



Mutational Profiling by Next-Generation Sequencing in Patients with Metastatic Non-Small Cell Lung Carcinoma: Our Experience

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Abstract



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Introduction According to GLOBOCAN 2020, overall lung cancer is the second most common cancer in both sexes (11.4%) accounting for highest cancer-related mortality (18%).¹ Adenocarcinoma subtype of non-small cell lung cancer (NSCLC) is the most common subtype and is divided into further molecular subtypes based on oncogenic driver mutations. Overall survival in these patients is poor with the use of conventional platinum-based double chemotherapy and various recent studies on targeted therapy studies have showed improved survival. Therefore, broad panel-based testing like next-generation sequencing (NGS) is strongly recommended to identify these targetable driver mutations.

Aims and Objectives The aim of this study was to evaluate the mutational profile in patients with metastatic NSCLC (mNSCLC) by NGS method.

Materials and Methods A hospital-based prospective observational study done on 88 patients under the Department of Medical Oncology, State Cancer Institute during a period of 1 year. All patients above 18 years of age diagnosed as mNSCLC having Eastern Cooperative Oncology Group performance status 0 to 2 and evaluated for mutational profiling by NGS method were included. Five gene panel tests including endothelial growth factor receptor (EGFR), echinoderm microtubule-associated protein-like 4 and anaplastic lymphoma kinase (EML4-ALK), BRAF, mesenchymal epithelial transition (MET), and ROS proto-oncogene 1 (ROS1) were used.

Results and Observations Majority of mNSCLC cases were in the age group of 41 to 50 years ($n = 30$, 34.1%) with average age at presentation being 53.74 years. Male: female ratio was 1.14:1 and most patients were nonsmokers. Adenocarcinoma subtype of mNSCLC cases had the highest mutational burden ($n = 55$, 62.5%). EGFR ($n = 32$, 56.14%) was the most common mutation followed by EML4-ALK ($n = 19$, 33.33%). Most

Keywords

- EGFR
- EML4-ALK
- lung cancer
- NGS
- oncogenic driver mutations

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common EGFR mutation was in Exon 19. Other rare mutations were ROS1 ($n = 4$), BRAF V600E ($n = 1$), and MET ($n = 1$). Skeleton was the most common site of metastasis across all driver mutations.

Conclusion EGFR and EML4-ALK were the commonest targetable mutations detected in the study. As there is very limited data from North Eastern region of India regarding mutational status in mNSCLC, this study opens up possibilities for further studies targeting multiple mutations to give us more comprehensive understanding of the mutational landscape of mNSCLC in this era of precision medicine.

Introduction

According to GLOBOCAN 2020 statistics, lung cancer is the most common cancer in men (14.3%) and third most common cancer in female (8.4%). Overall lung cancer is the second most common cancer in both sexes (11.4%) after female breast cancer (11.7%) but accounts for overall highest cancer-related mortality (18%).¹ In India, lung cancer is fourth most common cancer both in incidence and cancer-related death.² Histologically, lung cancer is divided into non-small cell (NSCLC) and small cell lung cancer (SCLC). NSCLC is the most common type accounting approximately 80 to 85% cases and includes adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma. Adenocarcinoma subtype accounts for more than half and is further defined according to different molecular subtypes by the identification of oncogenic drivers.

Over the last few decades, conventional platinum-based chemotherapy remains the mainstay of treatment in metastatic NSCLC, but it has produced only a modest increase in patient's overall survival, reaching a plateau, with response rates around 35% and median survival time of 10 to 12 months.³ With recent advances in the knowledge of NSCLC biology, various oncogenic driver mutations are identified that cause aberrant activation of intracellular signaling pathways associated with the sustained growth of lung cancer cells. This leads to the development of molecularly directed targeted therapies with significant improvement in patient's outcomes. Updated recommendations from the College of American Pathologists, International Association for the Study of Lung Cancer, Association for Molecular Pathology, and European Society of Medical Oncology have strengthened the 2013 guidelines and suggest genomic testing for endothelial growth factor receptor (EGFR), ALK, and ROS1 for all advanced NSCLC, regardless of patient's characteristics which was revised in 2018.⁴ The minimal conventional genomic study generally includes, sequentially or in parallel, at least, the EGFR, ALK, and ROS 1 mutational analysis. Sequential testing requires a substantial DNA amount, is time-consuming, caused sample exhaustion, and often leads to under genotyping and treatment delay.^{5,6} Therefore "National Comprehensive Cancer Network (NCCN)" strongly advises to perform broad panel-based testing like next-generation sequencing (NGS) in appropriate patients with stage IV NSCLC, because it is more efficient and

cost-effective than testing for one biomarker at a time for detection of rare mutations to which targeted therapies are available.⁷ NGS allows the sequencing of several genomic regions in a single test, in a single platform and even in samples with low DNA content. An NGS-based approach potentially provides a more sensitive and comprehensive genetic characterization of lung cancer, which may impact the therapeutic options and patient's prognosis.

Aims and Objective

The aim of this study was to evaluate the mutational profile by NGS method in patients with metastatic NSCLC, diagnosed in a Tertiary Hospital in North East India.

Materials and Methods

This study is a hospital-based prospective observational study performed on 88 patients evaluated and treated under the Department of Medical Oncology, State Cancer Institute, Guwahati during a period of 1 year from June 1, 2020 to May 31, 2021. All patients aged 18 years or above, who were diagnosed as metastatic NSCLC with Eastern Cooperative Oncology Group performance status (ECOG PS) 0 to 2 and gave consent for the study and underwent mutational study by NGS were included in the study. Patients with histology large cell lung cancer, SCLC, or ECOG PS 3 or more were excluded from the study.

Ethical clearance was obtained from the ethical committee of the institution prior to the study vide letter no. SCI/ECR/2020/15 held on April 29, 2020 at Seminar Hall State Cancer Institute, Guwahati

The data for the study was collected in a predesigned proforma. The patients were fully informed about the study and their informed consent was taken prior to their participation in the study. A detailed clinical history, physical examination, and relevant investigations were done. Non-smokers are those patients who never had a smoking history or smoked less than 100 cigarettes in lifetime. Ex-smokers are those who smoked more than 100 cigarettes in his lifetime but quit smoking 1 year back and current smokers are those who continue to smoke.

NGS was performed in fresh frozen paraffin embedded tissue block. DNA from samples was extracted and subjected to NGS using the ion S5 system. To achieve optimal

sensitivity, this assay requires a minimum of 10 to 100 ng DNA. High-quality nucleic acids that passed quality control checked were subjected to library preparation and analyzed for relevant genomic alternations. In this study, we have included five genes, for example, EGFR, EML4-ALK, BRAF, MET, and ROS1 that are under current recommendation from NCCN guideline for broad-based panel testing for metastatic NSCLC and they have specific actionable targeted therapy recommended against them. Sequencing was performed to achieve a minimum 500x depth of coverage. Reporting was done only for variant with a minimum coverage of more than 500x. The output sequences were aligned to the human reference genome hg19 (GRCh37). High-quality sequencing data was then analyzed using the optimized ION (a software used in NGS data analysis) torrent suite and the ION Reporter software to accurately detect rare somatic variants. The hotspots, indels, and fusions were analyzed with the help of the ION reporter software and variants were annotated according to the American College of Medical Genetics and American Medical Oncologist (AMO) guidelines.

NGS panel study for mutational analysis was not available in study the center, so it was done using a sponsored coupon form Novartis Healthcare under their patient support program.

Results

This study is a hospital-based prospective observational study performed on 88 patients in department of Medical Oncology, State Cancer Institute, Guwahati, during the study period of 1 year from June 1, 2020 to May 31, 2021. After considering the inclusion and exclusion criteria, a total of 88 cases were studied. The results and observations of this study are described in the following text.

Majority of cases ($n = 30$) of mNSCLC cases were in the age group of 41 to 50 years (34.1%). The next majority of patients ($n = 21$) were in the age group of 51 to 60 years (23.9%). Highest age was 75 years and lowest was 26 years in this study. Average age at presentation in the study was 53.74 years. Majority of the patients ($n = 47$) were male (53.4%). Females comprised of 46.6% of cases ($n = 41$). The male:female ratio in the study was 1.14:1. Out of 88 patients, 70 patients (79.5%) were from rural areas and 18 patients (20.5% cases) were from urban areas. Most patients in the study were nonsmoker in the study ($n = 47$, 53.4%), while 21.6% ($n = 19$) were ex-smoker and 25% ($n = 25$) were current

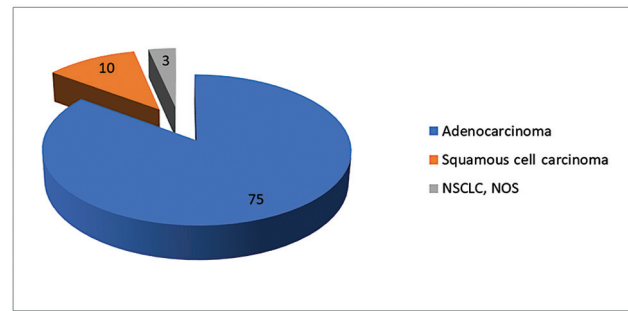


Fig. 1 Histopathological types of metastatic non-small cell lung carcinoma (NSCLC). NOS, not otherwise specified.

smoker. In this study, adenocarcinoma ($n = 75$, 85.2%) was the most common histological type followed by squamous cell carcinoma ($n = 10$, 11.4%) and NSCLC, not otherwise specified ($n = 3$, 3.34%) as shown in **Fig. 1**.

mNSCLC cases with adenocarcinoma histology had the overall highest mutational status ($n = 55$, 62.5%). Squamous cell carcinoma and NSCLC, not otherwise specified type had very rare mutational status ($n = 1$ each) as shown in **Table 1** and **Fig. 2**. Total 31 patients (35.3%) had no detectable mutation in the study.

As shown in **Table 1** and **Fig. 2**, EGFR ($n = 32$, 56.14%) is the most common mutation detected in mNSCLC followed by EML4-ALK ($n = 19$, 33.33%). ROS1 mutation was detected in four patients, while MET and BRAF V600E mutations were detected in one patient each. Among the mNSCLC cases where driver mutations were detected ($n = 57$, 64.77%), adenocarcinoma is the most common histology ($n = 55$, 96.49%). Again, among metastatic adenocarcinoma of lung ($n = 55$), EGFR is the most common mutation ($n = 31$, 56.33%) followed by EML4-ALK ($n = 19$, 33.33%). ROS1, BRAF V600E, and MET were the rare mutations detected in 5.45% ($n = 3$), 1.81% ($n = 1$), and 1.81% ($n = 1$) each.

Most common variant in EGFR mutation was Exon 19 ($n = 17$, 53.12%) followed by Exon 20 ($n = 8$, 25%) and Exon 21 ($n = 6$, 18.75%) as shown in **Fig. 3**. EGFR mutation in Exon 18 was the rarest mutation ($n = 1$, 3.13%). Two patients with EGFR Exon 20 have upfront T790M mutation. p. Glu746_Ala750 was the most common variant in EGFR Exon 19 mutation, while L858R was the commonest variant in EGFR Exon 21 mutation.

Among patients with mNSCLC with detectable driver mutation, skeleton is the overall most common site of

Table 1 Mutational pattern among mNSCLC cases

| Histology | Mutation detected | | Mutation not detected | |
|-------------------------|-------------------|----------------|-----------------------|----------------|
| | Number (n) | Percentage (%) | Number (n) | Percentage (%) |
| Adenocarcinoma | 55 | 62.5 | 19 | 21.6 |
| Squamous cell carcinoma | 1 | 1.1 | 10 | 11.4 |
| NSCLC, NOS | 1 | 1.1 | 2 | 2.3 |
| Total | 57 | 64.77 | 31 | 35.3 |

Abbreviations: mNSCLC, metastatic non-small cell lung cancer; NOS, not otherwise specified.

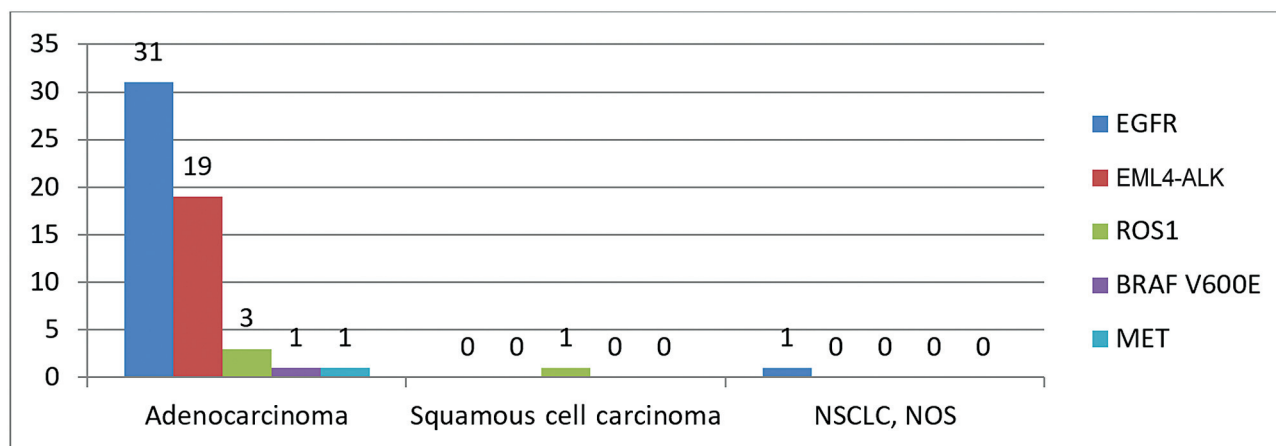


Fig. 2 Mutational landscape in metastatic non-small cell lung carcinoma (NSCLC). NOS, not otherwise specified.

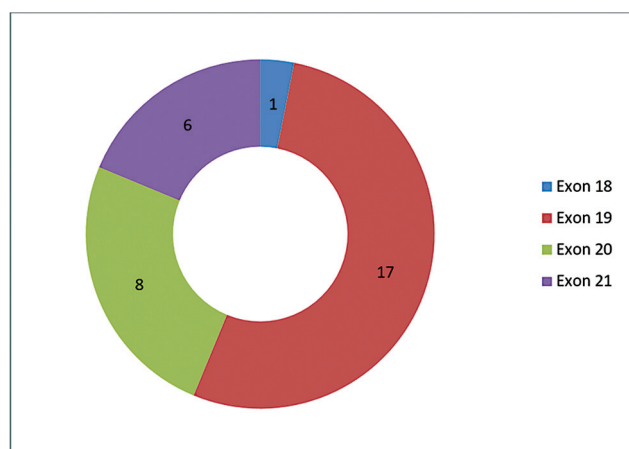


Fig. 3 Subtypes of epithelial growth factor receptor mutations.

metastasis ($n=39$) followed by pleural effusion ($n=13$), brain ($n=12$), and opposite lung ($n=9$). Also, skeleton is the most common site of metastasis across all subtypes of driver mutations, while brain is the most common site in the EML4-ALK mutated patients.

Discussion

This study is a prospective hospital-based study of mutational profile of metastatic NSCLC patients. NSCLC is the most common histological type of lung carcinoma that can be further divided into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. In this era of precision medicine, NSCLC is further divided based on driver mutation like EGFR, EML4-ALK, and ROS1. These targets are important not only for classification but also for therapeutic purpose and for prognostication.

Chun et al⁸ and Zhang et al⁹ in their study found that the median age of mNSCLC was 63 years (range, 34–83) and 63 years (range, 33–87), respectively, and in our study, we found that majority of cases ($n=30$) were in the age group of 41 to 50 years (34.1%) followed by 51 to 60 years ($n=21$, 23.9%) age group. Average age at presentation in the study was 53.74 years. There was male preponderance of mNSCLC cases in this

study like other studies from China⁹ and Portugal.¹¹ Smoking plays an important role in oncogenesis of lung cancer, but most mNSCLC cases in this study were nonsmoker like in other studies. In compared to the study by Zhang et al⁹ and Reis et al,¹² our study also found that adenocarcinoma was the most common histology for mNSCLC.

In this study, we have found that EGFR ($n=32$, 56.14%) is the most common mutation detected in mNSCLC followed by EML4-ALK ($n=19$, 33.33%). ROS1 mutation was detected in four patients, while MET and BRAF V600E mutations were detected in one patient each. As shown in **Fig. 4**, in most studies^{8,9,11,12} EGFR was found to be the most common driver mutation in NSCLC.

The incidence of EGFR mutations is much higher in the Indian population than in whites and is closer to the incidence observed in East Asian countries.¹³ The incidence of EGFR mutation differs by ethnicity, with 10 to 23% of adenocarcinomas in whites being driven by activating EGFR mutations, compared with 40 to 50% of adenocarcinomas in Asians as shown in **Fig. 5**.^{14–16}

Conclusion

In this study, an approach was made to know the mutational profile and clinical status of metastatic NSCLC patients attending Medical Oncology Department State Cancer Institute, Guwahati.

At the end of this study analysis, it was seen that mNSCLC here is common in younger age group compared with other studies, common in nonsmoker male, and adenocarcinoma is the most common histology. Most patients were residing in rural areas of the state. As most patients in stage IV disease presented with ECOG PS 2 (60.23%), palliative chemotherapy in them may further deteriorate the quality of life and ECOG PS, so searching for a targetable driver mutation in them is the current standard of care which in this study was done with NGS technique. We have found that EGFR is the most common driver mutation in them followed by EML4-ALK. Overall skeleton is the most common site of metastasis, while presence of brain metastasis may also indirectly suggest EML4-ALK derived mNSCLC.

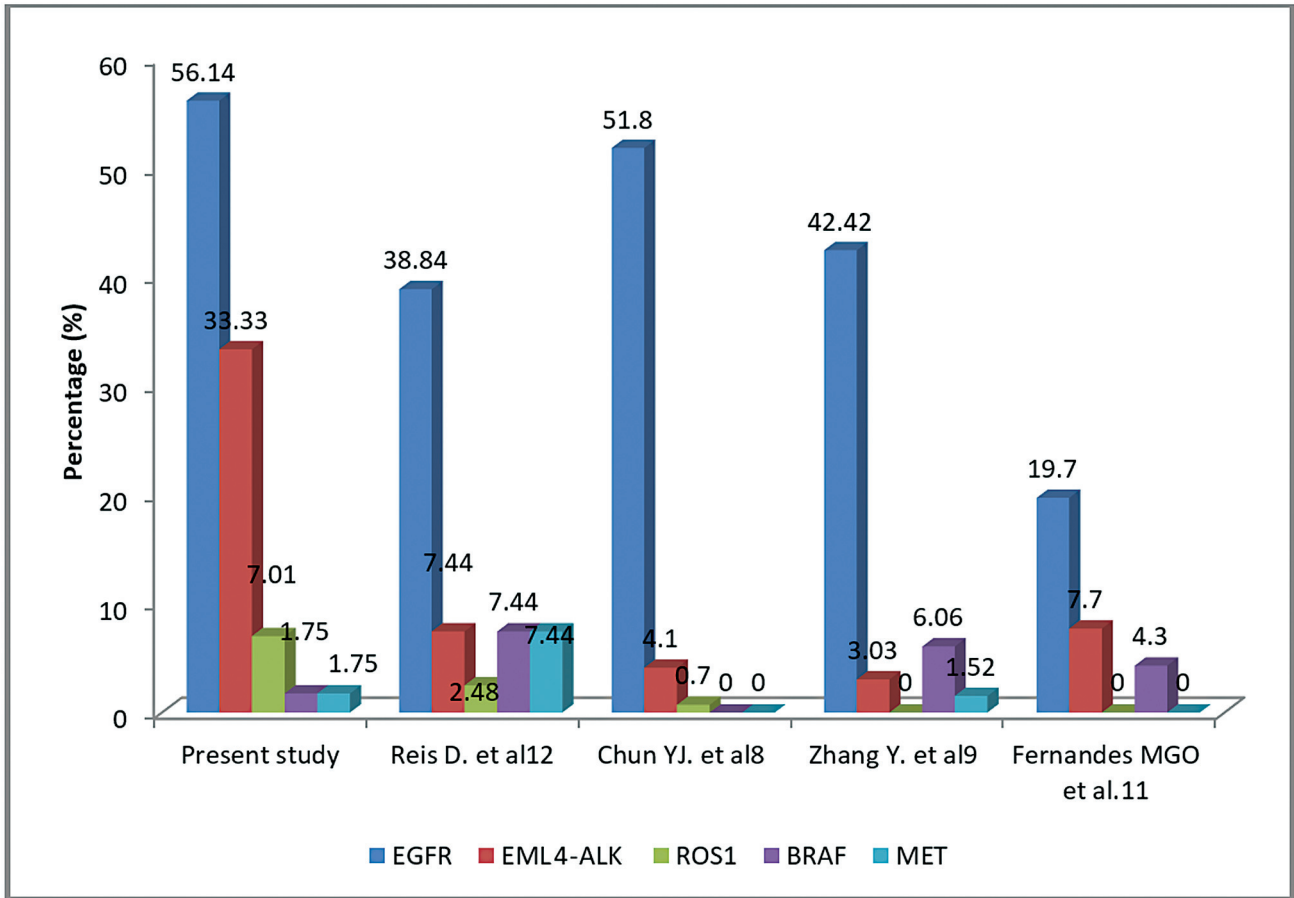


Fig. 4 Mutational landscape in metastatic non-small cell lung carcinoma (mNSCLC). EGFR, epithelial growth factor receptor.

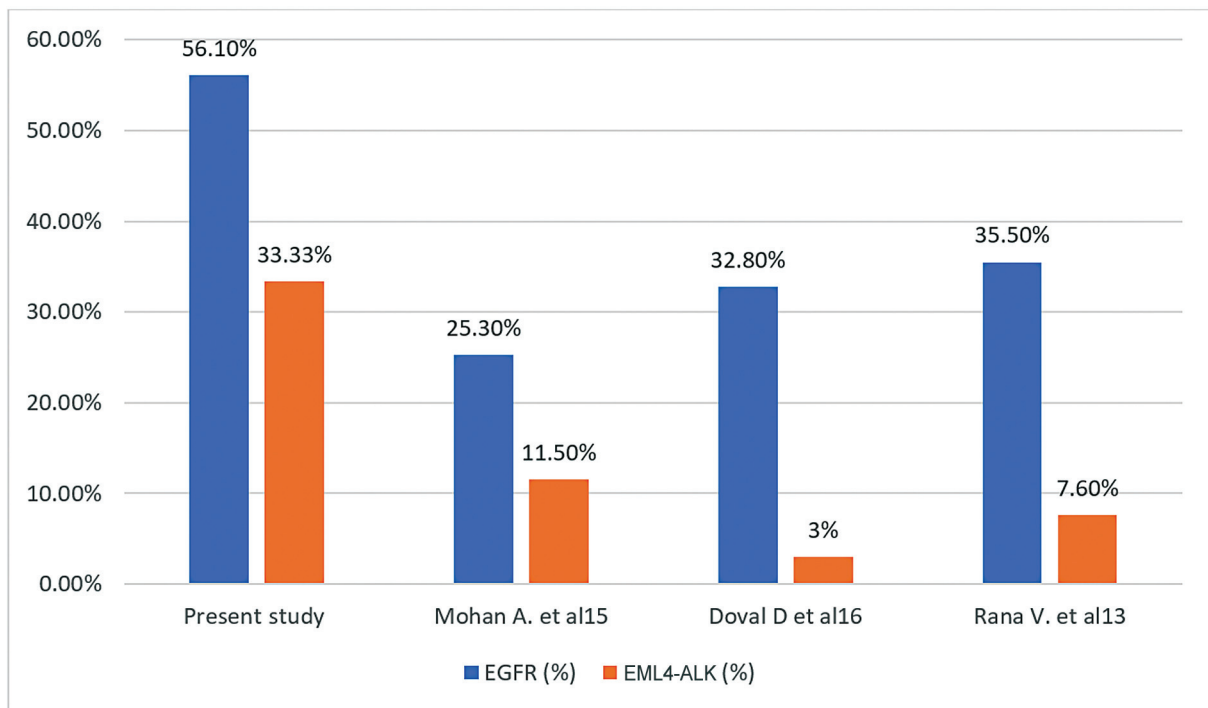


Fig. 5 Mutational pattern in studies from India. EGFR, epithelial growth factor receptor.

There is very limited data from North East region till now regarding mutational study in mNSCLC. Most current recommendation is based on western data. However, geographical and demographic differences of this part of world may have impact on the mutational profile in lung cancer patients from this region. About 64.77% of our mNSCLC patients have driver mutation detected by NGS method as per current recommendation of which 56.14% had EGFR and 33.33% had EML4-ALK mutation. Although this study comprises relatively a small sample size for a short period of time, it opens up wide range of possibilities for further studies. Larger prospective study with a large sample size targeting multiple mutations for a long duration is required to give us more comprehensive understanding of the mutational landscape of mNSCLC in this part of world in the current era of precision medicine.

Limitation of the Study

A limitation in our study is that it was conducted on a small sample size of 88 patients. Since the duration and study population were less, further studies with a large number of patients over a larger duration of period are required.

Funding

None.

Conflict of Interest

None declared.

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