RESEARCH ARTICLE

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Genomic Analysis and Clinical Correlation of Non-Small Cell Lung Cancer with Special Reference to Brain Metastasis

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Abstract

Background: Next-generation sequencing (NGS) has improved genomic analysis depth in precision oncology. This study analyzed genomic biomarker testing in stage IV NSCLC, focusing on brain metastasis and clinicopathological correlations. **Objective:** To study molecular markers and clinicopathological correlations in stage IV NSCLC patients, with and without brain metastasis. **Methods:** A total of 169 stage IV NSCLC patients were studied from April 2023 to May 2025. Demographic data, clinical presentations, and mutation analyses were assessed using NGS on tissue blocks or liquid biopsies. **Results:** Among 169 patients, 41.42% (n = 70) had brain metastasis (NSCLC-BM), while 58.58% (n = 99) had no brain metastasis (mNSCLC). Median ages were 51.5 and 56 years, respectively. Adenocarcinoma comprised 95.27% (n = 161) of cases. The cerebral hemisphere was the most common intracranial metastatic site, while skeletal involvement was the most common extracranial site. Headache was the predominant neurological symptom. *EGFR* mutations were the most common overall. *EGFR* > *TP53* > *ALK* > other mutations were observed in NSCLC-BM, while *EGFR* > *TP53* > *KRAS* > other mutations were seen in mNSCLC. Mutation analysis stratified by smoking history ($\chi^2(1) = 1.347$, $p = 0.245$) and sex ($\chi^2(1) = 0.0302$, $p = 0.862$) was not statistically significant. The benefit of gefitinib plus chemotherapy in *EGFR* exon 19 and exon 21 L858R mutations was greater in mNSCLC (log-rank $\chi^2(1) = 10.813$, $p = 0.001$) than in NSCLC-BM (log-rank $\chi^2(1) = 3.100$, $p = 0.078$). Median survival was 11 months (95% CI: 7.506–14.494) for NSCLC-BM versus 21 months (95% CI: 8.365–33.635) for mNSCLC, with a statistically significant difference (log-rank $\chi^2(1) = 8.639$, $p = 0.003$). **Conclusion:** NSCLC-BM showed higher genomic biomarker enrichment (80% vs. 68.68%) but poorer outcomes than mNSCLC. *EGFR* was the most common targetable mutation, followed by *ALK* in NSCLC-BM and *KRAS* in mNSCLC.

Keywords: brain metastases- metastatic non-small cell lung cancer- genomic analysis- next-generation sequencing

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Introduction

Lung cancer is the most frequently diagnosed cancer in males and females. It is also the most common cause of cancer-related mortality worldwide [1]. Lung adenocarcinoma, a prevalent subtype of NSCLC, poses a significant therapeutic challenge, especially in stage IV patients with brain metastasis. Brain metastasis is common among NSCLC, and approximately 10-40% of patients eventually develop brain metastasis [2].

Brain metastases are a critical complication of non-small cell lung cancer, affecting survival rates and requiring systemic therapy for management. In advanced stages, approximately 36% of patients with NSCLC develop brain metastases, which are associated with a poor prognosis and a median survival time of approximately 7 months [3]. Local therapies such as stereotactic radiotherapy, whole-brain radiotherapy, and surgery are employed to manage brain control [3]. The development of brain metastases signifies a grim prognosis due to the high frequency of recurrence following standard treatments, such as whole brain irradiation, which often fails to prolong overall survival [4]. The advent of next-generation sequencing has identified numerous genetic changes in NSCLC with many targeted medicines are being used in clinical practice [5]. Non-small cell lung cancer accounts for 80–85% of all lung cancer cases and usually shows no symptoms until it has advanced, leading to poor prognosis [6]. Therapeutic strategies are determined by analyzing the molecular hallmarks of non-squamous NSCLC, such as alterations in *EGFR*, *ALK*, *BRAF, KRAS, HER2, ROS*, and *MET* genes [7]. Despite improvements in systemic therapy, the survival rate of individuals diagnosed with stage IV disease remains low, with fewer than 5% surviving beyond 5 years [8].

With the treatment of metastatic disease relying on

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targetable driver mutations, genomic analysis has become important, providing deeper insights into the molecular underpinnings of this aggressive disease. Data on stage IV Non-small cell lung carcinoma with brain metastasis are limited; hence, a study of the genomic landscape in this subset is undertaken.

Materials and Methods

The current study was a ambi-spective observational study started from April 2023 to May 2025 at a tertiary cancer institute. Data were procured from patients followed up in the Medical Oncology outpatient department and those admitted in wards.

Patient selection

Inclusion criteria

1. Patient willing for genetic testing.
2. Histologically confirmed case of Non-small cell lung carcinoma.
3. Radiologically proven cases of metastatic Non-small cell lung carcinoma with and without Brain Metastasis.

Exclusion criteria

1. Patient not willing for genetic testing.
2. Patients with small cell lung carcinoma.

Given the observational nature of this study and the lack of comprehensive Indian data on genomic profiles of NSCLC with brain metastasis, this study was designed as an exploratory analysis. A post-hoc power analysis was performed to assess the adequacy of the sample size. Demographic, pathological, and genomic profiles; clinical data; and date of diagnosis were obtained from patient records. Clinical staging was conducted using Fluorodeoxyglucose Positron Emission Tomography/ Computed Tomography (FDG PET/CT) and magnetic resonance imaging (MRI), and response to therapy was assessed using Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) and Response assessment in neuro-oncology brain metastases (RANO-BM) criteria. Genomic profiling was performed using next-generation sequencing of tumor and liquid biopsy samples. For patients with mNSCLC and de novo NSCLC-BM, NGS was performed on tissue biopsy samples after confirming adequate tumor content. For patients with inadequate tumor content and metastatic NSCLC progressing to brain metastasis on treatment, liquid biopsy was performed.

Genomic analysis

Genomic profiling was performed using next-generation sequencing in the National Accreditation Board for Testing and Calibration Laboratories (NABL)-accredited reference laboratories following internationally validated protocols. Platform allocation was determined by sample availability and clinical indication: tissue-based analysis utilized the Ion Torrent platform, whereas liquid biopsy analysis employed the Illumina platform.

For tissue-based analysis, DNA and RNA were extracted from formalin-fixed, paraffin-embedded lung tumor biopsies with tumor cellularity ≥20%, using standard formalin-fixed paraffin-embedded (FFPE) extraction protocols (Qiagen, Hilden, Germany). DNA libraries were prepared using the Ion AmpliSeq™ colorectal and lung somatic panel (Thermo Fisher Scientific, Waltham, MA, USA) covering 22 clinically actionable genes, while RNA libraries utilized a targeted fusion panel (Thermo Fisher Scientific) interrogating *ALK*, RET, ROS1, and NTRK transcripts. Libraries were sequenced on the Ion S5™ semiconductor platform, achieving a mean sequencing depth of 6,963× for the DNA samples.

Sequenced data were aligned to the human reference genome (hg19) and analyzed for mutations using the Ion Torrent Suite™ and Ion Reporter™ software with standard quality filters. Quality control metrics, including on-target rates >98% and uniformity >95%, met established standards. Orthogonal Sanger sequencing confirmation was recommended for clinically significant variants but was not performed for any variants detected in this study.

Liquid biopsy analysis using the OncoMonitor™ assay (OneCellDx, Pune, India) was performed selectively in patients with inadequate tissue biopsy samples or those developing brain metastasis during treatment. Ten milliliters of peripheral blood were processed for circulating free DNA (cfDNA) and circulating tumor cell (CTC) detection. Plasma cfDNA was subjected to hybrid-capture sequencing targeting 126 selected clinically relevant genes on an Illumina NovaSeq 6000 platform with a mean coverage of ~10,000×. CTCs were enumerated by immunophenotyping ($EpCAM^+/CK^+/CD45^-$). Variants with allele fractions of ≥0.1% were reported using proprietary error-suppression algorithms.

Owing to financial constraints, patients underwent either tissue-based (Ion Torrent) or liquid biopsy (Illumina) testing, but not both. Genomic findings were integrated into treatment selection as part of routine clinical care.

Statistical analysis

The data were entered into a Microsoft Excel spreadsheet and analyzed using SPSS version 23. Descriptive statistics (frequencies, means, medians, and ranges) were used as baseline parameters. The chi-square test was used, and statistical significance was set at $p < 0.05$. The Kaplan–Meier method was used to prospectively assess survival probabilities.

Results

Clinicopathologic characteristics

Among 169 patients 41.42% (n=70) of patients had brain metastasis, while 58.58% (n=99) did not have brain metastasis. Males were more common (55.71%, n=39 vs. 53.53%,n=53) in the NSCLC-BM cohort, while females were more common (46.46%, n=46 vs. 44.28%, n=31) in mNSCLC cohort.

Adenocarcinoma was the only subtype observed in patients with brain metastasis (100%, n=70 cases), whereas in the group without brain metastasis, adenocarcinoma was highly predominant (91.91%, n=91 cases) followed by squamous cell carcinoma, adenosquamous cell carcinoma and NSCLC-NOS. In terms of smoking history,

non-smokers were more prevalent in both groups. The median pack-years for smokers in the brain metastasis group was 25, which was slightly lower than the 28.5 median pack years observed in the non-brain metastasis group (Table 1).

The most common extracranial sites of metastases were the bone (56.80%) and pleura/pleural fluid (31.95%), followed liver (20.70%), distant lymph nodes (12.72%), and adrenals (7.91%). In the NSCLC-BM cohort the most common intracranial sites of metastases were the cerebral hemisphere (90%), cerebellum (34.29%), brainstem (5.71%), and basal ganglia (2.86%) (Figure 1, 2).

Cough with expectoration and dyspnea were highly prevalent in both groups (47.99% and 44.44%, respectively in mNSCLC versus 38.57% in NSCLC-BM for both). Musculoskeletal symptoms were also frequently reported, particularly in the non-brain metastasis cohort (37.75%). Hemoptysis showed a comparable prevalence in both groups (Table 2).

In the NSCLC-BM cohort, 58 had de novo brain metastasis, while 12 had progressed to brain metastasis. The patients presenting with neurological symptoms were higher in cases who had progressed to brain metastasis than in those with de novo brain metastasis (75% vs. 58.62%), but the difference was not significant ($\chi^2(1) = 1.1258$, $p = 0.288$) (Table 3).

Headache was the most common neurological symptom, affecting a vast majority (74.41%) of patients with brain metastasis, consistent with increased intracranial pressure or direct tumor effects. Dizziness was observed in 16.27% of the patients. Less frequent neurological manifestations included altered sensorium, visual disturbance, and focal neurological deficits, each identified in 9.3% of symptomatic patients. Seizures were reported in smaller proportions (4.65%) (Table 4).

Mutation Analysis

Tissue biopsy was the predominant method used in

Table 1. Clinicopathologic Characteristics of NSCLC with Brain Metastasis (NSCLC-BM) and Metastatic NSCLC without Brain Metastasis (mNSCLC)

Clinicopathologic Characteristics	NSCLC With Brain Metastasis(41.42%, N=70)	NSCLC Without Brain Metastasis (58.58%, N=99)
1) Gender		
Male	55.71%(n=39)	53.53%(53/99)
Female	44.28%(n=31)	46.46%(46/99)
2) Age		
Median	51.50 years	56 years
Mean	52.37 years	55.24 years
3) Histologic subtype		
Adenocarcinoma	100% (n=70)	91.91% (n=91)
Squamous cell	0	5.05% (n=5)
Adenosquamous	0	2.02% (n=2)
NSCLC-NOS	0	1.01% (n=1)
4) Smoking history		
Smoker	38.57%(27/70)	36.36%(36/99)
Non-smoker	61.43%(43/70)	63.63%(63/99)
Median pack years	25	28.5

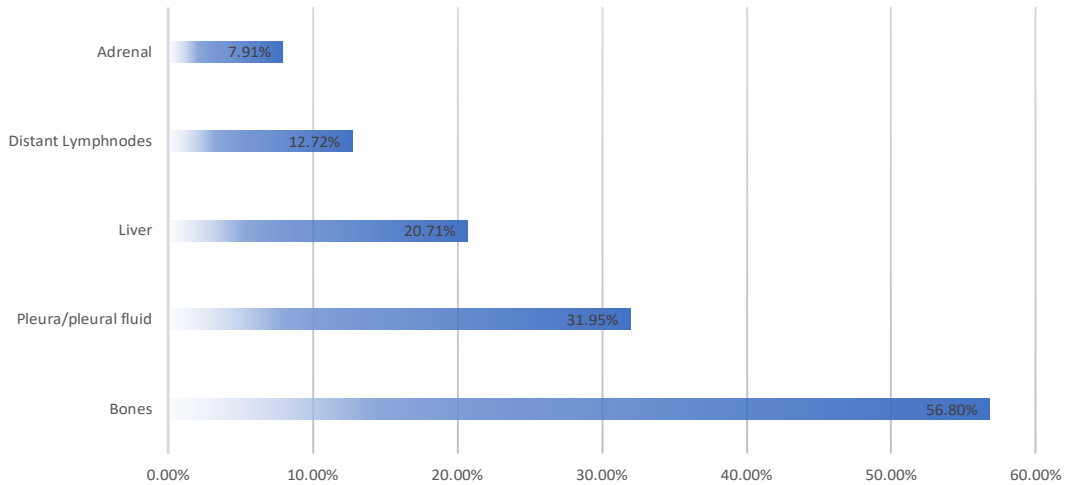

Figure 1. Prevalence of Extracranial Metastasis

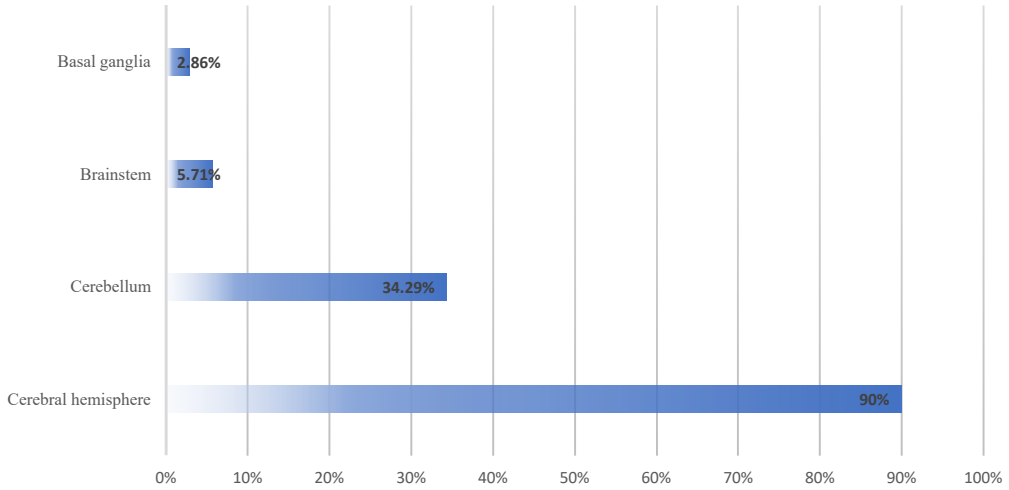

Figure 2. Prevalence of Intracranial Metastasis

Table 2. Prevalence of Non-Neurologic Symptoms

Symptoms	NSCLC-BM	mNSCLC
Cough with expectoration	38.57%	47.99%
Dyspnea	38.57%	44.44%
Musculoskeletal symptoms	24.29%	37.75%
Hemoptysis	7.14%	7.10%
Fatigue	7.14%	3.06%

Table 3. Probability of NSCLC-BM Patients Presenting with Neurologic Symptoms

Neurologic symptom	Denovo case brain metastasis	Progressed to brain metastasis	P value
Present	34 (58.62%)	9 (75%)	0.288
Absent	24 (41.37%)	3(25%)	

Table 4. Prevalence of Neurologic Symptoms in NSCLC-BM Patients

Neurologic symptom	Percentage
Headache	74.41%
Dizziness	16.27%
Altered sensorium	9.30%
Visual disturbance	9.30%
Focal neurological deficit	9.30%
Seizure	4.65%

both the NSCLC-BM (55.71%) and non-BM groups (60.60%). Liquid biopsy, a less invasive alternative, was performed in a substantial proportion of patients (44.28% in NSCLC-BM and 39.39% in non-BM) (Table 5).

Mutation positivity was observed in a high percentage of patients, with the NSCLC-BM cohort showing a numerically higher rate of 80% (n=56) compared to 68.68% (n=68) in the non-brain metastasis group. However, this difference was not statistically significant

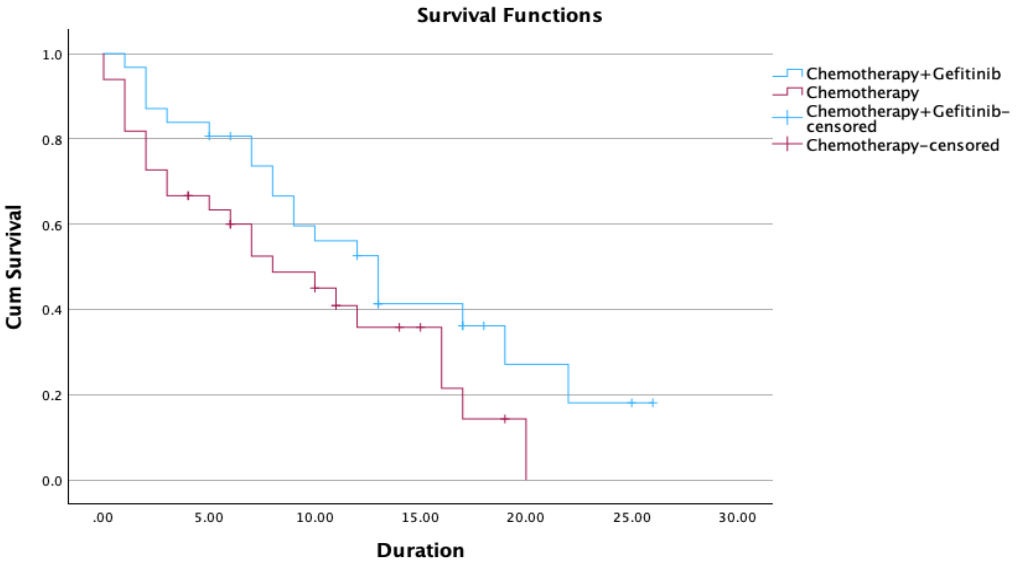

Figure 3. Survival Analysis of NSCLC Patients with Brain Metastases

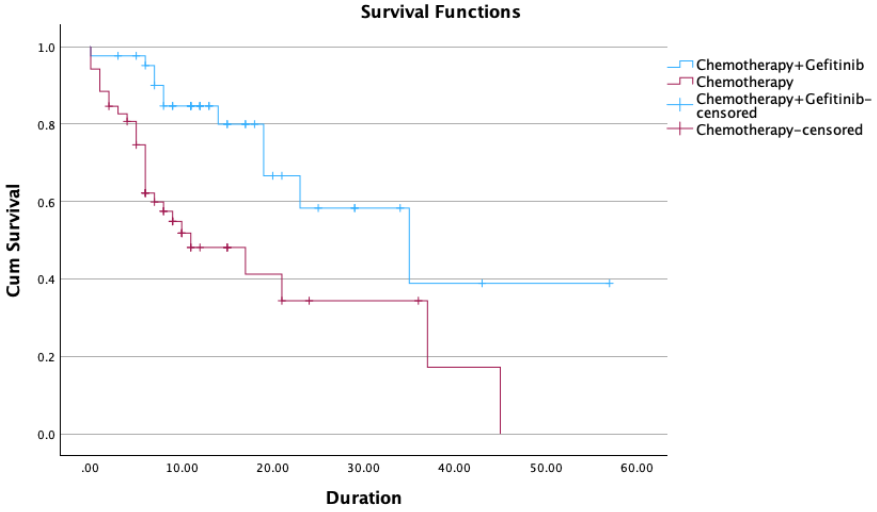

Figure 4. Survival Analysis of NSCLC Patients without Brain Metastases

Table 5. NGS Technique Utilized

NGS TECHNIQUE	NSCLC-BM	mNSCLC
Liquid biopsy	44.28% (31/70)	39.39% (39/99)
Tissue biopsy	55.71% (39/70)	60.60% (60/99)

($\chi^2(1) = 2.6863$, $p = 0.101$) (Supplementary Table 1).

EGFR mutations were most common in the NSCLC-BM and mNSCLC cohorts. *EGFR* exon 19 del was more common in NSCLC-BM than in mNSCLC (32.85% vs. 22.22%). *EGFR* exon 21 L858R was also frequent, showing a similar prevalence between cohorts (17.14% in NSCLC-BM vs. 19.19% in mNSCLC). The *EGFR* exon 20 T790M mutation was observed in a small proportion of patients (5.71% of NSCLC-BM patients and 3.03% of mNSCLC patients).

Other frequently identified mutations include *TP53*, *ALK*, *KRAS*, ROS1 and other mutations. *ALK* mutations showed a notably higher prevalence in the NSCLC-BM cohort (8.57%) than in the mNSCLC cohort (2.02%). *TP53* mutations had a comparable prevalence of 22.68% in NSCLC-BM and 20.20% in mNSCLC. *KRAS* mutations were more common in the mNSCLC cohort (8.08%) than in the NSCLC-BM cohort (2.86%) (Supplementary Table 2).

Mutation results in consideration of smoking history were more positive in non-smokers (76.42%) than in smokers (68.25%). However, this difference was not statistically significant ($\chi^2(1) = 1.347$, $p = 0.245$) (Supplementary Table 3).

EGFR mutations were the most common in both cohorts with respect to smoking history. *EGFR* mutations were more common in non-smokers than in smokers (59.43% vs. 46.03%). *EGFR* exon 19 del was more common in non-smokers than in smokers (31.13% vs. 19.04%), whereas *EGFR* exon 21 L858R was more common in smokers (19.04% vs. 17.92%). *EGFR* exon 20 T790M mutation and *KRAS* mutations were higher in smokers (7.94% and 9.52%, respectively) (Supplementary Table 4).

Analysis of mutation patterns by sex revealed that mutation positivity was similar between females (72.72%)

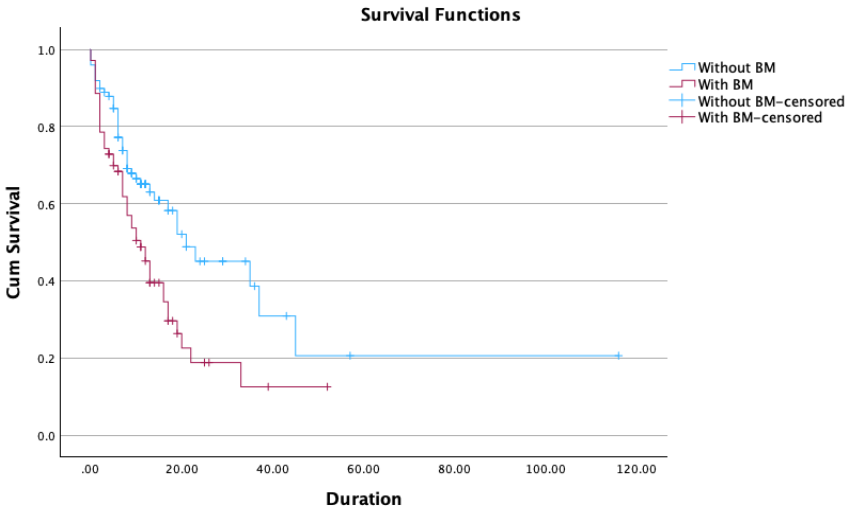

Figure 5. Survival Analysis of the Cohort with and without Brain Metastasis

and males (73.91%), with no statistically significant difference ($\chi^2(1) = 0.0302$, p = 0.862) (Supplementary Table 5).

Mutation analysis results by sex revealed distinct patterns of specific mutations. *EGFR* Exon 19 deletion was notably more prevalent in females (32.47%) than in males (21.74%). Conversely, *EGFR* Exon 21 L858R was observed more frequently in males (20.65%) than in females (15.58%). Furthermore, *ALK* mutation (7.61% in males vs. 1.30% in females) and *KRAS* mutation (7.61% in males vs. 3.90% in females) showed a higher prevalence in male patients, while *TP53* mutation was slightly more common in males (22.83% vs. 19.48%) (Supplementary Table 6).

Five of the 70 patients who had brain metastases were lost to follow-up. Of the 65 patients, 33 were *EGFR* exon 19 deletion and *EGFR* exon 21 L858R positive who received chemotherapy with gefitinib. Of the remaining 32 patients, one patient received crizotinib, one patient received osimertinib, one patient received erlotinib, and all others received chemotherapy. The median OS in patients who received chemotherapy with gefitinib was 13 months (95% CI 9.308-16.692) and the median OS in patients who received only chemotherapy was 8 months (95% CI 3.224-12.776), but the difference was not statistically significant (log-rank $\chi^2(1) = 3.100$, p = 0.078) (Figure 3). The median follow up of the cohort was 17 months, calculated using reverse Kaplan Meier curve method.

9 of the 99 patients without brain metastases were lost to follow-up. Of the 90 patients,41 patients were positive for *EGFR* exon 19 and *EGFR* exon 21L858R mutations and received chemotherapy with gefitinib. Of the remaining 49 patients, one patient received crizotinib, one patient received osimertinib, one patient received erlotinib, one patient received afatinib, and all others received chemotherapy. The median OS in patients who received chemotherapy with gefitinib was 35 months (95% CI 13.49-56.51) and it was 11 months in patients who received only chemotherapy (95% CI 2.533-19.467). The results were statistically significant (log-rank $\chi^2(1) = 10.813$, p = 0.001) (Figure 4). The median follow up of the cohort was 16 months, calculated using reverse Kaplan Meier curve method.

Analysis of the survival data of the cohort after stratifying patients into NSCLC-BM and mNSCLC patients, excluding five patients from the NSCLC-BM cohort and nine patients from the mNSCLC cohort who were lost to follow-up, the median survival in the NSCLC-BM cohort was 11 months (95% CI 7.506-14.494) and the mNSCLC cohort had a median survival of 21 months (95% CI 8.365-33.635). The difference in survival between the two cohorts was statistically significant (log-rank $\chi^2(1) = 8.639$, p = 0.003) (Figure 5). The median follow up of the cohort was 16 months, calculated using reverse Kaplan Meier curve method.

Discussion

Targeted therapies have emerged largely in cases of non-small cell lung carcinoma, leading to better outcomes, survival, and manageable toxicity. In resource-limited settings, such as India, data regarding the genomic profile of NSCLC patients are scarce because of limited accessibility to genomic profiling. Lung cancer cases are projected to rise dramatically to over 81,219 among males and 30,109 in females by 2025 in India [9], and brain metastases occurring in 17-21% of NSCLC patients at diagnosis according to recent Indian studies [10]. The present study provides comprehensive genomic profiling of 169 patients with stage IV NSCLC in India, analyzing the molecular and clinical characteristics distinguishing brain metastasis cases from those without central nervous system involvement.

Our cohort had a 41.42% prevalence of brain metastases among NSCLC patients, which exceeds the 17-21% prevalence reported in recent Indian studies [9] but aligns with international data of advanced NSCLC, which has shown a prevalence of brain metastases up to >40% [11]. The higher prevalence of brain metastases in our study may reflect the advanced presentation of NSCLC and a smaller sample size.

The median age at diagnosis was 51.5 years in the NSCLC-BM cohort and 56 years in the mNSCLC cohort, which corresponds to the data from other Indian studies, including the Tata Memorial cohort, which documented a median age of 50-59 years in the majority of patients [12]. The younger age at presentation in patients with brain metastases compared to patients without brain metastases reflects the aggressive biology and early presentation of the disease [12]. Adenocarcinoma was the predominant histology in the entire cohort, which is consistent with established knowledge. Data from the Indian registry demonstrated that adenocarcinoma was the most common histology in both males and females (34.3% and 52.7%, respectively) [9].

Bones were identified as the most common site of systemic dissemination, affecting 56.80% of the cases. Pleura/pleural fluid metastases were seen in 31.95% of the cases. Other extracranial metastatic sites included the liver in 20.71% of patients and distant lymph nodes in 12.72% of patients, indicating varied patterns of distant spread. Adrenal gland involvement was observed in a small proportion of patients (5.91%). Regarding intracranial metastases, the cerebral hemisphere was the most common site, identified in 90% of patients, underscoring its primary susceptibility to tumor dissemination. The cerebellum was the second most common cranial metastatic site, affecting 34.29% of the cases, suggesting a notable predilection for this region. Less frequent but clinically significant sites of cranial metastasis included the brain stem in 5.71% and the basal ganglia in 2.86% of patients. The distribution of these cranial metastases highlights the propensity for hematogenous spread of the disease and the heterogeneous nature of the brain involvement in NSCLC. These findings parallels the data from other studies [13].

61.43% (n=43) patients presented with neurological symptoms, which were more common in patients progressing to brain metastases. These findings suggest an advanced presentation of patients, which could have been contributed by late referrals and resource-constrained settings, with symptomatic cases leading to diagnostic

imaging. Similar findings have been reported in other studies [14].

Mutation analysis and brain metastatic tropism

Genomic analysis results are more positive in patients with brain metastases than patients those without brain metastases, but the difference was not statistically significant (p=0.09). This finding suggests a higher mutation burden in patients with brain metastasis [15], and a larger cohort may help to statistically prove this.

Our study identified *EGFR* mutations as the most prevalent genomic alteration, with exon 19 deletions showing a higher frequency in the NSCLC-BM cohort (32.85%) than in patients without brain metastasis (22.22%), while exon 21 L858R, exon 20 T790M, and other *EGFR* mutations showed comparable prevalence. A higher prevalence of *EGFR* mutations in patients with brain metastasis has been shown in previous studies [16], and this finding has treatment implications. Third-generation TKI such as Osimertinib, Lazertinib with amivantamab have shown better central nervous system activity in studies culminating in better survival [17].

ALK mutations demonstrated a higher prevalence in NSCLC-BM patients (8.57%) than in patients without brain metastasis (2.02%), which reinforces the higher prevalence of *ALK* mutations (25-40%) in patients with brain metastasis, which is consistent with the results of large-scale studies [18]. This marked CNS tropism of *ALK* mutations prompted the development of *ALK* inhibitor drugs, such as lorlatinib, which has better intracranial activity, leading to better survival outcomes in this group of patients. In our study, *KRAS* mutations were less prevalent in patients with brain metastasis(2.86%) than in those without brain metastasis (8.08%), which is in contrast to some studies that suggest *KRAS* mutations, particularly G12C, have a significant brain metastatic risk [19]. The findings of our study could not be substantiated, particularly due to the small sample size.

TP53 mutations were comparable between the two cohorts (22.68% in BM vs. 20.20% in non-BM), suggesting that *TP53* mutations are common in advanced NSCLC, irrespective of brain metastases, and may not drive tropism for brain metastasis. However, *TP53* mutations associated with NSCLC have a higher likelihood of distant metastasis [20].

Smoking and mutation profiles

In this study, there was a higher prevalence of *EGFR* mutations among non-smokers, which is in agreement with the available literature, suggesting an inverse relationship between smoking and *EGFR* mutations [21]. *ALK* mutations were comparable in smokers and non-smokers (4.76% vs. 4.72%) in our study, whereas in the literature, they have been found to be more common in young, non-smoker patients [22].

Other findings such as *KRAS* mutations were more common in smokers than in non-smokers (9.52% vs. 3.77%), which was contradictory to a larger study in which *KRAS* mutations showed a prevalence of 15% in non-smokers, 22% in former smokers, and 25% in current smokers [23]. *TP53* was more common in smokers (25.4%) than in non-smokers (18.87%), similar to the literature that has shown a higher prevalence of *TP53* in smokers than in non-smokers [24], demonstrating a causal relationship between *TP53* mutation prevalence and NSCLC.

Gender and mutation profile

EGFR mutations in female patients were more(66.23%) than in male patients (53.26%). While exon 19 deletion was more common in females(32.37 vs 21.74%),exon 21 L858R was common in males (20.65% vs. 15.58%); literature from larger studies has revealed a similar finding of a higher prevalence of *EGFR* mutation among female patients [25]. Mutations such as *ALK* and *KRAS* were common among males (7.61% each) compared to females (1.30% and 3.90%, respectively) in our cohort. The findings of our study contradict those of other available studies [26]. This finding warrants a large multi-institutional study.

Survival outcome and prognosis

Gefitinib is the only *EGFR* TKI readily available in our resource-limited setting. Patients with brain metastasis who received chemotherapy with gefitinib had a median OS of 13 months compared with 8 months in patients who only received chemotherapy. The difference in survival was not statistically significant (p=0.078). This observation aligns with recent studies [27], which indicate the limitation of first-generation TKI to cross the blood brain barrier and limited intracranial activity; the third-generation *EGFR*-TKI osimertinib has good intracranial activity [28].

Patients without brain metastasis who received chemotherapy with gefitinib had a median survival of 35 months compared to 11 months in patients who received chemotherapy only, and this difference was statistically significant (p=0.001). This parallels the findings of other similar studies [29], highlighting the role of targeted therapy benefiting from improved survival, quality of life, and manageable safety profiles.

In our study, the median survival of patients with brain metastasis was 11 months and the median survival of patients without brain metastasis was 21 months (p=0.03). The shorter survival among patients with brain metastasis signifies the prognostic impact of brain metastases. The survival finding in our study is consistent with recent studies [17]. The poor prognosis associated with brain metastases has led to research on targeted therapy and development of various molecules with better activity in the central nervous system.

Limitations and future perspective

The single-center design and sample size calculation, which was partly due to the exploratory nature of this genomic profiling study in an underrepresented population, and relatively small sample size, especially in some molecular subgroups, restricted the statistical analysis. Longer follow-up of the cohort will provide better insights into the prognosis, survival of the patients and more mature data. It is also necessary to acknowledge that the study was done in a resource limited setting. Therefore, the generalizability of these findings to real

world settings is limited.

In conclusion, this comprehensive genomic analysis of patients with NSCLC with and without brain metastases provides important insights into the molecular drivers of metastatic behavior. The higher prevalence of *EGFR* and *ALK* alterations in brain metastatic disease, coupled with distinct gender- and smoking-related mutation patterns, has important implications for risk stratification, surveillance strategies, and therapeutic selection. The continued development of CNS-penetrant targeted therapies offers hope for improved outcomes in this challenging patient population, although the significant survival disadvantage associated with brain metastases underscores the need for continued research and therapeutic innovations.

Author Contribution Statement

Dr KGA - Study conception and design, acquisition & analysis and interpretation of data, draft manuscript preparation, Supervision of the project, approved the submitted version, agreed both to be personally accountable for the author's own contributions and to be answerable to all questions related to the accuracy or integrity of any part of the work. Dr AHR - Study conception and design, acquisition & analysis and interpretation of data, draft manuscript preparation, Supervision of the project, approved the submitted version, agreed both to be personally accountable for the author's own contributions and to be answerable to all questions related to the accuracy or integrity of any part of the work. Dr VBM - Supervision of the project, analysis and interpretation of data, approved the submitted version, agreed both to be personally accountable for the author's own contributions and to be answerable to all questions related to the accuracy or integrity of any part of the work. Dr MCS - Study conception and design, analysis and interpretation of data, Supervision of the project, approved the submitted version, agreed both to be personally accountable for the author's own contributions and to be answerable to all questions related to the accuracy or integrity of any part of the work. Dr LKN - Supervision of the project, analysis and interpretation of data, approved the submitted version, agreed both to be personally accountable for the author's own contributions and to be answerable to all questions related to the accuracy or integrity of any part of the work. Dr LKR - Supervision of the project, analysis and interpretation of data, approved the submitted version, agreed both to be personally accountable for the author's own contributions and to be answerable to all questions related to the accuracy or integrity of any part of the work. Dr SCS - Supervision of the project, analysis and interpretation of data, approved the submitted version, agreed both to be personally accountable for the author's own contributions and to be answerable to all questions related to the accuracy or integrity of any part of the work. Dr GVG - Supervision of the project, analysis and interpretation of data, approved the submitted version, agreed both to be personally accountable for the author's own contributions and to be answerable to all questions related to the accuracy or integrity of any part of the work.

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Ethical Approval and consent to participate

Informed consent for treatment and open access publication was obtained or waived by all participants in this study. Medical ethics committee issued approval KMIO/MEC/2023/04/PG/MO/18. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent for publication

Written informed consent obtained.

Availability of data and material

The datasets generated and/or analyzed during the current study are not publicly available due to privacy of the study participant.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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