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# *PIK3CA* mutations as prognostic factor in squamous cell lung carcinoma

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ABSTRACT

*Objectives:* Mutation in the *PIK3CA* gene is reported frequent in squamous cell carcinomas of the lung, but its potential prognostic role is still obscure. We have studied the prognostic importance of *PIK3CA* mutations as well as the relation to other markers in a large number of early stage lung cancers of squamous carcinoma subtype.

*Patients and methods:* Tumour tissue was obtained from 308 consecutively operated lung cancer patients with squamous cell carcinoma in the period 2003–2013. DNA was isolated according to standard procedures, and mutation analysis was done with either the SnapShot method and/or using *PIK3CA* specific primers in the Cobas system. PD-L1-expression was analysed with immunohistochemistry

After thorough follow-up (median 67.6 months), overall survival and time to relapse was calculated.

*Results:* Tumour tissue from 102 females and 206 males were analysed. 167 (54.2%) were in stage I, 96 (31.2%) in stage II and 45 (14.6%) in stage III. *PIK3CA* mutation was found in 35 (11.4%) patients, most frequently in exon 20. There were no differences in sex, stage or smoking behaviour between mutated and non-mutated cases. Patients with *PIK3CA* mutations had a significantly longer overall survival ($p = 0.042$) and time to relapse ($p = 0.030$) than non-mutated cases, and the difference in time to relapse was also retained in stage I-cases ($p = 0.044$). PD-L1-expression was less frequent among mutated cases.

*Conclusion:* Our results indicate that *PIK3CA* mutations may confer a survival advantage in early stage squamous cell lung cancers, but further work is needed to confirm this finding.

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## 1. Introduction

Lung cancer remains the leading cause of cancer-related deaths in both genders [1]. Genetic aberrations are regarded both prognostic and predictive in NSCLC, and are found with various frequency and different significance in the subtypes. Hitherto, targets for therapy are found primarily in adenocarcinomas, mainly *EGFR* mutations and *ALK* translocations, but also aberrations with lower frequencies, involving *e.g. BRAF*, *ROS1* and *RET* [2]. *PIK3CA* mutations are potentially therapeutically targetable and are frequently found in squamous cell carcinomas of the lung, which accounts for a third of all the lung cancers, as well as in breast cancer [3], head and neck cancer [4] and other cancers [5], whereas it is relatively infrequent in lung adenocarcinomas [6].

The lipid kinase phosphatidylinositol-3 kinase family (PI3K) is involved in a multitude of normal cellular processes, including the activation of the serine/threonine kinase AKT, which in turn activates a number of factors including mTOR [7]. The PI3K-Akt-mTOR pathway is central in the regulation of multiple cancer-relevant regulatory processes as cell survival, cell growth and cell cycle progression, and somatic mutations in this pathway are frequently found in cancers, hence being attractive targets for therapy [8,9]. PI3K consists of a catalytic (p110) and a regulatory subunit (p85). There are various isoforms of each subunit, and the catalytic subunit are encoded by three genes, *PIK3CA*, *PIK3CB* and *PIK3CD* [10], of which *PIK3CA* is most frequently mutated in cancers.

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The most common mutation hotspots on the *PIK3CA* gene are located in the helical domain, typically E542K and E545K on exons 9, and less frequently in the kinase domain, H1047R on exon 20 [6]. The helical domain mutations are thought to exert their activating effect through "unlocking" the inactive conformation, whereas kinase domain mutations changes the interaction between the protein and the cellular membrane thus providing access to the kinase substrate [11].

The prognostic role of *PIK3CA* mutations is still debated, and some studies have shown *PIK3CA* mutations to confer a survival advantage in some cancers, *e.g.* breast [12,13], whereas studies in other cancers, including lung adenocarcinomas, have indicated a correlation with poor prognosis [14]. So far little if anything is known of the potential prognostic role of *PIK3CA* mutations in squamous cell carcinomas of the lung. Clinical trials targeting PIK3CA are already ongoing [15–17], thus knowing the prognostic role of this aberration is warranted. PIK3CA is a known regulator of PD-L1 in cancer cells, probably involving both transcriptional and post-transcriptional mechanisms [18,19]. Given the new immunotherapies interfering with the PD1-axis, it is of interest to study the correlation of PD-L1 expression and *PIK3CA* mutations.

We have studied the frequency and prognostic importance of *PIK3CA* mutations in a large number of early stage lung cancers of squamous carcinoma subtype, and correlated these findings to clinic-pathological features, including PD-L1 expression.

## 2. Patients and methods

Tumour tissue was obtained from 308 consecutively operated early stage lung cancer patients with squamous cell carcinoma at Oslo University Hospital, Norway, in the period 2003–2013. No pre-defined inclusion or exclusion criteria were defined. None of the patients had undergone neoadjuvant therapy, whereas adjuvant therapy was given according to international guidelines [20]. Smoking information was collected, and categorized as never-smoking (less than 100 cigarettes smoked in lifetime), former smokers (stopped smoking one year or more before diagnosis of lung cancer) or current smokers.

DNA was isolated from fresh frozen tumour tissue stored in a dedicated biobank (n = 91) or archival paraffin embedded formalin fixed tissue samples (n = 217) according to standard procedures, and mutation analysis was done with either the SnapShot method (Life Technologies, Foster City, CA, USA), or using *PIK3CA* specific primers in the Cobas 4800 platform (Roche Diagnostics, Basel, Switzerland). The tumour cell content in the specimens was found to be more than 70% in most samples. The SnapShot-analysed samples were also analysed on the Cobas-platform with identical *PIK3CA*-results. The SNaPshot assay for evaluation of multiple oncogenic mutations in *APC, AKT1, BRAF, CTNNB1, EGFR, FLT3, JAK2, KIT, KRAS, MAP2K1 (MEK1), NOTCH1, NRAS, PIK3CA, PTEN*, and *TP53* was performed by amplification using 13 multiplexed PCR reactions followed by single nucleotide base extension reactions. The products were separated by capillary electrophoresis and analysed using GeneMapper 4.0 as has been previously described [21,22].

Immunohistochemical staining for p40 (Calbiochem, Cat.No PC373) was performed in order to verify the squamous histology by using the EnVisionTM FLEX + detection system from Dako (Dako, Glostrup, Denmark) according to manufacturer's protocol. Nuclear staining was evaluated and intensity scoring reported as none (0), weak/intermediate (1+) and strong (2+), the latter two regarded positive. PD-L1-staining (Ventana SP142 assay) was evaluated on tumour-infiltrating immune cells (IC) or tumour cells (TC). Positivity was defined as detectable staining on ≥5% of either IC or TC.

**Table 1**
Distribution of mutations found in the SnapShot-analysed cohort (n = 91).

| | N | % |
|---|---|---|
| No mutation | 70 | 76.9 |
| PIK3CA | 10 | 11.0 |
| TP53 | 7 | 7.7 |
| KRAS + TP53 | 1 | 1.1 |
| PTEN | 1 | 1.1 |
| JAK2 | 1 | 1.1 |
| BRAF (G469A) | 1 | 1.1 |

**Table 2**
Patient characteristics of the SnapShot cohort.

| | No mutation (n = 70) | *PIK3CA* mut (n = 10) | Other mut (n = 11) |
|---|---|---|---|
| Age (median, range) | 66.4 (43.2–82.4) | 71.0 (65.1–81.3) | 66.0 (45.9–81.0) |
| | *N* (%) | *N* (%) | *N* (%) |
| *Sex* | | | |
| Females | 20 (28.6) | 3 (30.0) | 7 (63.6) |
| Males | 50 (71.4) | 7 (70.0) | 4 (36.4) |
| *Smoking* | | | |
| Never | 1 (1.4) | 0 (0.0) | 0 (0.0) |
| Former | 49 (70.0) | 5 (50.0) | 8 (72.7) |
| Current | 20 (28.6) | 5 (50.0) | 3 (27.3) |
| Packyears | 32.0 | 32.5 | 39.0 |
| *Stage* | | | |
| I | 38 (54.3) | 9 (90.0) | 6 (54.5) |
| II | 22 (31.4) | 0 (0.0) | 4 (36.4) |
| III | 10 (14.3) | 1 (10.0) | 1 (9.1) |

After thorough follow-up (median 67.6 months, range 18.0–109.6), time to relapse (TTR) and overall survival (OS) was calculated in GraphPad Prism 6 software (GraphPad Inc., La Jolla, CA) with the Kaplan-Meier method using Log-rank for significance assessment. The study was approved by the Regional Ethics Board who granted a waiver of informed consent.

## 3. Results

Fresh-frozen tumour tissue samples from 91 resected early stage non-small cell lung cancer patients with unequivocal squamous cell carcinoma were analysed with the SnapShot method which detects mutations in hot-spot regions of 15 different cancer genes. *PIK3CA* mutations were most frequently found, in 10 cases (11%), whereas a *TP53* mutation (only minor parts of the *TP53* gene are analysed in SnapShot) was found in 8 cases, of which one also harboured a *KRAS* mutation (Table 1). None of the *PIK3CA* mutated cases had additional aberrations. More of the *PIK3CA* mutated cases were in stage I (9 of 10 versus 38 of 70 of non-mutated cases, based on the 7th edition of TNM-classification), otherwise there were no major differences in clinical characteristics between the non-mutated, *PIK3CA* mutated and otherwise mutated cases respectively (Table 2).

In this primary data-set, there was a difference in time to relapse regarding mutation types, with only one relapse (47.8 months after surgery) among the ten *PIK3CA* mutated cases, while 7 of the 11 patients with other mutations relapsed (Fig. 1).

We therefore decided to study *PIK3CA* mutated squamous cell carcinomas more extensively, and in addition to the 91 samples, another 217 resected squamous cell carcinoma cases were analysed with the Cobas *PIK3CA* mutation test kit. The SnapShot-analysed cases were re-tested with the Cobas method. There was a 100% concordance between the two methods (not shown). Approximately 20% of these cases were stained for the squamous cell carcinoma marker p40, and 92% were deemed p40 positive, the rest were diag-

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