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ORIGINAL ARTICLE Lung Cancers

# Detection of clinically relevant epidermal growth factor receptor pathway mutations in circulating cell-free tumor DNA using next generation sequencing in squamous cell carcinoma lung

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## Abstract

**Background:** Limited repertoires of targets are available in the management of squamous cell carcinoma lung. In this study, we analyzed epidermal growth factor receptor (EGFR), RAS, BRAF mutations in lung cancer patients of squamous cell histology using next-generation sequencing (NGS) on the circulating cell-free DNA (cf-DNA). **Materials and Methods:** In this prospective observational study, patients with squamous cell carcinoma lung, either newly diagnosed or having a progressive disease on prior therapy were eligible. Cf-DNA was extracted from peripheral blood and analyzed for EGFR, KRAS, NRAS, and BRAF mutations using NGS. **Results:** Sixteen patients were enrolled over a period of 1 month. The mean cf-DNA quantity extracted from the plasma was 96.5 ng (range, 15–200 ng). Eight clinically relevant mutations in the EGFR pathway were identified. These include Exon 21 mutations in 4 patients, Exon 20 mutation in onepatient, complex mutations with coexisting Exon 21 and Exon18 in one patient and KRAS Exon 2 mutations in two patients. **Conclusion:** cf-DNA is a minimally invasive technique for detection of clinically relevant mutations in lung cancer patients. The use of novel advanced techniques such as NGS may help in detecting EGFR pathway mutations in patients with squamous cell carcinoma lung.

**Key words:** Circulating cell-free DNA, epidermal growth factor receptor mutations, KRAS, next-generation sequencing, squamous cell carcinoma lung

## Introduction

Lung cancer remains one of the common malignancies worldwide and is associated with significant mortality.[1] Plethoras of molecular abnormalities were detected in adenocarcinoma lung that had a significant impact on the prognostication and management of these patients. On the other hand, a limited target driven options for prognostication and management of squamous cell carcinoma lung exists. Recent studies identified molecular abnormalities such as SOX2 amplification, NFE2 L2 and KEAP1 mutations, PI3K pathway changes, fibroblast growth factor receptor 1 amplification, and discoidin domain receptor 2 mutations. These mutations may have numerous therapeutic implications in the future.[2] Earlier studies suggest that abnormalities in the epidermal growth factor receptor (EGFR) pathway are uncommon in squamous cell carcinoma lung[3] while recent evidence suggests that a subset of these patients harbor mutations in the EGFR pathway.[4] In this study, we attempted to identify mutations in the EGFR pathway in circulating cell-free DNA (cf-DNA) using next-generation sequencing (NGS) among the patients with squamous cell carcinoma lung.

## Materials and Methods

A prospective observational study was conducted in the Department of Medical Oncology from March 1 to March 31, 2017. All squamous cell carcinoma lung patients either newly diagnosed or patients with progressive disease on prior treatment were eligible. Patients with secondary malignancies or patients currently on chemotherapy were excluded from the study. Written informed consent was obtained in all patients for participation in the study. The study was carried out in accordance with the Declaration of Helsinki and good clinical practice guidelines.

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Clinical profile of the patient such as age, sex, addictions, history of prior treatments, stage, and site of metastasis, was documented. Tissue diagnosis from either the primary tumor site or metastatic site was made. All patients underwent staging investigations at baseline or at progression.

Intended for circulating cell-free tumor DNA extraction 20 ml of the blood sample was collected in Streck® tubes from the patient. Using Qiagen Kit cf-DNA was isolated, and the amount of tumor DNA extracted was quantified. EGFR, KRAS, NRAS, and BRAF genes were enriched using the amplicon-based method. EGFR mutations analyzed include Exon 3; Exon 7; Exon 15; Exon 18: G719X; Exon 19: deletions; Exon 20: S768I, Insertions, T790M; Exon 21: L858R, L861Q, Exon 2, 3, 4 of KRAS and NRAS and also Exon 15: L597R/Q/S/V, V600E/K/L/R, K601E; Exon 11: G466V of BRAF. The amplicons were subjected to library preparation, and the libraries were sequenced using the HiSeq 2500 instrument. The depth of sequencing was quantified. The sequences were aligned to the human reference genome using BWA program and processed using Picard and genome analysis toolkits. Curated somatic database somatic variants from published literature, the Cancer Genome Atlas were used to identify reported mutation as per the AMP-ASCO-CAP guidelines.

## Results

Sixteen patients of squamous cell carcinoma lung were enrolled in the study. The clinical profile of the patients is described in Table 1. The mean circulating cell-free tumor DNA extracted from the plasma was 96.5 ng (range, 15–200 ng). Genomic analysis by NGS on the extracted cf-DNA revealed clinically relevant mutations in the EGFR pathway among 8 (50%) patients. The



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most common mutation was Exon 21 Leu858Arg (4 patients). One patient had Exon 20 Thr790Met mutation. One patient had complex mutations with coexisting Exon 21 Leu858Arg and Exon18 Gly719Arg in the same sample. Two patients had KRAS Exon2 Gly12Cys mutation. The depth of analysis and mutation allele frequency are described in Table 2.

**Table 1: Patient characteristics (*n*=16)**

| Patients | Number (*n*) |
|---|---|
| Sex | |
| Male | 15 |
| Female | 1 |
| Median age (range) | 62 (37-69 years) |
| ECOG performance status | |
| 1 | 12 |
| 2 | 4 |
| Smoking | 15 |
| Beedi smoking | 13 |
| Cigarette smoking | 2 |
| Other addictions | |
| Alcohol consumption | 7 |
| Tobacco chewing | 2 |
| Biopsy | |
| CT-guided biopsy | 4 |
| USG-guided biopsy | 3 |
| Bronchoscopic biopsy | 6 |
| Lymph node biopsy | 2 |
| Skin biopsy | 1 |
| Stage | |
| IIIB | 6 |
| IV | 10 |
| Site of metastasis | |
| Contralateral lung nodules | 7 |
| Pleural/pericardial effusion | 4 |
| Bone | 3 |
| Adrenal | 1 |
| Brain | 1 |
| Skin metastasis | 1 |

ECOG=Eastern Cooperative Oncology Group, CT=Computed tomography, USG=Ultrasonography

Among the patients with Exon 21 mutation, two patients were treatment naïve, and two patients were having progressive disease (one post gemcitabine/carboplatin-based chemotherapy and another post gemcitabine/carboplatin and docetaxel chemotherapy). Patient with complex mutations had progressive disease post gemcitabine/carboplatin. Patient with Exon 20 T790M mutation had a hyper-progressive disease post-Nivolumab based regimen. While one patient with KRAS mutation was treatment naive another had progressive disease post gemcitabine/carboplatin-based regimen [Table 2].

Two patients with Exon 21 mutations who progressed on earlier lines of treatment received Gefitinib. One patient had progressive disease at 3 months, and another patient succumbed to the disease 2 months after starting gefitinib. Treatment Naïve patients with EGFR Exon 21 mutations ($n$ = 2) upfront received gemcitabine and carboplatin-based chemotherapy. Of this 1 patient is currently progression-free and another patient progressed 6 months post chemotherapy and at progression was started on gefitinib. Patient has a stable disease after 3 months of treatment and is still on gefitinib. Patient with Exon 20 T790M mutation was stated on nab-paclitaxel and succumbed to the illness 6 months later. Patient with complex mutations received docetaxel as second-line chemotherapy and had a progressive disease 4 months after the initiation of therapy and died.

## Discussion

The incidence of squamous cell carcinoma lung in India varies from 15.6% to 30%.[5,6] Worldwide the incidence of squamous cell carcinoma lung is decreasing.[7] Molecular sub-classification and target based tailored approach for squamous cell carcinoma lung is still not standardized or recommended.[8] There are currently limited advances and targeted treatment opportunities in the treatment of squamous cell carcinoma lung.[8] In adenocarcinoma lung patients activating EGFR mutations account for 10%–15% in the Caucasian population and 40%–50% in the Asian population.[9] The frequency of EGFR mutations in squamous cell carcinoma lung varies between 0%

**Table 2: Depth of analysis and mutation allele frequency**

| Patient number | DNA extracted (ng) | Hotspot average sequencing depth | Gene mutataed | Exon number | Amino acid variant | Depth of mutation analysis | Mutant allele (%) | First line regimen | Second line regimen |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 92 | 128078X | EGFR | Exon 21 | Leu858Arg | 18010X | 1.9 | Gemcitabine/carboplatin | Docetaxel |
| 2 | 100 | 870347X | EGFR | Exon 20 | Thr790Met | 32233X | 1.2 | Induction chemotherapy and radiotherapy | Nivolumab |
| 3 | 15 | 130773X | EGFR | Exon 21 | Leu858Arg | 1946X | 2.3 | Gemcitabine/carboplatin | - |
| 4 | 32 | 137878X | EGFR | Exon 18 | Gly719Arg | 8900X | 8.5 | Gemcitabine/carboplatin | - |
| | - | | EGFR | Exon 21 | Leu858Arg | 22202X | 1.5 | - | - |
| 5 | 95 | 167048X | EGFR | Exon 21 | Leu858Arg | 15839X | 1.6 | - | - |
| 6 | 145 | 472744X | KRAS | Exon 2 | Gly12Cys | 26229X | 16 | - | - |
| 7 | 190 | 161326X | EGFR | Exon 21 | Leu858Arg | 22162X | 1 | - | - |
| 8 | 70 | 417686X | KRAS | Exon 2 | Gly12Cys | 32255X | 29 | Gemcitabine/carboplatin | - |
| 9 | 70 | 793053X | | | - | - | - | Gemcitabine/carboplatin | Docetaxel |
| 10 | 25 | 349726X | - | - | - | - | - | Gemcitabine/carboplatin | - |
| 11 | 200 | 169027X | - | - | - | - | - | Gemcitabine/carboplatin | - |
| 12 | 45 | 228170X | - | - | - | - | - | - | - |
| 13 | 200 | 242459X | - | - | - | - | - | - | - |
| 14 | 130 | 164393X | - | - | - | - | - | - | - |
| 15 | 40 | 580006X | - | - | - | - | - | - | - |
| 16 | 95 | 155958X | - | - | - | - | - | - | - |

EGFR=Epidermal growth factor receptor

and 14.6%.[10] In the Indian population, the frequency of EGFR mutations in patients with adenocarcinoma lung ranges between 25%– and 40% while the frequency of EGFR mutations in squamous cell carcinoma lung in an earlier study was 4.5%.[11,12]

Most oncological societies favor EGFR mutational testing in squamous cell carcinoma lung in never smokers, small biopsy specimens, or mixed histology. The evidence regarding the responses to EGFR tyrosine kinase therapy in squamous cell carcinoma lung cancer patients harboring activating EGFR mutations is controversial. While some groups suggest a comparable median survival in EGFR mutation-positive adenocarcinoma and non-adenocarcinoma patients,[13] others suggest a poor survival in EGFR positive squamous cell carcinoma lung patients when treated with EGFR tyrosine kinase inhibitors (TKI).[12] Response to treatment with EGFR TKI in these patients is approximately half of what is seen in adenocarcinoma lung patients treated with EGFR TKI.[8] No prospective randomized clinical trial evaluated the benefit of EGFR TKI in patients with activating mutations in squamous cell carcinoma lung. With the limited treatment options available in these patients EGFR TKI may still hold to be one of the promising treatments.

KRAS mutations are relatively rare mutations in squamous cell histology than adenocarcinoma histology.[14-16] Due to the rarity of these mutations in squamous cell carcinoma lung, currently, KRAS mutation analysis is not recommended.[14] The prognostic and predictive implications of KRAS mutations in squamous cell carcinoma lung is controversial.[14-16]

In our study, we incorporated NGS for detecting clinically relevant mutations in the EGFR pathway in squamous cell carcinoma lung patients. NGS is more efficient and has a greater depth of sequencing than first generation sequencing technologies such as Sanger sequencing. Thus NGS offers an increased sensitivity in detecting desired mutations.[17] As the study population was heterogeneous NGS analysis was performed on cf-DNA extracted from the peripheral blood rather than on the tumor tissue as repeat biopsy at the time of progression was not performed in most of these patients.

The high frequency of activating mutations detected in squamous cell carcinoma lung noted in our study is the first as per our knowledge of the literature. The small and heterogeneous sample size may be the primary reason for this high frequency of mutation detection. Patients with progressive disease formed a significant proportion of our study population. The other reason might be that the earlier studies evaluated EGFR mutations using lesser sensitive techniques such as PCR.[18-21]

Our study is limited by the small sample size and the heterogeneous patient population including both treatment naïve and post-treatment patients. A prospective study using advanced techniques such as NGS and with a larger sample size may help in detecting the true percentage of patients with squamous cell carcinoma lung carrying activating EGFR mutations.

## Conclusion

Due to the limited treatment options in the management of squamous cell carcinoma lung detection of EGFR mutations may help further increase the treatment armamentarium. Cf-DNA is a minimally invasive technique for detection of clinically relevant mutations in lung cancer patients. The use of novel advanced techniques such as NGS may help in detecting EGFR pathway mutations in patients with squamous cell carcinoma lung.

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## Conflicts of interest

There are no conflicts of interest.

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