response”. However, the analysis reported in the Table 3 evaluated the clinical benefit (i.e. PR+SD). The Authors should correct the text or provide further analysis of PR vs. SD-PD.

- In the discussion the Authors stated that the studied VNRT polymorphism was associated with OS, “irrespective of the response to chemotherapy”. However, in order to really differentiate between predictive (predict the effect of chemotherapy) and prognostic (evaluating the natural course of the disease) value of this polymorphism, they should have evaluated its role in non-treated patients.

- The Authors reported that tumor samples of 34 patients were analyzed for EGFR mutations and that 21 were positive. However, since different mutations in EGFR have been associated with different outcome, they should add the type of mutations that were observed. Furthermore in the Discussion they stated that the “favourable median OS […] is probably due to […] the dominance of adenocarcinoma (which often exhibits EGFR activating mutations and is responsive to EGFR-tyrosine kinase inhibitors)”. However, they should add that gefitinib has been recently registered as first-line treatment of NSCLC patients with EGFR activating mutations. This approval is based on the data of the Phase III IPASS study, which demonstrated superior progression-free survival, greater objective response rate, improved tolerability and significant quality of life benefits for gefitinib compared to carboplatin/paclitaxel doublet chemotherapy in clinically selected first-line patients in Asia. In particular, PFS was significantly longer for gefitinib than doublet-chemotherapy in patients with EGFR activating mutation positive tumors, and significantly longer for chemotherapy than gefitinib in patients with EGFR mutation negative tumors. Similar data have been reported in the study of Mitsudomi et al., Lancet Oncol 2010. These results represent a milestone toward personalized medicine in NSCLC oncology and should be appropriately cited.

- The Conclusions should be rewritten. The Authors stated that these results “indicate the VNRT polymorphism may be associated with the progression of NSCLC and shed light on the positive association between the concentration of endothelial NO and survival of patients with NSCLC”. However, since no data on NO concentrations are evaluated in this study, this conclusion is not justified by the data presented.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests