STUDY OF CORRELATION BETWEEN EGFR MUTATION STATUS AND P-AKT, TTF 1 IN ADENOCARCINOMA LUNG AND TO COMPARE THE QUALITY OF LIFE BETWEEN PATIENTS ON TKI AND CHEMOTHERAPY

This dissertation is submitted to

The Tamilnadu Dr MGR Medical University, Chennai

in partial fulfilment of the regulations for

D.M.Medical Oncology(Branch VII)

Degree Examination of

August 2013

CANCER INSTITUTE (W.I.A)

Adyar, Chennai-600020

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CERTIFICATE

This is to certify that this dissertation on "**STUDY OF CORRELATION BETWEEN EGFR MUTATION STATUS AND P-AKT, TTF 1 IN ADENOCARCINOMA LUNG AND TO COMPARE THE QUALITY OF LIFE BETWEEN PATIENTS ON TKI AND CHEMOTHERAPY**" is a bonafide work done by the candidate with **Reg No 16104303,** in the Department of Medical Oncology, College of Oncological sciences, Adyar, Chennai,, under my overall supervision and guidance, to my satisfaction.

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CONTENTS

INTRODUCTION

Lung cancer is one of the most common cancers in the world. Lung cancer accounts for 15 % of all newly diagnosed cancers worldwide and is the leading cause of cancer deaths.[1] According to Madras Metropolitan Tumour Registry (MMTR), it is the most common cancer among males accounting for around 11% of all cancers .[2]

For treatment purpose lung cancer is divided broadly into 2 groups, small cell lung cancer (SCLC) and non small cell lung cancer (NSCLC). [3] Overall survival of lung cancer is poor with 5 year overall survival for all stages of lung cancer put together is around 15 %.[4] More than half of the lung cancer patients present in advanced stage and median survival of these patients is around 6- 8 months.

Till early 21st century, cisplatin based doublet chemotherapy was standard of care in patients with metastatic carcinoma lung who are in good performance status, which produced objective response of 20-30% and median survival of 6-8 months.[5]

The real path breaking discovery in the management of advanced NSCLC came with the invention of epidermal growth factor receptors (EGFR) inhibitors, which changed the natural history of lung cancer especially in the subset of lung cancer with EGFR mutation.

IDEAL-1 and IDEAL-2 studies confirmed the effectiveness of EGFR inhibitors as second and third line chemotherapy in relapsed carcinoma lung with acceptable toxicity profile. [6, 7] IPASS trial published in 2009 was conducted on patients from East Asia and around 60 % of patients were positive for EGFR mutation and these patients when treated with gefitinib had significant progression free survival (PFS) compared to those treated with chemotherapy. [8] Thus EGFR inhibitors have become the drug of choice in patients with advanced lung cancer who have positive EGFR mutation.

The most common method used for the detection of EGFR mutation was through direct sequencing of DNA. [9] But due to low sensitivity presently this method is not preferred worldwide. The newer and more sensitive method for detecting EGFR mutation which is being used worldwide is RT- PCR using ARMS (amplified refractory mutation system) technique. The most important drawback of this method is the cost involved in EGFR mutation testing and because of which, this test is difficult to perform in countries with limited resources where the access to EGFR testing is limited. [10, 11]

Any surrogate tests which are cost effective and have high sensitivity and specificity to detect EGFR mutation is the need of the day .Various immunohistochemistry (IHC) markers have been tried as surrogate tests to detect EGFR mutation , some of the promising being P-AKT,P- EGFR and TTF-1.

There is a limited data regarding EGFR mutation status in Indian patients with lung cancer and their outcome after treatment with chemotherapy/TKI. In the study by Louis et al done at cancer institute between January 2009 to December 2010, where TKI was prescribed depending on factors like female sex, never smoker and poor performance status .In this study 1 year PFS was better in TKI arm compared to chemotherapy. [12]

Present study is designed as continuation of the study by Louis et al, where in EGFR mutation testing is done in all patients of lung cancer and treatment is based on result of mutation analysis. Also this study is intended to find out the correlation between EGFR mutation detected through RT-PCT by using ARMS technique and P- AKT , TTF-1 done through IHC ,which can act as surrogate tests in patients with metastatic carcinoma lung who requires EGFR mutation testing.

One of the important factors to be considered in the treatment of metastatic lung cancer is improvement in quality of life. Present study also aims to compare quality of life between tyrosine kinase inhibitors and standard chemotherapeutic agents.

AIMS AND OBJETIVES

PRIMARY AIMS

- To find out correlation between EGFR mutation status with P-AKT,TTF-1.

- To find out the quality of life of patient on tyrosine kinase inhibitor and chemotherapy.

SECONDARY AIMS

- To find demographic profile of patients with adenocarcinoma of lung presenting at our hospital.

- To find out the EGFR mutation status of these patients and response to treatment.

REVIEW OF LITERATURE

Lung cancer or bronchogenic carcinoma mainly includes cancer originating from bronchi or lung parenchyma. Lung cancer accounts for 15% of all newly diagnosed cancer and is the most common cause of death worldwide and is responsible for 1.38 million deaths annually, as of 2008. [1] In United States the death due to lung cancer is more than the combined death from prostate, colon and breast cancer.

In south India lung cancer is the most common cause of death in males and 6th most common cause of death among females. According to Madras Metropolitan Tumour Registry (MMTR),lung cancer is the most common cancer in males accounting for 10.9% of all cancer among males and seventh most common cancer among females accounting for 3.3% of all cancers in females.[2]

Majority of the lung cancer are seen in patients above the age of 60 years and 30 % of lung cancer are seen in patient above 70 year.

The 4 major sub types of lung cancer include

Adenocarcinoma

Squamous cell carcinoma

Large cell carcinoma

Small cell carcinoma. [13]

Adenocarcinoma is the most common subtype of lung cancer followed by squamous cell carcinoma. [14]

For treatment purpose lung cancer can be classified as small cell lung cancer (SCLC) and non small cell lung cancer(NSCLC) and NSCLC comprises of 85 % of all lung cancers.[3]

The most common cause for lung cancer is smoking which accounts for 80-90 % of lung cancers. Around 90% of cancer death in males and 70 % of cancer deaths in females were attributed to smoking [15]. Passive smoking which is inhalation of smoke from others smoking is also an etiological factor for lung cancer. Persons living with the smoke, in the same house has 20-30 % increased risk of developing lung cancer and person working with the smoker, in the same smoking environment has 16-20 % increased risk of lung cancer.[16] A recent meta-analysis showed the relative risk of developing lung cancer is 1.15 to 1.31 in never-smoking women due to passive smoking from spouses.[17]

Other etiological factors for lung cancer include exposure to asbestos , heavy metals like uranium, nickel chromate, beryllium and radon gas. Previous exposure of radiation to lung also is a risk factor for developing lung cancer.

Lung cancers are aggressive and metastasize via lymphatic and haematogenous routes to bone, brain, adrenal gland and liver if left untreated.

Treatment of Advanced Lung Cancer

Treatment of lung cancer depends on histology, performance status and stage of the disease. Over all the prognosis of lung cancer is poor and 5 year

overall survival is around 15 % .The best survival seen after complete surgical resection of stage 1A NSCLC patient which is as high as 70 %. On the other hand survival of patient of SCLC, extensive disease is as less as < 1%. [4]

Over 80 % of all diagnosed case of lung cancer are of advanced stage, and will not be amenable for curative treatment. The aim of treatment in this set of patients is to improve the quality of life and to improve overall survival.

As already stated the overall survival of metastatic carcinoma lung is dismal with median survival of 6-8 months. But with the advent of newer chemotherapeutic agents and targeted therapy median overall survival has improved in the last decade. Presently 1/3rd of patients with stage 3B and 4 are alive at 1 year and 2 year overall survival is around 10-21%.[18]

Two large randomized control trial conducted in 1970-80's clearly showed superiority of cisplatin containing combination chemotherapy over best supportive care (BSC).

Canadian trial which composed of 150 patients, randomly assigned patients into cisplatin+vindesine or cisplatin+adriamycine+cyclophosphamide (CAP) or best supportive care. In these trial patients receiving cisplatin based chemotherapy had median survival of 33 weeks compared to 23 weeks of BSC group. [19]

Another large trial Big Lung Trial, 725 patients were randomly assigned to 3 cycles cisplatin based chemotherapy to BSC, confirmed chemotherapy improved survival by 8 months .[5]

With these trials cisplatin based doublet chemotherapy became standard of care in patients with advanced lung cancer in good performance status, which showed objective response of 20- 30% and median survival of 6- 8 months.

Updated meta-analysis of chemotherapy for advanced lung cancer in 1995, which included 2714 patients enrolled in 16 randomized control trials showed significant benefit of chemotherapy compared to best supportive care with hazard ratio (HR) of 0.77 which is equivalent to absolute increase in survival of 9%, from 20% to 29%, and increase in median survival of 1.5 months (from 4.5 months to 6 months favoring chemotherapy). [20]

One more meta-analysis in which cisplatin based chemotherapy was compared with carboplatin based chemotherapy, which included 2968 patients with advanced carcinoma lung, cisplatin based chemotherapy resulted in higher response rate (30% v/s 24%) but overall survival and treatment related mortality was similar in cisplatin and carboplatin arms.[21]

Recently published ASCO guidelines for chemotherapy in advanced lung cancer has clearly stated that good performance status is the most important prognostic factor, and only patients in good performance status can achieve prolonged survival and improvement in quality of life when started on chemotherapy.

EGFR Inhibitors in Advanced Lung Cancer

The major progress in the treatment of lung cancer has come from the development of molecular targets, mainly with the invention of tyrosine kinase inhibitors which targets EGFR pathway. This is a result of the progress made in understanding the disease biology, signaling pathways involved in lung cancer. Treatment of metastatic lung cancer with TKI like erlotinib or gefitinib in patients who have EGFR mutation has significantly improved Progression free survival(PFS), overall survival (OS) and quality of life (QOL)

Phase 1 studies in early 2000 have showed that TKI's are well tolerated and have tolerable side effects. IDEAL-1 trial (Irissa dose evaluation in early lung) tried gefitinib in second line setting, showed response rate of 18 %. IDEAL 2 trial which was conducted in USA, gefitinib was administered in patients who had earlier been exposed to cisplatin and docetaxol showed response rate of 11%. [6, 7] On the basis of above two trials, FDA approved gefitinib in treatment of metastatic adenocarcinoma in patients with platinum and docetaxel failure. The evidence for using gefitinib in upfront setting has come from IPASS trial in which patients were recruited from various countries of East Asia. [8] In this trial 1217 patients of advanced adenocarcinoma of lung who were never smoker (< 100 cigarettes in their lifetime) or former light smokers (stopped smoking > 15 years back and had history of smoking for < 10 pack years) were randomly assigned to geftinib or paclitaxel and carboplatin in 1:1 ratio in upfront setting.

The primary objective was to show non inferiority of gefitinib to paclitaxel/carboplatin which was met in the trial. 1 year progression free period was 24.9 % and 6.7 % respectively for gefitinib and chemotherapy. Response rate was also significantly higher with gefitinib (43 % v/s 32.2%). EGFR mutation data was available for 437 patients, 261 patients (59.7%) were EGFR mutation positive. The PFS was significantly better in patients treated with gefitinib treated patients compared to chemotherapy treated patients who were EGFR positive with hazard ratio of 0.48. In EGFR mutation negative patients PFS was better in patients treated with chemotherapy compared to patients receiving gefitinib.

In OPTIMAL trial, which was conducted in China, 165 patients who were EGFR mutation positive were randomly assigned in 1:1 ratio to either erlotinib or gemcitabine and carboplatin. The median PFS was significantly longer in erlotinib arm compared to chemotherapy arm (13.4 months v/s 4.6 months with hazard ratio of o.16). The toxicity profile was also favoring erlotinib arm. [22]

In EURTAC trial, which was conducted in 42 hospitals from France, Italy and Spain, 174 patients who were EGFR mutation positive were randomized in 1:1 ratio to either erlotinib or chemotherapy(cisplatin with gemcitabine or docetaxol). The median PFS of erlotinib arm was 9.7 months compared to 5.2 months in chemotherapy arm with hazard ratio of 0.37 .[23]

On the basis of above trials EGFR inhibitors have become the treatment of choice in patients who have EGFR mutation in first line setting.

Quality of Life

With small survival benefit with most chemotherapeutic agents with regards to overall survival, the focus in metastatic lung cancer should be on improvement in quality of life. Randomized trials have clearly demonstrated the improvement in quality of life, with chemotherapeutic agents compared to BSC.

In a recently published North East Japan study group 002 trial, the quality of life was better in patients treated with gefitinib compared to chemotherapy and this was maintained for long time, indicating gefitinib was well tolerated compared to chemotherapeutic agents.[24]

In IPASS study Health-related quality of life was one of secondary endpoints . In EGFR mutation positive, the gefitinib treatment was associated with a significant improvement in global quality of life (total score reported at FACT-L) compared to chemotherapy.[8]

EGFR Signaling Pathway

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein with extracellular ligand binding domain and intracellular domain having tyrosine kinase activity. On ligand binding the receptor dimerizes, which leads to activation of tyrosine kinase domain which instead

activates RAS/RAF/MAPK pathway and PI3K-Akt pathway which regulate cell proliferation, survival and transformation.

The persistent activation of tyrosine kinase domain is believed to play role in neoplastic activity and hence EGFR activation is believed to play critical role in neoplastic process. [25, 26]

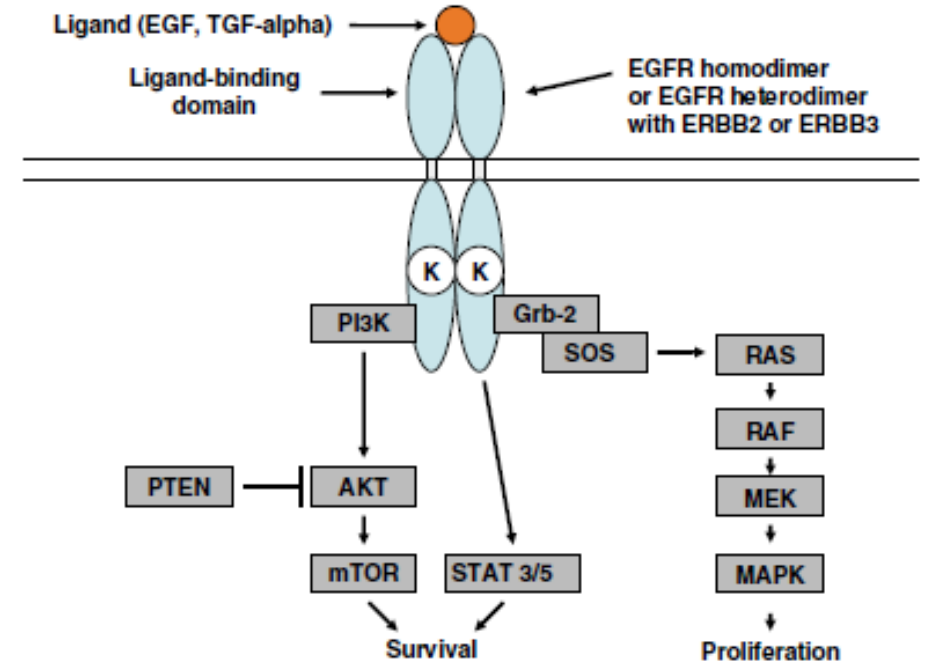


Figure 1 Schematic of EGFR signaling pathway. Erlotinib and gefitinib block signaling at the level of the kinase domain (K). They are ineffective if the signaling pathway is activated by mutations downstream of this point, such as in *KRAS* or *BRAF*.

EGFR is found over expressing in many tumors including lung cancer, head and neck cancer, pancreatic cancer, carcinoma gall bladder and so forth. In lung cancer it is over expressed in 43- 89 % of cases. [27]

The inhibition of EGFR by using tyrosine kinase inhibitors induces apotosis and reduces proliferation of neoplastic cells. Thus the EGFR inhibitors are new promising targeted therapeutic agents in the treatment of lung cancer. Erlotinib and gefitinib are commercially available tyrosine kinase inhibitors which competitively inhibits ATP binding site of tyrosine kinase domain which arrests EGFR signaling pathway.

Lynch et al found that lung cancer patients who are having mutation in tyrosine kinase domain of EGFR respond to gefitinib where as patients with no mutation did not respond to gefitinib.[28] Similar results were demonstrated by paez et al also.[29] In both the above studies EGFR mutations were found in Exon 18, 19 and 21. The two most common EGFR mutation which accounts for > 90% of all EGFR mutations are short inframe deletion of exon 19 and point mutation of exon 21, that results in replacement of leucine to arginine at codon 858(L858R). Other less common mutation sensitive to TKI are G719 in exon 18 and L861 in exon 21. In two retrospective studies deletion 19 compared to exon 21 mutation had good prognosis.

The most common method used for EGFR mutation is direct sequencing. The main drawback of direct sequencing is low sensitivity and increased risk of contamination.[9] The newer and more sensitive method of detecting EGFR mutation is reverse transcriptase polymerase chain reaction (RT-PCR) using specific probes or amplified refractory mutation system (ARMS) technique.[10,11]

Because of the cost and time involved in PCR, surrogate markers using IHC tests to detect EGFR mutation will make difference in the early detection of EGFR mutation which will instead make a difference in management of lung cancer especially in countries like India.

Various IHC markers have been tried which act as surrogate marker to EGFR mutation. The important markers are downstream proteins in EGFR pathways in the phosphorylated forms like P-AKT, P- EGFR and TTF-1.

P- AKT is a serine threonine protein kinase which regulates many of key mechanism responsible for carcinogenesis like apoptosis, angiogenesis and cell cycle propagation. [30]

In a study by Amit shah et al where he studied 82 lung cancer specimen P-AKT protein levels above normal was associated with favorable outcome. In multivariate analysis P- AKT was associated with high tumor grade. Cytoplasmic positivity and nuclear positivity was present in 96 % and 43 % cases respectively. The nuclear positivity of P-AKT was associated with nodal metastasis and poor survival compared to cytoplasimic positivity.[31]

Cappuzo et al evaluated 103 patients in which p-AKT was positive in 51 patients and p- AKT in NSCLC is more strongly correlated with female gender, absence of smoking history and bronchoalveolar histology and is associated with better response rate and overall survival when treated with gefitinib.[32]

Ikeda S et al studied 130 cases of adenocarcinoma of lung. EGFR mutation was detected in 32% of cases and P-AKT by IHC was positive in 51% of cases, he showed that there is a correlation between p-AKT positivity and EGFR mutation especially (L858R mutation) with p=0.040. He also suggested that the activation of Akt is dependent on EGFR mutation. [33]

TTF-1(Thyroid transcription factor 1) is required for development and maturation of thyroid and lung. TTF-1 is expressed in type 2 pneumocytes and non ciliated bronchial epithelial cells. TTF-1 plays important role in sustainment of lung cancer. [34]

TTF-1 is present in approximately 75% of adenocarcinoma and is expressed in only 5% squamous cell carcinoma. Some recent studies have shown that TTF-1 is expressed in majority of patients of EGFR mutated lung cancer.

Mary J Fiedler et al evaluated 216 patients of carcinoma lung and found EGFR mutation in 11.6% of patients.TTF 1 was positive in 71% adenocarcinoma patients. TTF 1 was strongly associated with EGFR mutation (p=0.0060).He stated that TTF is an indirect marker for EGFR mutation and patients with positivity of TTF should strictly be screened for EGFR mutation. [35]

Julian Vincenta et al from his study, evaluated 810 NSCLC patients from 2004 to 2010, demonstrated EGFR mutation in 114 patients (14%). TTF 1 was positive in 92% patients. He concluded that negative predictive value of

TTF-1 was >95 % when correlating to EGFR mutation and also suggested that this can be used as surrogate marker and chemotherapy can be started if it is urgent in TTF-1 negative patients and in case of TTF-1 positive patients it is better to wait for EGFR mutation report.[36]

Sun PL et al evaluated 382 Korean patients with non small cell lung cancer. EGFR mutation was positive in 196 patients (51.3%) and EGFR mutation was most prevalent in BAC subtype of lung cancer. He also demonstrated strong correlation between EGFR mutation and TTF1 positivity ($p<0.001$). [37]

Somaih et al evaluated 693 adenocarcinoma, in which TTF status was known in 301 patients and EGFR mutation was positive in 224 patients. Only 2 patients out of 224 patients who were EGFR positive had TTF negative. He hypothesized that adenocarcinoma of lung that is TTF-1 negative have 99 % probability of having EGFR mutation negativity and can be started on chemotherapy. [38]

Indian Data on Outcome of Advanced Carcinoma Lung

Recent retrospective analysis by Rajappa et al from India where he analysed 194 patients with stage 3 and 4 NSCLC quoted overall survival was ranging from 6.2 to 8.7 months with cisplatin based doublet chemotherapy. One and two year overall survival in this study was 29.8% and 9.7 %. The survival was significantly better in subset of patients who were non smokers

and in females. He also stated that the survival with first generation platinum doublet was similar to second generation platinum doublets. [39]

Prevalence of EGFR Mutation in India

Only limited data is available regarding EGFR mutation status in Indian population. The only largest study which has analyzed EGFR mutation status from India was published by R Sahoo et al.

In this analysis of 220 patients of NSCLC, in which majority was stage 3 and 4 disease, EGFR mutation was found in as high as 51.6 % of patients. The 2 most important mutations found were deletion 19 (52%) followed by missence mutation in exon 21 (26%). Mutation 19 and 21 were significantly more in non smokers and females. [40]

Louis et al in his paper analyzed 120 patients of stage 3B and 4 NSCLC who presented at cancer institute, Chennai. Patients were stared on upfront gefitinib based on parameters like female sex, non smoking status, adenocarcinoma histology and poor performance status. PFS and OS of the population were 5 months and 7.5 months respectively. PFS was significantly better with non smokers, female sex and upfront treatment with gefitinib.12 months PFS of upfront gefitinib verses chemotherapy were 26 % and 16 % respectively. Toxicity profile favored gefitinib compared to chemotherapy.[12]

Another publication by Aggarwal et al in which EGFR mutation of 55 non smokers were analyzed .EGFR mutation was positive in 27 of 49 cases.

EGFR mutation was strikingly more common in south Indian patients compared to north Indian patients (65 %v/s 33 %).[41]

With these limited data is clear evidence that EGFR mutation status and stratification of treatment depending on EGFR mutation status is not practiced universally and any data addressing the above issue will be encouraging.

MATERIALS AND METHODS

Study Design and Center

Prospective, observational, single institutional study conducted in patients of adenocarcinoma lung, under medical oncology department at Adyar Cancer Institute, Chennai. For IHC correlation of EGFR mutation status with p-AKT, TTF 1, 28 patients who were diagnosed having adenocarcinoma lung at our institute during 2009 -2010 were analyzed retrospectively. However they were excluded for survival and quality of life analysis.

Study Duration

The study was conducted between June 2010 to January 2013

Inclusion criteria

- Adenocarcinoma of lung by tissue diagnosis
- ECOG performance status <= 3
- Age > 18 years

Exclusion criteria

- Lung cancers with non adenomatous histology
- ECOG performance status 4
- Pregnant and lactating patient
- Age < 18 years

Patients who met eligibility criteria were included in the study. Detailed history with special emphasis on smoking history was taken. The patients underwent thorough clinical examination, and base line blood investigation including haemogram, renal and liver function tests. Patients also underwent chest X Ray, CT scan of the chest, ultrasound abdomen and bone scan. PET scan was done wherever necessary based on patients symptoms.

EGFR mutation was done by using scorpian probe based ARMS – PCR technique in Triesta Lab, Bangalore from tissue samples, either from formalin fixed paraffin embedded tissue(FFPE) or FNAC (fine needle aspiration cytology) obtained either from tumour tissues / metastatic nodes or from cell blocks from pleural fluid which was positive for malignant cells.

IHC was done on tumour tissue samples by using thyroid transcription factor 1 (TTF 1 ,Leika-product code NCL-TTF-1) and phospho AKT(p-AKT, cell signaling technology ,product code#4060P)

After tissue diagnosis and staging investigation, patients having advanced carcinoma lung who were EGFR mutation positive either received erlotinib/gefitinib and if found to be EGFR mutation negative received chemotherapy .The choice of chemotherapy was based on cancer institute treatment protocol guidelines.

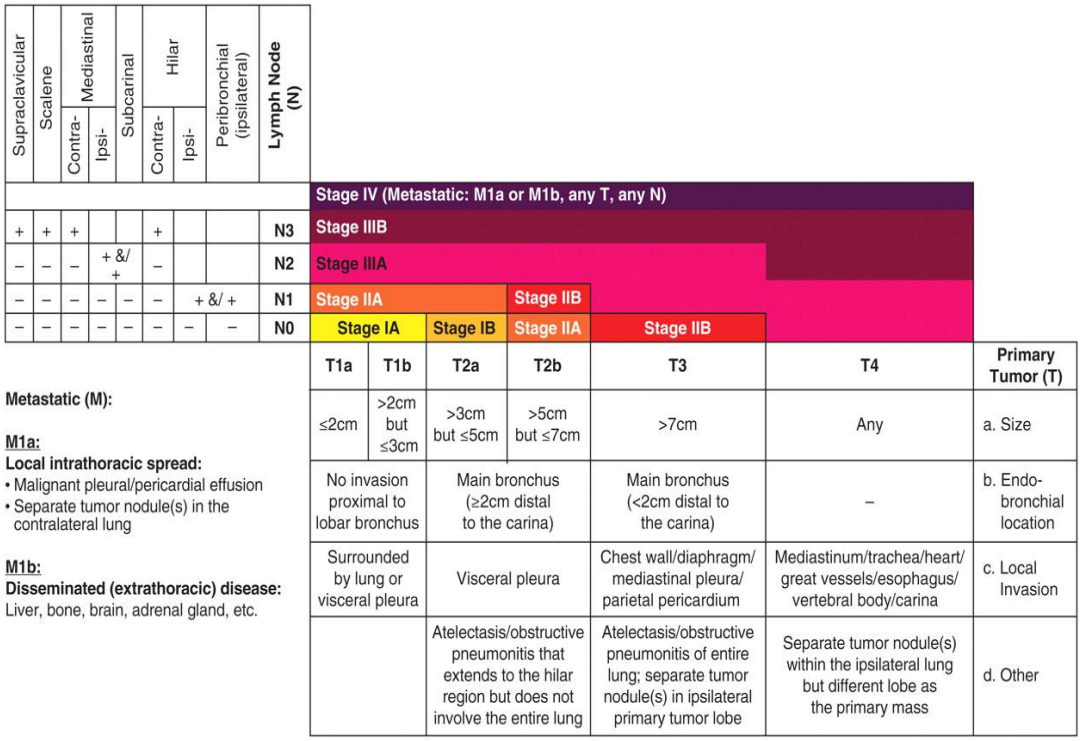
Patients were followed up monthly, where detail history regarding improvement / worsening of symptoms were noted with special emphasis on chemotherapy related toxicities [toxicity grading was done according to National Cancer Institute Common Terminology Criteria for Adverse Events(NCI- CTCAE)] .Patients were also examined in detail for signs of progression.[42]

Patients underwent response evaluation every 3 monthly with CT scan chest, ultrasound abdomen. PET scan and bone scan were done wherever necessary based on patients symptoms. Complete response (CR), partial response (PR) and progressive disease was defined according to response evaluation criteria in solid tumor (RECIST) criteria. [43]

In case of progressive disease, chemotherapy regimen was changed as per cancer institute treatment protocol. In case of significant chemotherapy related toxicities (especially grade 3-4 toxicities), either drug dosage was reduced or drug regimen was changed according to cancer institute treatment guidelines.

Quality of life (QOL) assessment was done at baseline and at 3 months. The QOL questionnaire was filled by patients in the presence of interviewer with in prescribed time.

Patients were staged according to American joint committee for cancer (AJCC) 7th edition



Supraclavicular	Scalene	Mediastinal: Contra-	Mediastinal: Ipsi-	Subcarinal	Hilar: Contra-	Hilar: Ipsi-	Peribronchial (ipsilateral)	Lymph Node (N)	T1a	T1b	T2a	T2b	T3	T4	Primary Tumor (T)
									Stage IV (Metastatic: M1a or M1b, any T, any N)						
+	+	+			+			N3	Stage IIIB						
–	–	–	+ &/ +		–			N2	Stage IIIA						
–	–	–	–	–	–	+ &/ +		N1	Stage IIA			Stage IIB			
–	–	–	–	–	–	–	–	N0	Stage IA		Stage IB	Stage IIA	Stage IIB		
									≤2cm	>2cm but ≤3cm	>3cm but ≤5cm	>5cm but ≤7cm	>7cm	Any	a. Size
									No invasion proximal to lobar bronchus		Main bronchus (≥2cm distal to the carina)		Main bronchus (<2cm distal to the carina)	–	b. Endo-bronchial location
									Surrounded by lung or visceral pleura		Visceral pleura		Chest wall/diaphragm/ mediastinal pleura/ parietal pericardium	Mediastinum/trachea/heart/ great vessels/esophagus/ vertebral body/carina	c. Local Invasion
											Atelectasis/obstructive pneumonitis that extends to the hilar region but does not involve the entire lung		Atelectasis/obstructive pneumonitis of entire lung; separate tumor nodule(s) in ipsilateral primary tumor lobe	Separate tumor nodule(s) within the ipsilateral lung but different lobe as the primary mass	d. Other

Metastatic (M):

M1a:
Local intrathoracic spread:
- Malignant pleural/pericardial effusion
- Separate tumor nodule(s) in the contralateral lung

M1b:
Disseminated (extrathoracic) disease:
Liver, bone, brain, adrenal gland, etc.

Current smokers were defined as those who smoked > 100 cigarettes/ beedies in their life time and those patients when asked question "currently do you smoke every day", answered "yes" or "some days".

Nonsmokers were defined as patients who had smoked <100 cigarettes in their lifetime and former light smokers were defined as those who had stopped smoking at least 15 years previously and smoked a total of ≤10 pack-years of cigarettes. [44]

Methodology of EGFR Mutation

1 DNA Extraction

Genomic DNA was extracted from different types of clinical materials such as FNAC smears, pleural effusion smears and cell blocks, core biopsies of lung and SCLN biopsies. The DNA was extracted as per the manufacturer's protocol (QIA Amp DNA Minikit).

2 DNA Quantification

DNA were quantified by using RNAse-P (Part no: 4316831,Applied Biosystems) as per the kit insert. 1000 pg of DNA was utilized for each test.

3. Real-time PCR

Exons 18,19,20 and 21 of the EGFR gene were screened for hotspots(oncogenicdriver mutations) using Scorpions ARMS Realtime PCR technology and analyzed using DxS ARMS-PCR kit (Product Code: EG-03 and EG-04). 29 somatic mutations that included the resistance mutation T790M were screened in this assay for all the samples. EGFR mutation was considered positive if patients sample was tested positive for 1 of the 29 mutation.

Methodology of Immunohistochemitry

Immunohistochemistry was performed on formalin-fixed, paraffin embedded sections, FNAC slides or pleural effusion smears and cell blocks. Five micrometer paraffin sections on APES coated glass microslides were used.

The following steps were carried out:

1. Dewaxing and Rehydration: Sections were deparaffinized and rehydrated by consecutive submersions in Xylene (twice for 8 mins), absolute ethanol (twice for 3 mins) and hydrated in water (5 min).
2. Blocking of Endogenous Peroxidase action: Endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide for 10 mins at room temperature and slides were washed in Tris Buffer Saline (TBS pH 7.6).
3. Antigen retrieval: The sections were then subjected to antigen retrieval in Tris EDTA buffer by using pressure cooker .
4. Incubation with specific primary antibody: After cooling down to room temperature, the sections were washed in TBS and incubated with the specific antibodies(P- AKT or TTF-1) overnight at room temperature in a humid chamber. Following day, the slides were washed with TBS for 5 mins three times to remove the unbound primary antibody.
5. Incubation with Labelled Polymer (EnVision^{+} System-HRP) (DAKO): Tissue sections were then incubated at room temperature for 2 hours with Labelled Polymer. The slides were then washed with TBS for 5 mins three times to remove unbound polymer.
6. Incubation with substrate-chromogen: The sections were then incubated with liquid DAB+Substrate-chromogen for 5-10 mins.

7. Counterstaining and mounting: The sections were then counterstained with haematoxylin, dehydrated, cleared and mounted using DPX.

Negative control slides were processed under same conditions as above omitting the primary antibody.

Quality of Life Assessment

The QOL was assessed by using standardized CANCER INSTITUTE - QUALITY OF LIFE QUESTIONNAIRE (CI-QOL-Q) Version II. [45] This is a modified version of the EORTC –QOL 30 questionnaire and has 41 questions that has been standardized for the Indian population. This mainly assesses the quality of life in 11 dimensions. They are

- General well being
- Physical well being
- Psychological well being
- Interpersonal relationship
- Sexual and personal ability
- Cognitive well being
- Optimism and belief
- Economical well being
- Informational support
- Patient physician relationship
- Body image.

The norms of QOL scale version 2 is as follows:

Score	Quality of life
below 99	very low
99-117	low
118-146	average
147-165	high
Above 165	Very high

QOL was also assessed by using EORTC-QOQ-C30 (version 3) and site specific EORTC-QCQ-LC13, which mainly assesses improvement in symptomatology in patients suffering from carcinoma lung.

The quality of life was assessed at baseline and was compared to the quality of life at 3 months for patients on TKI and chemotherapy.

STATISTICAL ANALYSIS

SPSS version 17.0 (SPSS Inc) was used for statistical analysis. Chi square test was used to find out correlation between categorical variables and survival. Kaplan Meier survival plot was used for estimating the progression free survival (PFS) and Overall survival (OS).Student 'T' test was used to compare mean differences of quantifiable variables. Log rank test was used in measurement of independent risk factor affecting PFS and OS in univariate analysis and was also used to estimate differences in survival curve. Cox –Ph model was employed for measurement of independent risk factor affecting PFS and OS in multivariate analysis. Kappa co-efficient was used to assess the level of agreement between EGFR mutation by PCR with P-AKT, TTF 1 by IHC.

Cofactors investigated in the univariate and multivariate analysis included age, gender, performance status, co morbidities, smoking status and EGFR mutation status.

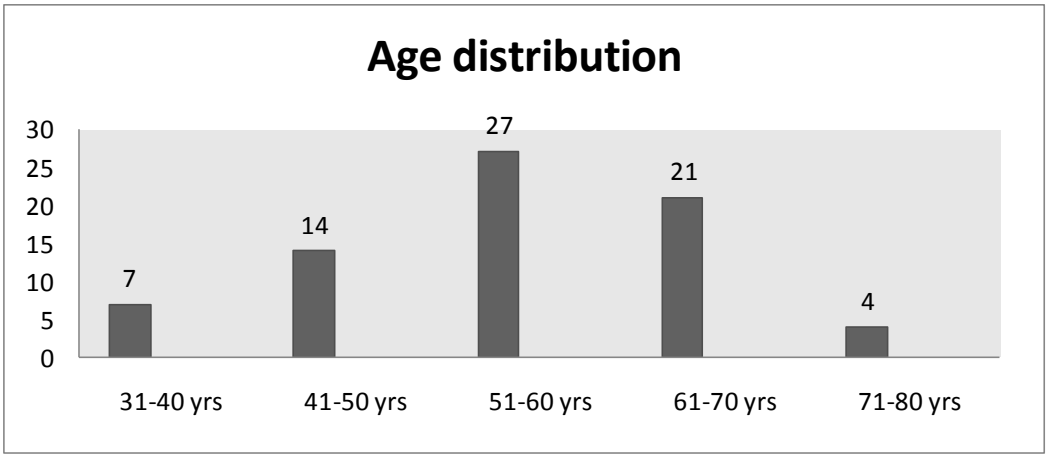
QOL assessment was done by using the descriptive statistics, paired sample't' test was used to compare the mean differences in QOL scores. The QOL data was also analyzed by using SPSS software version 17.

RESULTS

A Total of 101 patients were included in the study, of which 73 patients were followed up prospectively. The first case was enrolled on June 2010 and last case was enrolled on January 2013. 28 patients of adenocarcinoma lung were analyzed retrospectively for IHC correlation between EGFR mutation and P- AKT, TTF-1 .These patients were excluded from survival analysis and quality of life analysis.

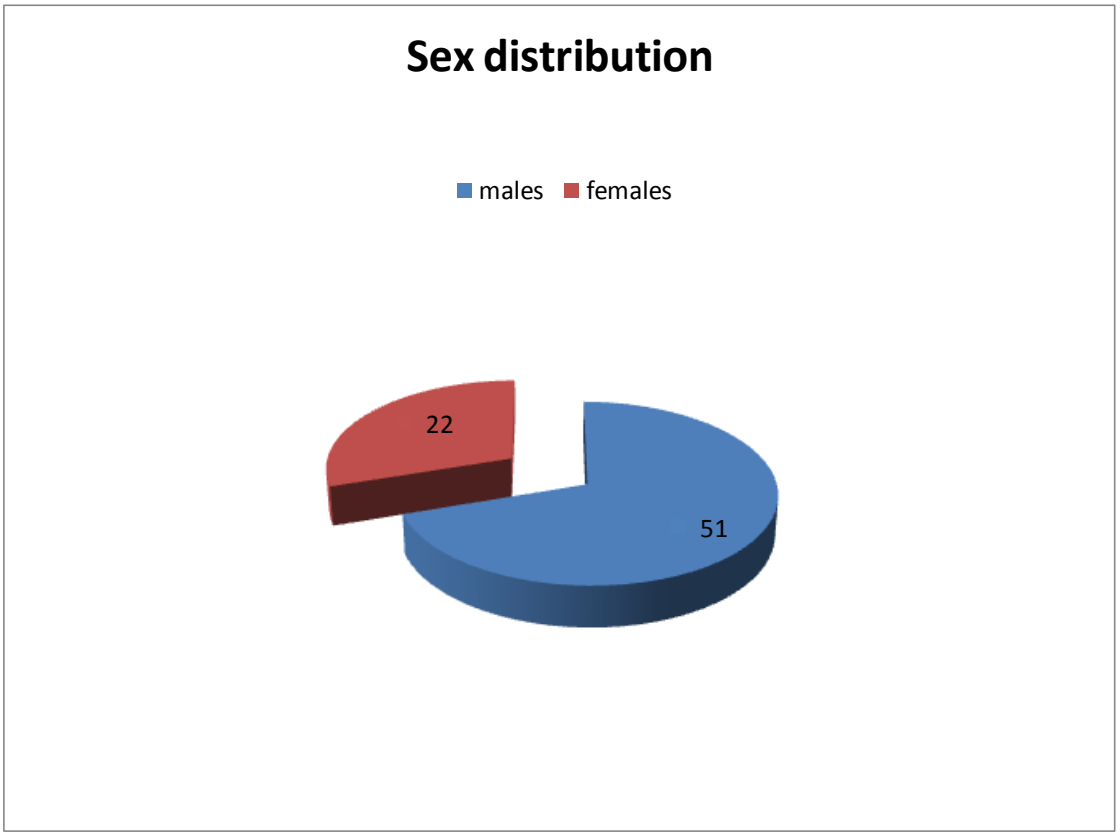
Age Distribution

The youngest patient in our study was 35 years and oldest being 78 years. The median age of study population was 55 years .There were 30 patients below age of 55 years and 43 patients above age of 55 years. The age distribution is represented in bar diagram below.



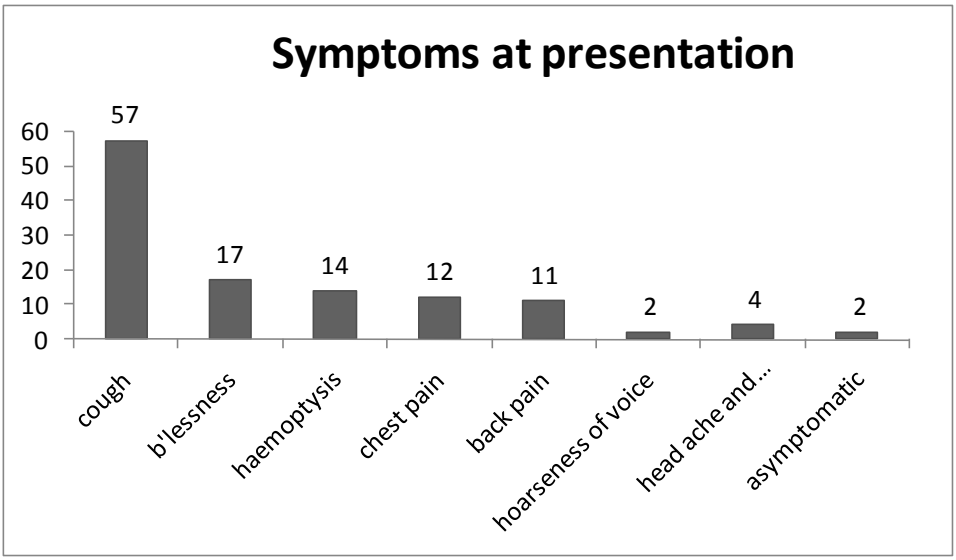
Sex Distribution

Among 73 patients included in the study 22 patients were females and 51 patients were males.



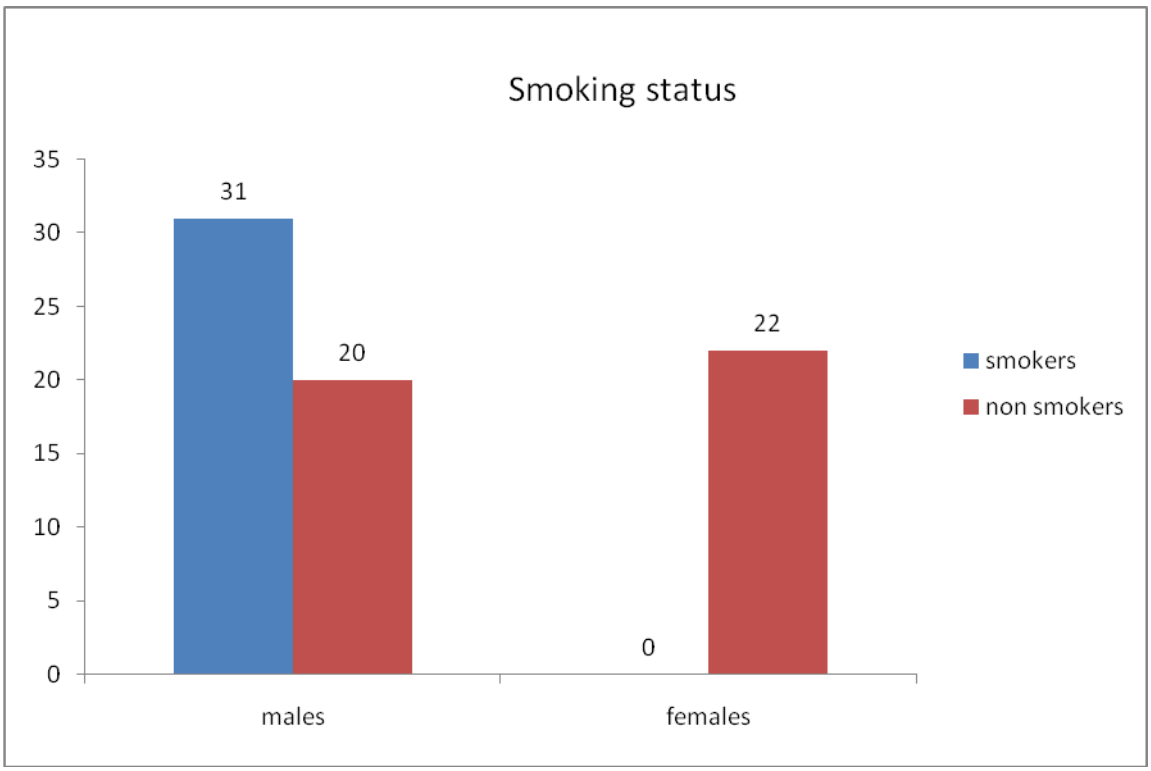
Presenting symptoms

The presenting symptoms has been shown by the bar diagram below. The most common symptom at presentation was cough which was present in 57 patients. Headache/ focal neurological deficit was present in only 4 patients at presentation.



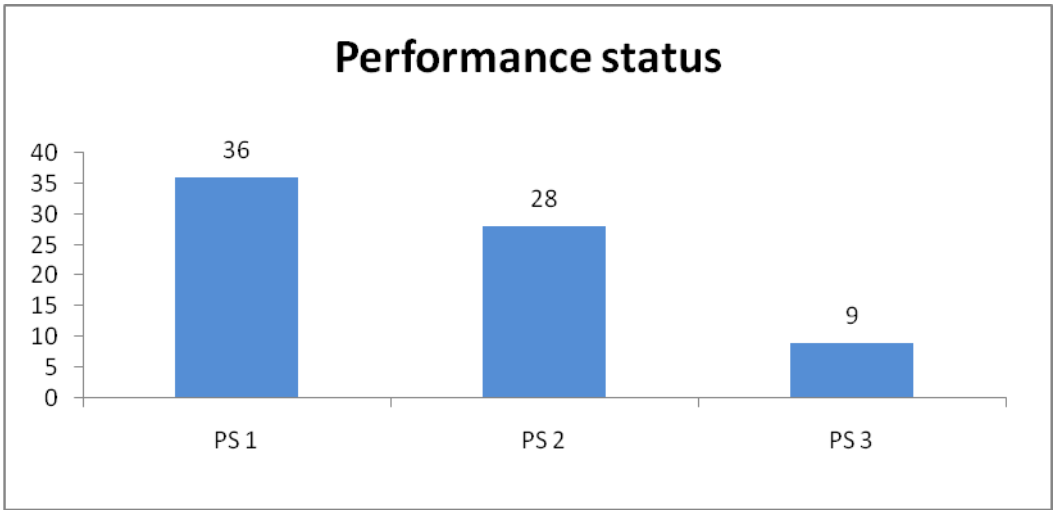
Smoking Status

Among 73 patients enrolled in the study , 31 patients were smokers and 42 patients were non smokers. The median duration of smoking was 24 pack years.



Performance Status at Presentation

Of the 73 patients who were followed up prospectively 36 patients were in performance status 1, 28 patients were in performance status 2 and nine patients were in performance status 3.



Comorbidities

23 patients out of 73 prospectively studied patients had co morbidities. The various co morbidities were as follows

Co morbidities	**Number of patients**
Diabetes	9
Hypertension	6
Ischemic heart disease	3
Diabetes and hypertension	3
Diabetes , hypertension and IHD	2

Stage at Presentation

Among 73 patients who were prospectively studied the stage of presentation is as follows

Stage	Number of patients
1	1
3a	1
3b	9
4	62

Metastatic Pattern at Presentation

Among the 62 patients who had metastatic disease the pattern of metastasis at presentation is as follows

Site	Number
Bone	27
Bilateral lung nodule/pleural effusion	22
Brain	6
Adrenal	8
Liver	6
Spleen	1
Uterus	1
Bone marrow	1

The two most common site of metastasis were bone, bilateral lung nodule/ pleural effusion accounting for 43.5% and 35.4% of metastasis respectively.

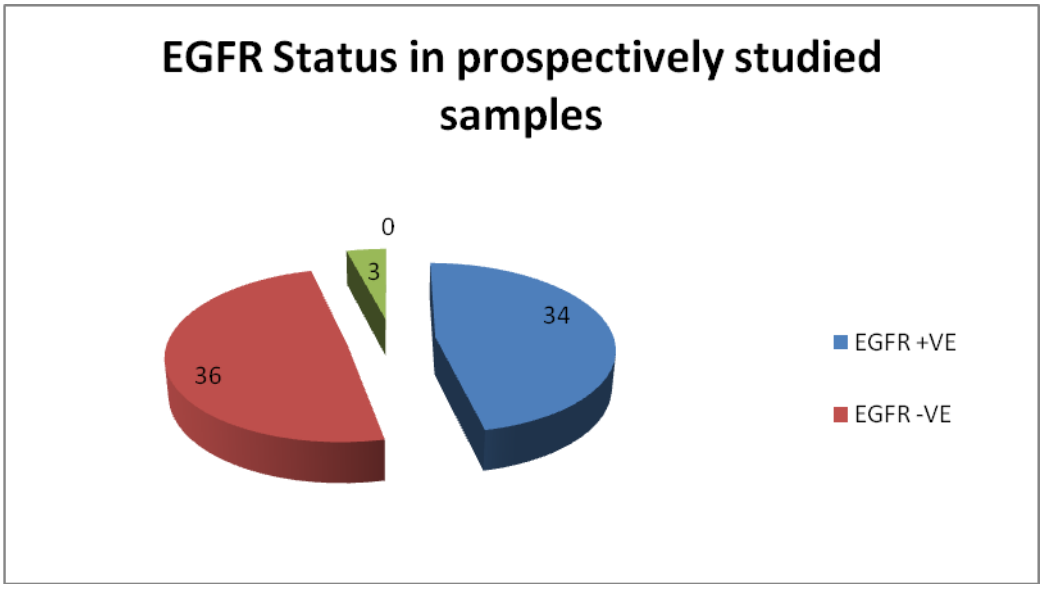
Tissue Diagnosis

Tissue diagnosis in our study population was obtained as follows:

Procedure	Number
Bronchoscopic biopsy	4
Bone marrow biopsy	1
FNAC of lung lesion/SCL node	6
Surgery of primary lesion	2
Pleural fluid cytology	3
SCL node biopsy	8
CT guided trucut biopsy	49

EGFR Mutation Status in Prospectively Studied Samples

Among the 73 prospectively studied patients EGFR mutation status is as follows. EGFR mutation was positive in 34 patients (48.2%), negative in 36 patients(51.6%) and sample was inadequate in 3 patients. 13 out of 22 females(59%) and 21 out of 51 males(41%) were EGFR mutation positive.



Types of EGFR Mutation

Among the 34 EGFR positive patients, mutation pattern is as follows:

Mutations	Number
Deletion (Exon 19)	23
L858R(Exon 21)	9
G719X(Exon 18)	1
L858R(Exon 21)+ G719X(Exon 18)	1

Chemotherapeutic Regimens

Among 73 patients one patient who was a case of stage 1 lung cancer after surgery was kept under follow up, one patient expired before starting the chemotherapy and one patient lost follow up before starting chemotherapy. Among the 70 patients the various chemotherapeutic agents received at presentation is as follows:

Chemotherapeutic regimen	numbers
Pemetrexed + carboplatin/cisplatin	22
Gemcitabine+carboplatin	7
Etoposide+ cisplatin	4
Oral etoposide	2
Paclitaxel+ carboplatin	1
Gefitinib	23
Erlotinib	11

Toxicity profile of Tyrosine kinase inhibitors

Toxicity profile of TKI's were as follows

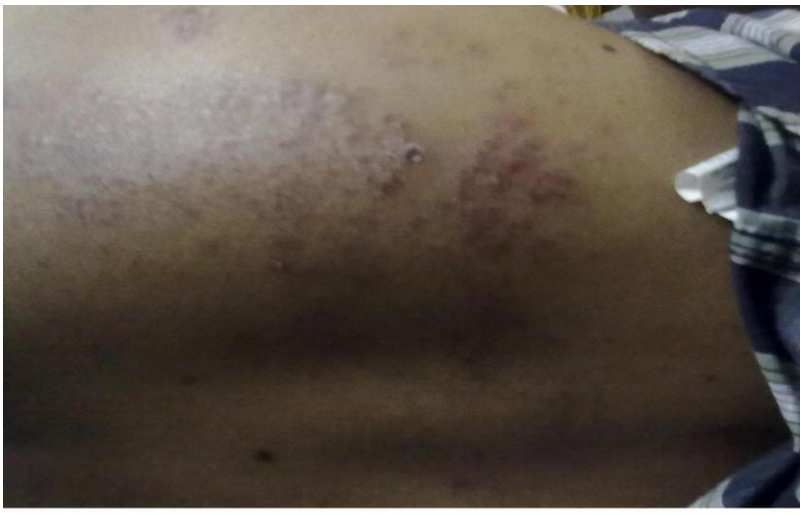
Toxicity	Number
Diarrhoea	4
Skin rash	16
Interstitial fibrosis/ILD	1
Mucositis	2
Increased SGOT/SGPT(>5 times ULN)	3
Hand foot syndrome	2
Conjunctivitis	1

Among the four patients who had diarrhoea, three had grade 2 diarrhoea and one had grade 3 diarrhoea and all were managed conservatively. The TKI was continued in all 4 patients under close observation.

The major side effect of TKI was skin rash. Among the 16 patients who developed skin rash, three patients had grade 1, five patients had grade 2, six patients had grade 3, two patients had grade 4 skin rash. Both patients who developed grade 4 skin rash were on erlotinib.

Skin rash was the major problem in patients who were started on erlotinib . Among 11 patients who were started on erlotinib , nine developed skin rash. Seven patients among 22 patients who were started on gefitinib developed skin toxicity. Erlotinib dose was reduced from 150mg to 100 mg in four patients, and in four patients erlotinib was changed to gefitinib. Gefitinib induced rash was managed conservatively and the drug was continued in all the patients once rash subsided.

One patient had gefitinib induced interstitial lung disease and the drug was discontinued. One patient on gefitinib had conjunctivitis which was managed with temporary stopping of drug and topical steroid drops. Gefitinib was restarted once conjunctivitis subsided under close observation.



* Picture showing erlotinib induced rash on the back

Toxicity Profile in Chemotherapy Arm

The major toxicities in chemotherapy arm are as follows

Toxicities	numbers
Fatigue	12
Vomiting(> grade 2)	5
Neurtopenia (>grade 2)	13
Thrombocytopenia (> grade 2)	5
Peripheral neuropathy	1
Mucositis	2
Diarrhoea(> grade 2)	2

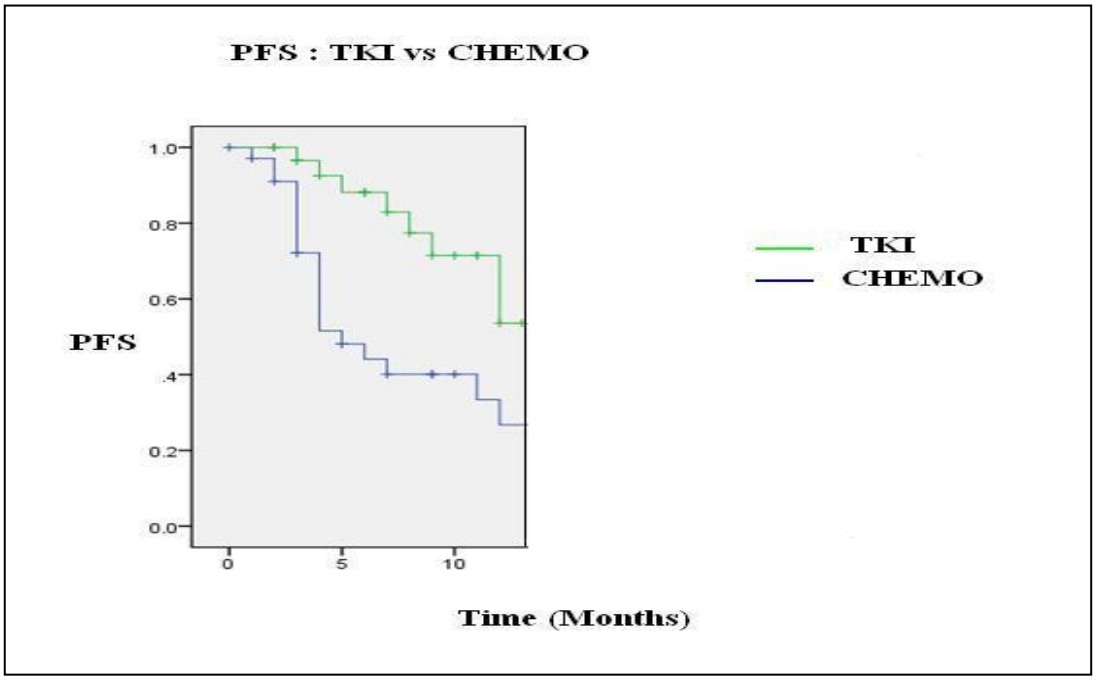
The most common side effect in chemotherapy arm was neutropenia and fatigue. Fatigue was most commonly observed in patients receiving pemetrexed chemotherapy. Among 12 patients who had fatigue, 10 had grade 2 fatigue and two had grade 3 fatigue. Among 13 patients who had neutropenia, eight had grade 2 neutropenia, three had grade 3 neutropenia and two had grade 4 neutropenia.

There was no treatment related mortality in both TKI and chemotherapy arm.

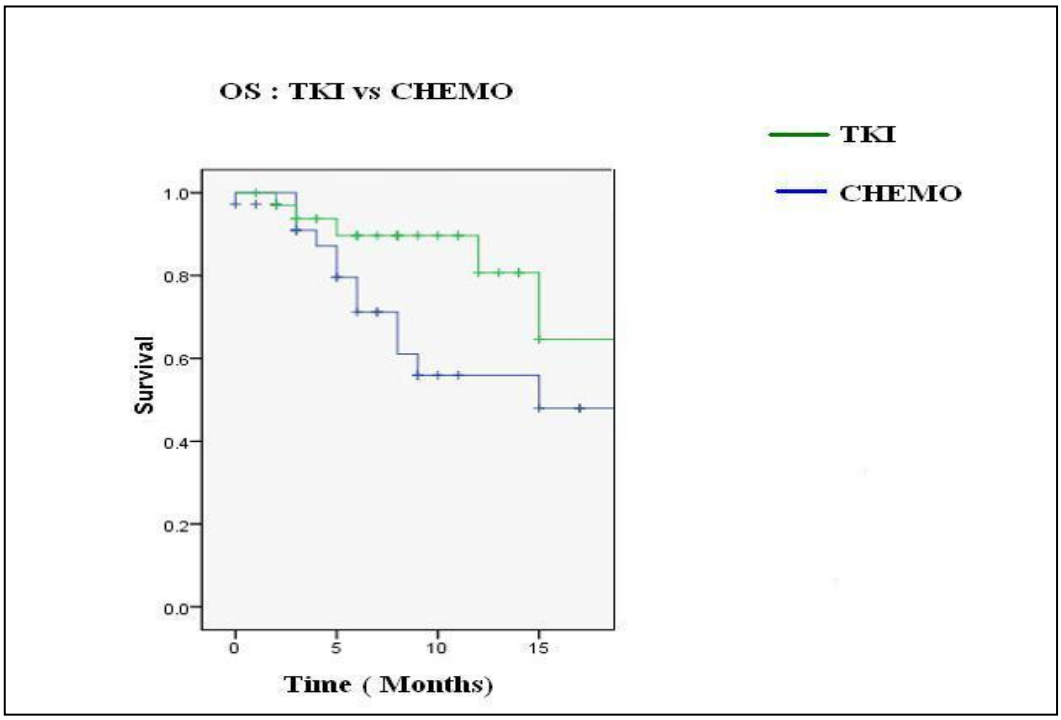
Survival analysis

70 patients were eligible for all the survival analysis. The one year progression free survival and overall survival of whole study population was 51.1% and 72.6%.

The median duration of follow up in chemotherapy group was 6 months and that with TKI group was 8 months. The PFS was more in TKI arm as compared to chemotherapy arm, that is 7.8 months v/s 6.28 months but this was not statistically significant(p= 0.22). One year PFS in TKI group compared to chemotherapy group was 61.2 %v/s 42.4% with p=0.37 which again was not statistically significant.



The one year overall survival in patients with TKI was 80.7% v/s 55.9% in patients on chemotherapy, with p=0.08 which again was not statistically significant.



There was no difference in survival between patient with 2 most commonly found mutations, that is del exon 19 and L858R in exon 21.

Correlation between Age and Survival

One year overall survival in patients > 55 years and those patients with age < 55 years was 66.1 % v/s 82.9% which was statistically not significant (p=0.61)

Correlation between Smoking and Survival

The one year overall survival of smokers was 32.3% compared to 94.4% in non smokers which was statistically significant (p= 0.001)

Correlation between Smoking, EGFR Mutation and Survival

ONE YEAR OVERALL SURVIVAL

	EGFR +VE	**EGFR - VE**
SMOKERS	53.3%(6)	33.5%(23)
NON SMOKERS	95.5%(28)	92.3%(13)

*figures inside brackets indicate number of patients

Patients who were non smokers and who were EGFR positive had highest survival. Smoking emerged as strongest factor for survival after adjusting for EGFR mutation status. Smokers had 5 fold increased risk of dying compared to non smokers.

Correlation of Sex and Survival

Females had 1 year survival of 90.2 % and males had 1 year survival of 61.7% which was not statistically significant (p=0.08)

Correlation between Sex, EGFR Status and Overall Survival

ONE YEAR OVERALL SURVIVAL

	EGFR -VE	EGFR +VE
MEN	49.2% (27)	81.6% (21)
WOMEN	74.1% (19)	100%(13)

*figures inside brackets indicate number of patients

Thirteen women who were EGFR positive had 1 year survival of 100% after starting TKI, the worst outcome was with men who were EGFR negative (49.2%).

Correlation between Co morbidities and Survival

Patients without co morbidities had 1 year survival of 82.2% and those with co morbidities had survival of 52.8%. This was not statistically significant (p=0.17).

Correlation between Performance Status and Survival

Survival according to performance status is shown below

Performance status	Overall survival(n)
PS-1	92.8%(35)
PS-2	59.2%(28)
PS-3	0(7)

Survival difference between patients in PS1 and PS2 was statically significant (p=0.006) .Among the patients who presented in PS 2 the 1 year overall survival with TKI and chemotherapy was 75.8% v/s 49.5 % which was statistically significant (p=0.006).

Summary of Univariate Analysis.

Factor at baseline	**Subgroups**	**No. of patients**	**1 year Survival**	**p value**
Age	<55 years	29	82.8%	0.61
	>55 years	41	66.1%	
Sex	Male	48	61.7%	0.08
	Female	22	90.2%	
Smoking	**Positive**	**29**	**32.3%**	**0.001**
	Negative	**41**	**94.4%**	
Comorbidities	Yes	21	52.8%	0.17
	No	49	82.2%	
Performance status	**PS 1**	**35**	91.8	**0.006**
	PS 2	**28**	**59.2**	
	PS 3	**7**	**0**	
EGFR mutation	Yes	34	89.7%	0.08
	No	36	55.9%	
Types of EGFR mutation	Deletion (exon 19)	23	95%	0.441
	L858R(exon 21)	9	80%	

In univariate analysis smoking history and performance status were the only factors which significantly affected overall survival.

Even in multivariate analysis (Cox regression model), smoking and performance status emerged as strongest predictors of outcome.

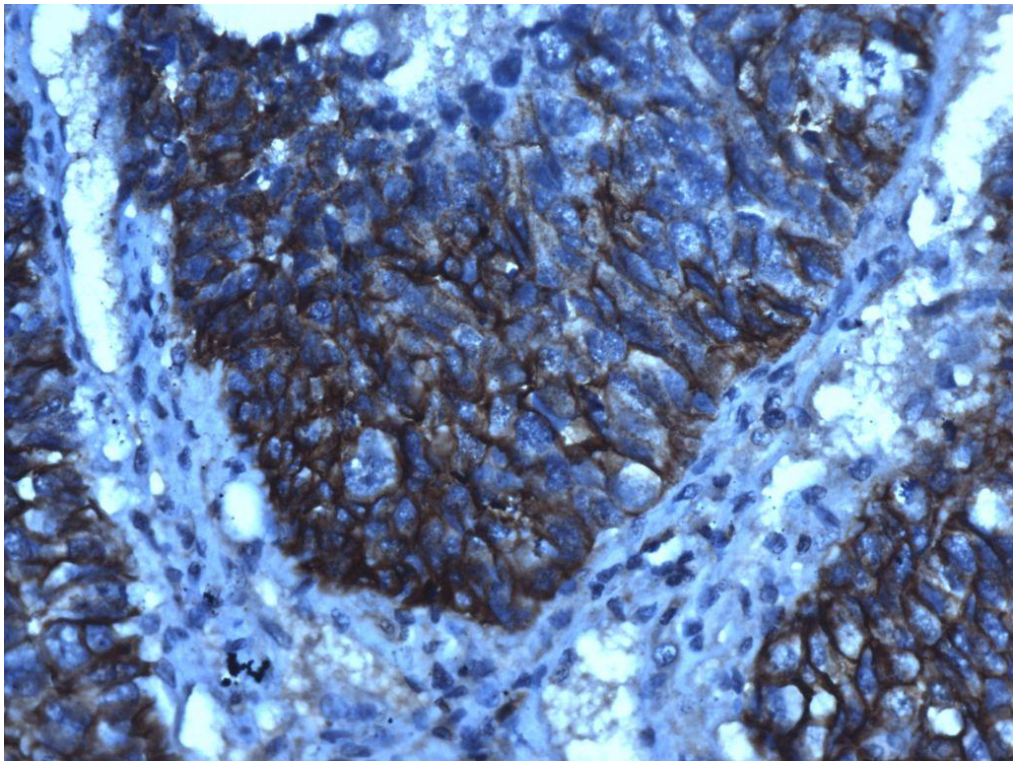
Correlation between EGFR Mutation Status and P-AKT by IHC

Total of 85 patients out of 101 patients were eligible for analysis. 16 patients were not eligible for analysis due to inadequate tissue sample to do IHC/EGFR mutation through PCR.

EGFR mutation was positive in 34 out of the above 85 patients, P- AKT was positive in 51 out of above 85 (60%).

	EGFR +ve	**EGFR -ve**	**Total**	**P value**
p-AKT +ve	29(85.3%)	21(42%)	50	<0.0001
p-AKT -ve	5(14.7%)	30(58%)	35	
Total	**34**	**51**		

The sensitivity and specificity of P-AKT by IHC compared to EGFR mutation done through RT-PCR was 85.3% and 58.8% respectively. The positive predictive value and negative predictive value of the test was 58% and 85.5% respectively. The kappa co-efficient for P- AKT was 0.409, which is a measure of agreement (>0.5 is taken as significant)



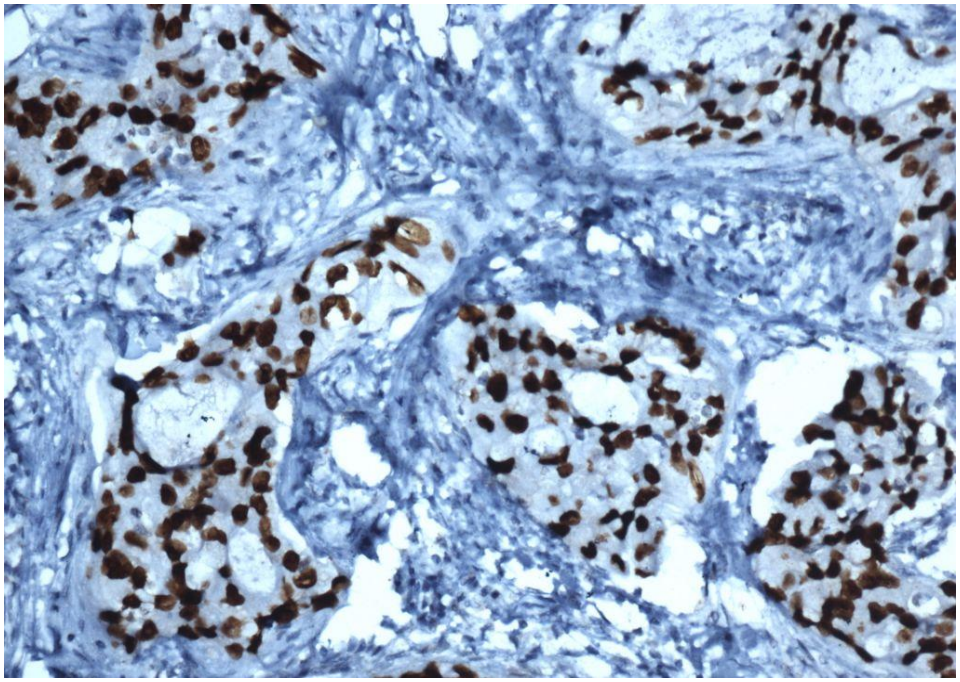
IHC X 40. Tumor cells showing membrane and cytoplamic positivity to P- AKT.

Correlation between EGFR Mutation Status and TTF-1

TTF-1 was positive in 68 of the 85 eligible patients (80%) and was negative in rest of the 17 patients (20%).

	EGFR +ve	**EGFR -ve**	**Total**	**P VALUE**
TTF-1 +ve	33(97.1%)	35(68.6%)	68	<0.00001
TTF-1 -VE	1(2.9%)	16(31.4%)	17	
Total	**34**	**51**		

The sensitivity and specificity of the test was 97.1% and 31.4% respectively. The positive predictive value and negative predictive value of the test was 48.5% and 94.1% respectively. The kappa co-efficient was 0.224.



IHC X40 : Tumor cells showing nuclear positivity to TTF-1.

Correlation between P-AKT, TTF-1 and EGFR mutation

	EGFR +ve	**EGFR -ve**	**Total**	**P value**
TTF +ve and P-AKT -ve	3(11.1%)	13(54.2%)	16	0.002
TTF +ve and P-AKT +ve	24(88.9%)	11(55.8%)	35	
Total	**27**	**24**		

In correlation study of p-AKT with EGFR mutation status and TTF-1 with EGFR mutation status, even though negative predictive value was very high, the positive predictive value was very low and they had high incidence of false positivity.

In correlation of EGFR mutation status with subset of patients having TTF-1 positive and P-AKT positive compared to TTF-1 positive and P-AKT negative had sensitivity and specificity of 88.9% and 54.2 % respectively and had positive predictive value of 68.6% and negative predictive value of 90%.The false positivity was as low as 31.4%. Kappa co-efficient was 0.439. The positive predictive value was significantly higher and false positivity was much lesser compared to TTF- 1 and P-AKT assessed seperately with EGFR mutation status.

Correlation of P-AKT and Survival

Patients who were P- AKT positive had one year overall survival of 76.4% compared to patients who were p-AKT negative who had overall survival of 61.7% which was statistically not significant (p=0.79).Among those who had P-AKT positivity, patients who were started on TKI had better outcome than those started on chemotherapy (88.2% to 65%), even though this was statistically not significant (p=0.214).

QOL Assessment

43 patients of 73 patients completed both base line and 2nd assessment which was 3 months after starting the treatment. The CI-QOL scores are as follows

CI –QOL-Q scoring at base line	CI –QOL-Q scoring at 2nd assessment

Score	Frequency	Percent		Frequency	Percent
Very low	2	4.7%		5	11.6%
Low	11	25.5%		8	18.6%
Average	24	55.8%		22	51.2%
High	6	14.0%		5	11.6%
Very high	0	0		3	7%
Total	43	100%		43	100%

The mean average score at base line was 128 and that at 2nd assessment was 126, which indicates that the lung cancer in our study had average quality of life.

Among the 43 patients,20 patients were on TKI and the remaining 23 were on chemotherapy. The comparison between TKI arm and chemotherapy arm between baseline and 2nd assessment is as follows

		MEAN	SD	P Value
CI-QOL Q ASSESSMENT1	TKI	128	21.78	0.931
	CHEMO	128.52	17.4	
CI-QOL Q ASSESSMENT2	TKI	132.83	25.51	0.126
	CHEMO	122.24	18.98	

The mean score in patients on TKI and chemotherapy was same at base line , but during 2nd assessment TKI patients had better quality of life compared to patients on chemotherapy, but this improvement was not statistically significant(p=0.126).

The mean EORTC C30 score in TKI group and chemotherapy group at baseline assessment was 56.28 v/s 57.56 , and same during 2nd assessment was 57.22 v/s 60.12 , none of these were statistically significant.

There was a statistical improvement in global health scale (which deals with overall health and overall QOL in the past 1 week) between TKI group and chemotherapy group during second assessment.

		MEAN	**SD**	**P Value**
GHS ASSESSMENT1	TKI	6.28	1.23	0.277
	CHEMO	6.78	1.23	
GHS ASSESSMENT2	TKI	10.24	3.03	0.031
	CHEMO	8.38	2.30	

There was no statistical difference between TKI arm and chemotherapy arm in site specific EORTC-QCQ-LC13 questionnaire, which focuses with improvement in symptomatology of patients with lung cancer.

DISCUSSION

Lung cancer is one of the commonest cancers worldwide and is the leading cause of death among both males and females throughout the world. Around 85 % lung cancer present in advanced stage, and the overall survival of metastatic lung cancer has been dismal.

The discovery of tyrosine kinase inhibitors, has added new dimension in the treatment of EGFR mutated advanced carcinoma lung, improving PFS and OS of patients with EGFR mutation.

The incidence of EGFR mutation is thought to be 15% in western population and around 50 % in Asian population. [8, 22, 23] The major problem in EGFR mutation testing is the technique used in testing. The DNA sequencing method which was being used commonly has been replaced by newer technique because of low sensitivity and contamination problems. [9]

The most common method which is used extensively in recent times for EGFR mutation testing is ARMS- PCR technique. But this test is very expensive and takes 7- 10 days for the results to be available. Hence in patients who need treatment immediately, waiting for EGFR report will delay the treatment.

In Indian scenario any surrogate tests for EGFR mutation which is cost effective, highly sensitive and less time consuming will be very useful.

This study was carried out to find out answers for following questions

- Data regarding EGFR mutation status in Indian population is limited. This study is designed to detect EGFR mutation status by using ARMS- PCR method, also to assess the response to treatment which was started as per EGFR mutation status.
- In this study we tried to know whether any correlation exists between EGFR mutation detected through ARMS-PCR method and P-AKT and TTF-1 which was performed through IHC method, which is less expensive and less time consuming.
- We also tried to compare quality of life in patients treated with chemotherapy and EGFR inhibitors by using questionnaire standardized to Indian patients.

In our study, the median age of presentation was 55 years which is a decade younger to those patients presented in EURTAC study, which was carried out in European population but is comparable with median age of presentation of IPASS study and OPTIMAL study which was carried out on Asian population. [8, 22, 23]

In our study, 70 % patients were males and only 30 % were females which may be probably due to decreased smoking habit among females. 31 out of 73(42.4%) patients included in the study were smokers which is higher than that quoted in EURTAC and OPTIMAL study (35% and 41.8% respectively). [22, 23]

Most of the patients in our study presented in good performance status and around 50% of patients presented in PS-1.The most common symptom at presentation in our study was cough.

Around 84% of all patients included in the study were having metastatic disease and most common site of metastasis was either bone or lung (pleural effusion or bilateral lung nodule). This figure is comparable with that quoted by Sahn HA et al. [46]

Brain metastasis was present in six out of 62 patients with metastatic disease, accounting for 9.6% of all metastasis. Sen et al has quoted that 25 % metastatic lung cancer presents with brain metastasis. [47]

Prevalence of EGFR mutation in our study population was 48.2%. In IPASS study which was done from East Asia, where only selected population of non smokers with adenocarcioma lung were included ,the EGFR mutation was present in 59.7% patients. In a study conducted by Sahoo R et al, in Indian patients, EGFR mutation was present in 51.8% of patients. [8,39]

The most common mutations found in our study were deletion in exon 19 and mutation at exon 21(L858R). Together these two mutations accounted for 94% of total EGFR mutation. Above 2 mutations were the predominant mutations in IPASS study and study by Sahoo R et al accounting for 96% and 78% of the mutations respectively. [8, 40]

Among 34 EGFR mutation positive patients, 23 patients received gefitinib and 11 patients received erlotinib. Among these 34 patients, four patients were started initially on chemotherapy and were changed subsequently after 1st cycle of chemotherapy to TKI once EGFR reports were available.

Among 36 patients who were EGFR negative, 22 patients received pemetrexed+carboplatin/cisplatin. Other chemotherapy which were administered were gemcitabine/carboplatin, etoposide/cisplatin, oral etoposide and paclitaxel/carboplatin. Three EGFR negative patients who were initially started on TKI were subsequently changed to chemotherapy once EGFR results were available.

The major toxicity with TKI'S was skin rash which was present in 47 % of patients. All patients were treated with topical clindamycine cream and antihistaminics.

In our study grade 3 or 4 skin rash was present in eight patients (23.5%) which is much more than that quoted in IPASS and OPTIMAL study (3.1 % and 2 % respectively). [8, 22]

We also noticed that erlotinib was largely responsible for skin toxicity in TKI group. Nine of the total 11 patients who received erlotinib developed skin toxicity. Two patients who developed grade 4 skin toxicity in our study were on erlotinib. Four patients were managed by reducing the dose of

erlotinib from 150 mg to 100 mg and the other four patients required change of treatment to gefitinib. This toxicity due to erlotinib resulted in significant treatment interruptions.

All the patients who developed skin toxicity with gefitinib were managed with topical clindamycine ointment and antihistaminics and were restarted on the drug once rash subsided. The treatment interruption due to gefitinib induced skin toxicity was negligible.

The differential toxicity due to gefitinib and erlotinib may be because erlotinib is prescribed at a dose close to its maximal tolerable dose(MTD) where as gefitinib is prescribed at a dose much lesser than MTD.

Diarrhoea was observed in only four patients treated with TKI'S (29.4%) which is less than that observed in IPASS study (46.6%) .

The major toxicities with chemotherapy were fatigue and haemotological toxicity,mainly neutropenia. Fatigue was mostly observed in patients receiving pemetrexed chemotherapy. Fatigue was the main side effect in patients receiving pemetrexed / cisplatin chemotherapy in LUX LUNG 3 trial which was observed in 46.2 % patients. [48]

The one year progression free survival and overall survival of whole study population was 51.1% and 72.6% respectively. One year overall survival for carcinoma lung (including all stages), enrolled in year 2002-2003 from hospital based cancer registries in Chennai , was 51.8%.[49]In the study conducted by Rajappa et al the one year overall survival of advanced lung cancer in Indian patients was 29.2% with cisplatin based doublet chemotherapy which is much less than that observed in our study indicating that treatment based on EGFR mutation status has significantly increased survival.[39]

The PFS in TKI group was 7.8 months compared to 6.28 months in chemotherapy group. Even though this was not statistically significant it is clear from our study that EGFR positive patients who were started on upfront TKI has improved PFS compared to EGFR negative patients started on standard chemotherapy. One year PFS in TKI group was 61.2 % compared to 42.4% of chemotherapy group which again was not statistically significant.

The various studies showing PFS of TKI compared to chemotherapy are:

Study	Chemotherapy	TKI	Significance
IPASS study(no randomization based on EGFR status).[8]	5.6 months	5.7 months	Not significant
	But in EGFR mutated pts chemotherapy TKI had better PFS compared to chemotherapy		Significant with HR of 0.74
OPTIMAX (in EGFR mutated pts).[21]	4.6 months	12.1 months	Significant P=0.0001
LUX-LUNG 3 (in EGFR mutated pt).[47]	6.9 months	11.1 months	Significant P=0.0001
EURTAC trial(EGFR mutated pts).[22]	5.2 months	9.7 months	Significant P=0.0001
Robert A. Louis.[40]	5months	7 months	Significant P=0.024
Our study	6.28 months	7.8 months	Not significant P=0.14

One year overall survival was better in TKI arm compared to chemotherapy arm (80.7% v/s 55.9 %) even though it was not statistically significant. Longer follow up is required for accurate calculation of overall

survival. In the study by Louis et al, conducted at our institute, one year OS with chemotherapy arm was 40.4 % compared to 44.4 % in TKI arm. [12]

In univariate analysis and multivariate analysis various factors influencing overall survival of carcinoma lung like age, sex, smoking status, EGFR mutation status, type of EGFR mutation, comorbidities and performance status at presentation were analyzed. Both in univariate and multivariate analysis, only smoking status and performance status at presentation emerged as independent risk factors which influence overall survival.

In IPASS trial only age had emerged as independent risk factor for survival in univariate analysis. None of the other factors influenced survival. In the study by Louis et al female sex, non smokers and upfront treatment with gefitinib had impact on PFS in univariate analysis but in multivariate analysis none of the factors emerged significant [8, 12]

A subset of patients who were 1) non smokers and EGFR positive 2) females who are EGFR positive started on TKI had the best survival outcome in our study. None of the 13 females who were EGFR positive died within one year accounting to 100% one year overall survival in this subset.

Smoking when adjusted for EGFR mutation status emerged as strongest risk factor for survival, that is smokers even though EGFR positive after starting on TKI had inferior survival compared to patients who were non smokers, EGFR negative and who were started on chemotherapy (53.3% v/s 92.3%).In our study smokers had 5 fold more risk of dying compared to non smokers.

In our study P-AKT by IHC was positive in 68% of the cases. There was a correlation between P- AKT and EGFR mutation with p value of <0.0001.The sensitivity and specificity of the test was 85.5% and 58.8% respectively. Only five out of 34 EGFR positive patients were P- AKT negative resulting negative predictive value of 85.5%. There was a trend towards agreement between EGFR mutation status and P- AKT, with kappa value of 0.409.

But for a test to become a strong surrogate method so as to replace the existing gold standard method, should have high sensitivity and specificity. In our study p- AKT has specificity of 58.8% and false positive rate of 42 %. Hence even though there is strong correlation between EGFR mutation status and P- AKT, it cannot replace the RT- PCR technique in detection of EGFR mutation status.

In study by Ikeda et al, p- AKT by IHC was positive in 51% cases and correlation between EGFR mutation and p- AKT was significant with p=0.002. In this study also, there was a high number of false positivity with P-AKT.

In our study, patients who were P- AKT positive had 1 year overall survival of 76.4% compared to 61.7% in patients with p- AKT negative and this was not statistically significant (p=0.79). In patients who were P-AKT positive when treated with TKI compared to chemotherapy had better overall survival (88.2% v/s 65%) which was again not statistically significant .This was contrast to study of Capuzzo et al, where p-AKT positive patients when treated with gefitinib had better response rate ,time to progression compared to P- AKT negative patients.[32]

In our study TTF -1 was positive in 80% of cases, there was statistically significant correlation between EGFR mutation positivity and TTF-1 positivity (p=<0.0001).The sensitivity and specificity of the test was 97.1% and 31.4% respectively. Only one out of 17 TTF-1 negative patients was EGFR mutation positive, accounting for negative predictive value of 94.1%. The kappa co-efficient was 0.224. This sensitivity and negative predictive value is consistent with study of Vincent et al and Sommiah et al who quoted negative predictive value of TTF-1 to be > 95%. [36, 38]

P- AKT and TTF-1 has high negative predictive value, which means chance of having EGFR mutation positive in patients who are p- AKT, TTF-1 negative is very less. In patients where P-AKT and TTF-1 negative, EGFR mutation testing can be avoided and started on chemotherapy when there is urgency in starting treatment.

The problem of false positivity and low sensitivity with TTF-1 and P-AKT can be partly overcome by testing for above two IHC simultaneously. In patients having TTF-1 positive and P-AKT positive, when compared to TTF-1 positive and P-AKT negative had sensitivity and specificity of 88.9% and 54.2 % in detecting EGFR mutation .The positive predictive value of 68.6% .False positivity was 31.4% which is much lesser compared to P-AKT and TTF-1 when they were tested separately.

Improvement in quality of life should be one of the important aims in the treatment of metastatic lung cancer, where survival is lesser than one year. Therapeutic interventions which improve the quality of life like general well being, physical well being and psychological well being should be strongly encouraged in the treatment of metastatic carcinoma lung.

There is limited data from India where quality of life has been measured using questionnaire standardized for Indian patients. In this study

we used cancer institute QOL questionnaire which has been standardized for Indian patients. [45]

At presentation our study population had average QOL with score of 128. After treatment patients who were EGFR positive and started on TKI had better QOL compared to patients who were EGFR negative and started on chemotherapy (mean QOL score of 122.22 v/s 132.83) even though it was statistically non significant. In our study there was significant improvement in global health scale during first assessment in TKI arm compared to chemotherapy arm (mean of 10.24 v/s 8.38, p=0.031). Our results clearly show that patients who are started on TKI upfront had better quality of life compared to those started on chemotherapy.

Similar to our study, in OPTIMAL trial which assessed quality of life by using FACT-L, TOI , LCS questionnaire had significant improvement in QOL with TKI compared to chemotherapy arm. In IPASS study health related quality of life was better in TKI arm in patients who were EGFR positive, and in chemotherapy arm in patients who are EGFR negative. [50,51]

CONCLUSIONS

- Present study is the first prospective study of lung cancer from India, where in , treatment decision was made on EGFR mutation status and treatment outcomes were assessed.

- EGFR mutation status in our population was 48.2%.

- The one year PFS and OS of study population was 51.1% and 72.6% respectively.

- The high PFS and OS in our study, strongly support the need for EGFR mutation testing in all patients of advanced carcinoma lung and importance of starting treatment depending on EGFR mutation status.

- Patients who were EGFR mutation positive and started on TKI's in the upfront setting had better PFS, OS compared to patients who were EGFR mutation negative and started on standard chemotherapy.

- Female patients who were EGFR mutation positive had 100% one year

- overall survival with TKI treatment indicating a clinically favorable subset.

- Factors significantly affecting overall survival of carcinoma lung were smoking status and performance status at presentation**.**

- The major toxicity with TKI's in our study was skin toxicity and the incidence of skin toxicity was more with erlotinib compared to gefitinib.

- P- AKT and TTF-1 by IHC has strong correlation with EGFR mutation status which was performed through ARMS- RT PCT. But due to low specificity and high false positivity these IHC's cannot replace RT- PCR method in detection of EGFR mutation status. The false positivity can be partly reduced by using P-AKT and TTF-1 simultaneously, where in patients who are positive for both P-AKT and TTF-1 had lower false positivity in detecting EGFR mutation.

- Due to high negative predictive value of P-AKT and TTF-1, in patients who are negative for these IHC studies, EGFR mutation testing can be avoided and patients can be started on chemotherapy if there is urgency in starting the treatment.

- The QOL was assessed by using cancer institute- quality of life questionnaire which is standardized for our population. The QOL was better with TKI treatment compared to other chemotherapy.

The limitation of our study is the small sample size, short duration of follow up and different regimens of chemotherapy used in chemotherapy arm.

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Proforma

NAME **AGE** **SEX**

UHID

CONTACT NO **ADDRESS**

OCCUPATION **SMOKING** **EXP TO CARCINOGEN**

SYMPTOMS **DURATION** **PS**

1

2

3

TISSUE DIAGNOSIS-

SUBTYPE

FINAL DIAGNOSIS

EGFR MUTATION STATUS **TYPE OF SAMPLE SENT**

IHC- EGFR receptor

p- AKT

TREATMENT GIVEN

1 . TKI A)ERLOTINIB DOSE b)GEFTINIB DOSE

2.I V CHEMOTHERAPY -

SIDE EFFECT OF TKI

QOL

1st follow up(AFTER 3 MONTH)

PS

CHEST X RAY

CT CHEST

OTHERS

1 CR 2 PR 3 SD 4 Pr D

CHANGE IN TREATMENT REASON

DOSE INTURRUPTION CAUSE

SIDE EFFECT OF TKI

2st follow up(AFTER 6 MONTH)

PS

CHEST X RAY

CT CHEST

1 CR 2 PR 3 SD 4 Pr D

CHANGE IN TREATMENT **REASON**

DOSE INTURRUPTION **CAUSE**

SIDE EFFECT OF TKI

3rdt follow up(AFTER 9 MONTH)

PS

CHEST X RAY

CT CHEST

1 CR **2 PR** **3 SD** **4 Pr D**

CHANGE IN TREATMENT **REASON**

DOSE INTURRUPTION **CAUSE**

SIDE EFFECT OF TKI

QOL