"SNPs in PI3K/PTEN/mTOR and brain metastases in NSCLC" --Letter

Abolfazl Avan¹, Mina Maftouh¹, Amir Avan¹,², Carmelo Tibaldi,³ Paolo A. Zucali,⁴ Elisa Giovannetti¹

Authors’ Affiliations: ¹Department of Medical Oncology, VU University Medical Center, Amsterdam, the Netherlands; ²Department of New Sciences and Technology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; ³Department of Oncology, Azienda USL-6 of Livorno, Livorno, Italy; ⁴Department of Medical Oncology and Hematology, Istituto Clinico Humanitas, Rozzano, Milan, Italy.

Corresponding Author: Elisa Giovannetti, Department of Medical Oncology, VU University Medical Center, CCA room 1.42, De Boelelaan 1117, 1081 HV Amsterdam, the Netherlands. Phone: 31-20-4442267; Fax: 31-20-4443844; E-mail: e.giovannetti@vumc.nl

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We have read with interest the study by Li and colleagues (1) on single nucleotide polymorphisms (SNPs) predicting brain metastases in non-small-cell lung cancer (NSCLC). Patients with the GT/GG genotype of AKT1-rs2498804, CT/TT of AKT1-rs2494732, and AG/AA of PIK3CA-rs2699887 had higher risk of brain metastasis. These data support the feasibility of studying germinal polymorphisms for risk stratification. However, several points should be discussed in more detail.

Firstly, the choice to focus on NSCLC is valuable, as treatment with prophylactic cranial irradiation is a matter of debate in this tumor. However, according to the “rule of tens”, in a multivariate analysis there should be a minimum of 10 events per predictor variable. As 16 SNPs were analysed, a total of 160 patients would have to have brain metastases in order to prevent overfitting, since the results from an overfitted model are not generalisable to other populations. Another critical obstacle to the successful development of a genotype-based test is the high number of spurious associations (2). To avoid false-positive associations, a Bonferroni correction considering all the studied SNPs would require a P<0.05/16=0.003 for statistical significance.

Emerging SNPs need to be validated in independent cohorts, and our dataset from stage-IIIB/IV NSCLC (3) confirmed the association of AKT1-rs2498804, but not of PIK3CA-rs2699887, with brain metastases (Table 1). These conflicting data might be explained by several factors, such as ethnicity, sample size, different clinical settings, histotypes, stage and treatment. However, Li and colleagues did not describe treatment details of their cohort, while all our patients received gefitinib. Similarly, although 50% of their patients were never-smoker, no information was provided on EGFR mutations or ALK translocations. Since almost half of ALK+ patients treated with crizotinib have central nervous system relapse (4), we wonder whether the same results would have been obtained after adjustment for EGFR/ALK genetic aberrations and targeted
treatments. Ultimately, the role of AKT1-rs2498804 in clinical decision-making should be validated within prospective trials in homogeneous patient cohorts.

In addition to pharmacogenetic trials, further research is needed to unravel the functional significance of these polymorphisms. Specifically, AKT1-rs2498804, located at 3′-UTR region, may affect gene expression through changes in transcription factors binding sites, microRNA target sequences, and/or splicing variants. Although this SNP has not been tested in experimental models (5), it could be evaluated at least in silico, using publicly available software such as PROMO (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3) to detect transcription factor binding sites, MicroSNiper (http://cbdb.nimh.nih.gov/microsniper) for prediction of SNP effects on microRNA targets, and Human Splicing Finder (http://www.umd.be/HSF1) to study pre-mRNA splicing. In particular, additional studies should investigate whether differential expression levels of candidate microRNAs might explain the fact that this polymorphism was not associated with metastasis at sites other than the brain. Finally, direct evidence of the effects on protein expression could be obtained by immunohistochemistry of NSCLC primary and metastatic tissues.

In conclusion, we thank Li and collaborators for their study, but we believe that additional parameters are essential to validate candidate SNPs beyond already available clinical factors for predicting brain metastases in NSCLC.

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References


### Table 1. Polymorphisms and brain metastases in advanced NSCLC patients treated with gefitinib

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Patients, N</th>
<th>Events, N (%)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT1:rs2498804</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>36</td>
<td>3 (8)</td>
<td></td>
</tr>
<tr>
<td>GT+GG</td>
<td>54</td>
<td>17 (31)</td>
<td>0.010</td>
</tr>
<tr>
<td>PIK3CA:rs2699887</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>79</td>
<td>15 (19)</td>
<td></td>
</tr>
<tr>
<td>AG+AA</td>
<td>11</td>
<td>5 (45)</td>
<td>0.062</td>
</tr>
</tbody>
</table>

*P values were calculated by Fisher exact test.

Note. Polymorphic loci in AKT1 and PIK3CA were assessed in DNA isolated from blood samples and/or paraffin-embedded tumors from 90 stage-IIIb/IV NSCLC patients, treated with gefitinib (ClinicalTrials.gov ID-NCT00831454), as described (3).
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