1.0 INTRODUCTION

1.1 Lung cancer – general overview

Lung cancer is the leading cause of cancer mortality worldwide, ranking number one for both sexes. In 2013, Surveillance, Epidemiology, and End Results (SEER) database estimated 228,190 new cases of lung and bronchus cancer and 159,480 people would die of this cancer, representing 27.5% of all cancer deaths. Based on the SEER data from 2003 to 2009, 57% presented with distant disease, and the percentage surviving five years was 16.6%. With the SEER data 2006-2010, the median age at diagnosis was 70, and more males were diagnosed than females across all races (1). According to the American Cancer Society, non-small-cell lung cancer (NSCLC) accounts for 85-90% of lung cancers while small cell lung cancer accounts for 10-15% of all lung cancers. NSCLC has three major subtypes: squamous cell carcinoma (25-30%), adenocarcinoma (40%), and large cell carcinoma (10-15%). About 20% of NSCLC are NOS (Not Otherwise Specified), such as adenosquamous carcinoma and sarcomatoid carcinoma (2). Since the 1960s, there had been a decline in the proportion of squamous cell carcinomas and an increasing proportion of adenocarcinomas. Several studies suggested this shift was a result of the changes in the characteristics of cigarettes, especially the development of cigarette filters. People smoking low yield filtered cigarettes tend to compensate for the lower nicotine levels by inhaling more deeply and frequently, leading to greater exposure of the peripheral lung to the carcinogens in tobacco smoke, and in part to the increased concentration of nitrosamines that preferentially produce adenocarcinoma (3-8). Adenocarcinoma currently accounts for approximately 39% of all cases of NSCLC in smokers, 35% in

never smokers. Squamous cell carcinoma accounts for 42% of NSCLC in smokers and 33% in never smokers (9).

In South Africa, there is limited formal reporting of incidence / prevalence of lung cancer. Most recent National Cancer Registry data from 2006 showed 1388 males (ASR 8.75 per 100,000; LR 1 in 93) and 680 females (ASR 3.52 per 100,000; LR 1 in 225) newly diagnosed with lung cancer histologically. There was no reporting on smoking status, histology subtypes or molecular abnormalities in lung cancer (10).

1.2 Lung cancer in never smokers – a new entity

Although the majority of lung cancer is related to tobacco smoking, approximately 10-15% of worldwide lung cancers occur in lifelong never smokers (3, 11). Never smokers have been estimated to constitute about 30-40% of all lung cancer patients in east Asian countries, such as China, Korea and Japan (12). If it is regarded as a separate category, lung cancer in never smokers (LCINS) causes approximately 300,000 deaths per year, making it the seventh leading cause of cancer deaths in the world, ahead of deaths from cancers of the cervix, pancreas, or prostate (12, 13).

The main histological subtype of LCINS is adenocarcinoma and two thirds of patients with LCINS are women (14, 15). Over the past few decades, many genetic, environmental, hormonal, and viral factors were proposed as risk factors of LCINS. Some environmental risk factors of importance included environmental tobacco smoke, radon, asbestos, and indoor air pollution, such as cooking-oil fumes and coal burning (3, 11).

With the recent understanding of cancer biology and the development of molecular pathology, there is a renewed interest in the problem of LCINS after the observation that the response rate with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors(TKIs), such as gefitinib and erlotinib, are higher in never smokers than smokers with NSCLC (12, 16). Moreover, activating mutations in the EGFR-TK domain are associated more frequently with adenocarcinoma, never smokers, female gender, and east-Asian ethnicity (11,15, 17-19).

Higher EGFR mutation rate of between 30% to 60% were reported in East Asia (20, 21), where mutation rate was usually 10-23% in western countries (22-28). This could be due to a higher proportion of female never smokers in East Asia where smoking was traditionally discouraged in females.

The specialized cancer agency of the World Health Organization, the International Agency for Research on Cancer (IARC), announced on the 17th October 2013 that it has classified outdoor air pollution as carcinogenic to humans and the world's leading experts convened by the IARC Monographs Programme concluded that there is sufficient evidence that exposure to outdoor air pollution causes lung cancer.

Particulate matter, a major component of outdoor air pollution, was evaluated separately and was also classified as carcinogenic to humans. The IARC evaluation showed an increasing risk of lung cancer with increasing levels of exposure to particulate matter and air pollution (29).

However, the lung cancer risk associated with air pollution is much lower than that associated with smoking. A meta-analysis (ESCAPE study) by Raaschou-Nielsen et al

showed a statistically significant association between the risk for lung cancer and particulate matter (PM) with diameter of less than 10 μm (HR 1.22; 95% CI 1·03-1·45; per 10 μg/m^3 increase). However, unlike smoking, everybody is exposed to air pollution, therefore everybody is at risk. Air pollution was also associated with adenocarcinoma in this study (30).

World Health Organization estimated that fine particulate matter cause about 16% of lung cancer deaths, 11% of COPD deaths, and more than 20% of ischaemic heart disease and stroke (31). Estimates based on the latest WHO mortality data from 2012 showed that lung cancer represented 6% of all outdoor air pollution-caused deaths, and 6% of all indoor air pollution-caused deaths (32).

Although severe air pollution has become a major problem in many Chinese cities, the association between EGFR mutation and NSCLC caused by air pollution has not yet been described to date.

1.3 Epidermal growth factor receptor and signaling pathways

Growth factors and their receptors play an important role in the pathogenesis of NSCLC. Epidermal growth factor receptor (EGFR) is frequently overexpressed in human tumours, including NSCLC (16). Ligands including EGF, TGF-α and epiregulin bind and activate receptors that belong to the c-erbB (EGFR) family of tyrosine kinase receptors. This comprises four distinct members: the epidermal growth factor receptor 1 (EGFR-1, c-erbB-1, HER1), c-erbB-2 (HER2-neu), c-erbB-3 (HER3), and c-erbB-4 (HER4). These receptors (with the exception of HER2) possess an extracellular ligand-

binding domain, a lipophilic transmembrane domain, and an intracellular domain in which resides the tyrosine kinase activity.

Ligand binding to the extracellular domain of EGFR drives homo- or hetero-dimerization of the receptors and subsequent activation of the intrinsic tyrosine kinase domain. Receptor activation leads to phosphorylation of specific C-terminal tyrosine residues that provide docking sites for proteins containing src homology 2 (SH2) or phosphotyrosine binding domains, which are important intermediates for the activation of intracellular signaling pathways. c-erbB receptors are able to activate several signaling pathways, including the RAS/RAF/MAPK pathway, which plays an important role in regulating cell proliferation, migration and differentiation, and the PI3K/AKT/mTOR pathway, which controls cellular survival and antiapoptotic signals. Increased downstream signaling occurred when EGFR were overexpressed or activated by mutations (33).

1.4 EGFR tyrosine kinase inhibitors and EGFR activating mutations

EGFR TK inhibitors such as gefitinib and erlotinib, compete for the ATP binding in the TK domain of the receptor, thus blocking EGFR autophosphorylation and downstream signaling. Both gefitinib and erlotinib have been shown in several phase III trials to be predictive markers in NSCLC in patients harboring the activating mutations in EGFR (21, 28). There are four main types of EGFR activating mutations: point mutations in exon 18 (G719A, G719C, G719S), deletions in exon 19, insertions in exon 20, and point mutations in exon 21 (L858R and L861Q). The exon 19 deletions and the exon 21 point mutation (L858R) are the two most common mutation types (90% of all EGFR

mutations) and are associated with improved outcomes to EGFR TKI therapy (17-19). These mutations account for 62.2% and 37.8% respectively in the Spanish screening trial for EGFR mutations conducted between April 2005 to November 2008, where 2105 lung cancer patients in 129 institutions in Spain were screened (23). EGFR mutations were found in 350 of 2105 patients (16.6%) and mutations were more frequent in women (69.7%), never smokers (66.6%) and adenocarcinomas (80.9%).

1.5 Development of TKI and studies before 2004

The development of TKIs preceded the discovery of EGFR mutations. TKIs were initially developed to overcome EGFR overexpression. It was developed as an alternative treatment option for lung cancer in the second / third line setting, after first line platinum combination chemotherapy. IDEAL 1 and IDEAL 2 trials were dose finding trials for gefitinib (34, 35). The results of Iressa Dose Evaluation in Advanced Lung Cancer trial (IDEAL 1) was published by Fukuoka et al in the Journal of Clinical Oncology in 2003. It was an international phase II trial of gefitinib for previously treated patients with advanced NSCLC. It was conducted in Europe, Australia, South Africa and Japan. Patients were randomized to receive either 250mg or 500mg of gefitinib once daily. Efficacy was similar between the two doses. Objective tumor response rates were 18.4% and 19% among the 250mg/d and 500mg/d groups respectively although the Japanese patients had an objective response rate of 27%. Symptom improvement rates were 40.3% and 37%. Adverse events at both dose levels were generally mild (grade 1 or 2) and consisted mainly of skin reactions and diarrhea. Drug-related toxicities were more frequent in the higher-dose group. Fukuoka et al concluded that gefitinib showed clinically meaningful antitumor activity and provided symptom relief

as second- and third-line treatment in advanced NSCLC. At 250mg/d, gefitinib had a favorable adverse event profile (34).

The IDEAL 2 trial was of the same design as IDEAL 1 but was conducted in America, and the patients had to have at least 2 chemotherapy regimens prior to enrollment to the trial. The results reported by Kris et al were similar to those in IDEAL 1. Symptoms improvement was 43% in patients receiving 250mg of gefitinib once daily and in 35% of patients receiving 500mg of gefitinib once daily. Partial radiographic responses occurred in 12% of patients receiving 250mg/d and in 9% of patients receiving 500mg/d (35). These were the first phase II trials to show meaningful anti-tumor activity with gefitinib with favorable side effects profiles.

Subsequent to that, various first-line phase III clinical trials were developed to evaluate the effect of combining TKIs with platinum combination chemotherapy. INTACT 1 was a phase III randomized, double-blind, placebo-controlled, multicentre trial in chemotherapy naïve patients with unresectable stage III or IV NSCLC, comparing cisplatin and gemcitabine plus either gefitinib 500mg/d, gefitinib 250mg/d or placebo. Trial patients continued gefitinib or placebo post chemotherapy until disease progression. There was no difference in efficacy end points between the three treatment groups. The median OS were 9.9 months, 9.9 months and 10.9 months (p=0.4560), the median PFS were 5.5 months, 5.8 months, and 6.0 months (p=0.7633), and response rates were 49.7%, 50.3% and 44.8%, respectively (36).

INTACT 2 was similar in design to INTACT 1 but the chemotherapy used were carboplatin and paclitaxel. There was no difference in median OS (8.7 months, 9.8

months and 9.9 months for gefitinib 500mg/d, 250mg/d and placebo, respectively; p=0.64). TTP and RR were similar between the three arms (37). Both INTACT 1 and INTACT 2 were published in March 2004 in the Journal of Clinical Oncology by Giaccone et al and Herbst et al, respectively. Adding gefitinib to first line chemotherapy had no added benefit in overall survival or response compared to chemotherapy alone.

TRIBUTE was a phase III first-line trial of erlotinib 150mg/d or placebo combined with carboplatin and paclitaxel chemotherapy, followed by maintenance monotherapy with erlotinib or placebo in advanced NSCLC. The result was published in September 2005 in the Journal of Clinical Oncology by Herbst et al. Median OS was 10.6 months for the erlotinib arm and 10.5 months for the placebo arm (HR 0.99; 95% CI 0.86-1.16; p=0.95). There was no difference in RR or TTP between the two arms. However, smoking status was first described to have OS advantage in this study where never smokers experienced improved OS in the erlotinib arm (22.5 months versus 10.1 months for placebo) (38).

The mutations in the EGFR and KRAS were sequenced in the TRIBUTE trial and this retrospective subset analysis was published by Eberhard et al in the Journal of Clinical Oncology at the same time as the original TRIBUTE results in September 2005. EGFR mutations were detected in 13% of tumors and were associated with longer survival irrespective of treatment ($p<0.001$). Among erlotinib treated patients, EGFR mutations were associated with improved response rate ($p<0.05$) (26).

Gatzemeier et al published the results of a phase III study of erlotinib or placebo in combination with cisplatin and gemcitabine in advanced NSCLC (Tarceva Lung Cancer

Investigation Trial - TALENT). Patients continued erlotinib or placebo until unacceptable toxicity or death. There were no difference in median OS between the erlotinib and placebo groups (43 weeks versus 44.1 weeks; HR 1.06; 95% CI 0.90-1.23; p=0.49). TTP and RR were the same between the two groups. In a small group of patients who had never smoked, OS and PFS were increased in the erlotinib group. Median OS in never-smokers (n = 10) was 11.4 months with placebo, but was not reached with erlotinib (n = 8). Median PFS was longer with erlotinib (7.9 months) than with placebo (5.4 months; HR 0.195; p=0.02) (39).

These trials did not show added benefit of TKI to first line platinum based chemotherapy. However, a subset of patients had emerged that may benefit from TKI treatment - never smokers and those with EGFR mutations.

1.6 Discovery of EGFR activating mutations and increase response to TKI

In 2004, EGFR activating mutations in the epidermal growth factor receptor conferring activity to gefitinib and erlotinib were described by Lynch et al (17). These mutations were in-frame deletions or amino acid substitutions clustered around the ATP-binding pocket of the tyrosine kinase domain of EGFR within exon 18 to exon 21. Over 90% of EGFR mutations were in-frame deletions in exon 19 (over 20 variants) and point mutations in exon 21 with amino acid substitution of arginine for leucine at position 858 (L858R). Most of these mutations occurred in never smokers and adenocarcinoma but also in women and East Asians (17-19). These results gave evidence to the molecular pathogenesis of a distinct subset of lung cancers. How to treat these patients with mutation-positive lung cancer at different stages of disease was unknown and because

of the sensitivity of these distinct subset of EGFR mutated lung cancer to EGFR TKIs, many subsequent trials were done to determine the best way of treating these lung cancers by either EGFR TKI therapy alone or incorporating EGFR TKIs with standard chemotherapy. Subsequently, screening for these mutations became the focus of many lung cancer trials.

To find out if Europeans had the same EGFR mutations as in East Asians, Rosell et al conducted a screening trial in Spain. EGFR mutations were found in 350 of 2105 patients (16.6%). Mutations were more frequent in women (69.7%), never smokers (66.6%), and adenocarcinomas (80.9%), $p<0.001$. These mutations were exon 19 deletions (62.2%) and L858R mutation (37.8%). Those who had EGFR mutations were eligible for erlotinib treatment. The median PFS and OS for patients who received erlotinib were 14 months and 27 months, respectively. The duration of response was similar for patients receiving first line erlotinib (14 months; 95% CI 9.7-18.3) and second-line erlotinib (13 months; 95% CI 9.7-16.3; $p=0.62$). Median OS for patients receiving first-line erlotinib was 28 months (95% CI 22.7-33), and for those receiving second-line therapy was 27 months (95% CI 19.9-34.1; $p=0.37$). A better response was associated with the exon 19 deletions than with L858R mutation (OR 3.08; 95% CI 1.63-5.81; $p=0.001$). Rosell et al. concluded that screening for EGFR mutations with subsequent customization of erlotinib treatment, is feasible and improves outcome (23).

1.7 Second- / third-line TKI studies versus placebo

For pretreated patients who failed first- or second-line chemotherapy, two randomized phase III trials comparing efficacy of erlotinib or gefitinib to placebo were conducted (24,

40). Both trials showed benefit in a subgroup of patients of certain clinical characteristics (women, non-smokers, Asian, adenocarcinoma).

The BR21 study published by Shepherd et al enrolled patients with stage IIIB or IV NSCLC, with performance status from 0 to 3, who had received one or two prior chemotherapy regimens, were randomly assigned in a 2:1 ratio to receive oral erlotinib, at a dose of 150 mg daily, or placebo. This study achieved its primary endpoint, OS (6.7 months versus 4.7 months; HR 0.70; 95% CI 0.58-0.85; $p<0.001$). In the subgroup analysis, being Asian, having adenocarcinoma and never smoker status were associated with better survival (24).

Tsao et al reported the molecular and clinical predictors of outcome on this study. In univariate analyses, survival was longer in the erlotinib group than in the placebo group when EGFR was expressed (HR for death 0.68; 95% CI 0.49-0.95; $p=0.02$) or there was a high number of copies of EGFR (HR 0.44; 95% CI 0.23-0.82; $p=0.008$). In multivariate analyses, adenocarcinoma ($p=0.01$), never having smoked ($p<0.001$), and expression of EGFR ($p=0.03$) were associated with an objective response. In multivariate analysis, survival after treatment with erlotinib was not influenced by the status of EGFR expression, the number of EGFR copies, or EGFR mutation. Although the response rate with erlotinib among patients with EGFR mutations were more than twice that among patients with wild-type EGFR, the difference was not statistically significant due to small numbers (41). These results from BR21, lead to the registration of erlotinib for the treatment of NSCLC patients in the second- / third-line settings, regardless of clinical or molecular features.

ISEL was a placebo-controlled phase III study comparing gefitinib as second-line or third-line treatment for patients with locally advanced or metastatic NSCLC. Patients were randomly assigned in a ratio of 2:1 to either gefitinib (250 mg/day) or placebo, plus best supportive care. The study failed to meet its primary endpoint of overall survival and survival in those with adenocarcinoma. Median OS did not differ significantly between the two treatment groups (5.6 months for gefitinib and 5.1 months for placebo; HR 0.89; 95% CI 0.77-1.02; p=0.087) or among those with adenocarcinoma (6.3 months versus 5.4 months; HR 0.84; 95% CI 0.68-1.03; p=0.089). Preplanned subgroup analyses showed significantly longer survival in the gefitinib group than the placebo group for never-smokers (median OS 8.9 months versus 6.1 months; HR 0.67; 95% CI 0.49-0.92; p=0.012) and patients of Asian origin (median OS 9.5 months versus 5.5 months; HR 0.66; 95% CI 0.48-0.91; p=0.01). Although treatment with gefitinib was not associated with significant improvement in OS, benefit was noted among never-smokers and patients of Asian origin (40).

Hirsch et al published the molecular predictors of outcome in the ISEL study. The result showed EGFR gene copy number was a predictor of clinical benefit from gefitinib. High EGFR gene copy number was a predictor of a gefitinib-related effect on survival (HR 0.61 for high copy number and HR 1.16 for low copy number; comparison of high versus low copy number HR, p=0.045). EGFR protein expression was also related to clinical outcome (HR for positive 0.77; HR for negative 1.57; comparison of high versus low protein expression HR, p=0.049). Of note, patients with EGFR mutations had higher response rates than patients without EGFR mutations (37.5% versus 2.6%), however there were insufficient data for survival analysis. EGFR mutations in this study were

linked to higher proportion of patients with adenocarcinoma, never smokers, Asian origin, and female gender (27).

1.8 Second-line studies with TKI versus chemotherapy

SIGN (Second-line Indication of Gefitinib in NSCLC) was a phase II trial that investigated oral gefitinib (250 mg/d) or docetaxel in patients with advanced NSCLC who had previously received one chemotherapy regimen. The primary objective was assessment of symptom improvement (using the FACT-L Lung Cancer Subscale). Secondary objectives included quality of life (FACT-L Total Score), response rate (using RECIST 1.0), overall survival and safety. Similar efficacy was observed with gefitinib and docetaxel, 36.8% and 26.0% symptom improvement rates, 33.8% and 26.0% quality-of-life improvement rates, 13.2% and 13.7% objective response rates, and 7.5 months and 7.1 months median overall survival, respectively. Fewer drug-related adverse events were observed with gefitinib compared with docetaxel (all grades: 51.5% versus 78.9%; Common Toxicity Criteria grade 3/4: 8.8% versus 25.4%). There were no withdrawals or deaths due to drug-related adverse events with gefitinib, while three patients withdrew and three died due to adverse events in the docetaxel group that were possibly drug related. Cufer et al concluded that gefitinib was similar to docetaxel in terms of efficacy, but with a more favorable tolerability profile (42).

INTEREST was a phase III global NSCLC study comparing patients previously pretreated with one or more platinum-based chemotherapy to gefitinib (250 mg/d) or docetaxel. The primary objective was overall survival. The conclusion of non-inferiority of gefitinib compared with docetaxel was confirmed for overall survival (7.6 months

versus 8.0 months; HR 1.020; 95% CI 0.905-1.150). Superiority of gefitinib in patients with high EGFR-gene-copy number was not proven (8.4 months versus 7.5 months; HR 1.09; 95% CI 0.78-1.51; p=0.62) (43).

In 2010, Douillard et al reported on the molecular predictors of outcome in the INTEREST trial. EGFR mutation positive patients had longer PFS (7 months versus 4.1 months; HR 0.16; 95% CI 0.05-0.49; p=0.001) and higher ORR (42.1% versus 21.1%; p=0.04) favoring gefitinib treatment. Patients with high EGFR copy number also had higher ORR favoring gefitinib (13% versus 7.4%; p=0.04) (28).

The ISTANA (IRESSA as Second-line Therapy in Advanced NSCLC-KoreA) phase III study, conducted in South Korea compared gefitinib with docetaxel in patients with advanced or metastatic NSCLC pretreated with platinum-based chemotherapy. The primary endpoint was PFS. A significant number of patients were male (62%), never smoker (41%) and adenocarcinoma (68%). PFS was longer for gefitinib compared with docetaxel (6 months PFS was 32% versus 13%; HR 0.729; 90% CI 0.533-0.998; one-sided p=0.0441). Gefitinib significantly improved ORR (28.1% versus 7.6%; two-sided p=0.0007). In the final analysis of OS, the hazard ratio was 0.870 (14.1 months versus 12.2 months; 95% CI 0.613-1.236; two-sided p=0.4370). No significant differences were seen in the quality of life or symptom improvement rates between the two treatment groups (44).

The Japanese phase III study (V-15-32) compared gefitinib (250 mg/d) with docetaxel in advanced/metastatic NSCLC who had failed one or two chemotherapy regimens. The primary objective was OS to demonstrate non-inferiority for gefitinib to docetaxel. Non-

inferiority in overall survival was not achieved (HR 1.12; 95.24% CI 0.89-1.40) according to the predefined criterion (upper CI limit for HR≤1.25). However, no statistically significant difference in overall survival was apparent (p=0.330) between treatments. The median overall survival and the 1-year survival rates were 11.5 months and 47.8%, respectively, for gefitinib and were 14.0 months and 53.7%, respectively, for docetaxel. Gefitinib significantly improved objective response rate and quality of life versus docetaxel. Progression free survival, disease control rates, and symptom improvement were similar for the two treatments. For EGFR mutation positive patients, the ORR was 67% with gefitinib and 46% with docetaxel. EGFR mutation positive patients appeared to have better PFS than EGFR mutation negative patients on both treatments (gefitinib HR 0.33; 95% CI 0.11-0.97; docetaxel HR 0.15; 95% CI 0.04-0.57). The authors concluded that gefitinib remains an effective treatment option for previously treated Japanese patients with NSCLC (45).

These four clinical trials (INTEREST, SIGN, ISTANA, V-15-32) comparing gefitinib versus docetaxel in second line treatment were analysed in a meta-analysis (46). The result showed no difference in OS or PFS between gefitinib and docetaxel in pretreated patients, but a higher response rate, better toxicity profile and better quality of life associated with gefitinib treatment.

Similarly, three phase III trials comparing erlotinib and chemotherapy in pretreated NSCLC patients were conducted (47-49). TITAN (Tarceva In Treatment of Advanced NSCLC) study assessed the efficacy and tolerability of second-line erlotinib versus chemotherapy. It was a phase 3 global study for locally advanced, recurrent, or metastatic NSCLC received up to four cycles of first-line platinum doublet

chemotherapy, after which patients with disease progression during or immediately after chemotherapy were randomly assigned (1:1) to receive erlotinib 150 mg/day or chemotherapy (standard docetaxel or pemetrexed regimens). The primary endpoint was overall survival in the intention-to-treat population. Median OS was 5.3 months with erlotinib and 5.5 months with chemotherapy (HR 0.96; 95% CI 0.78-1.19; log-rank p=0.73). No significant differences in efficacy were noted between patients treated with erlotinib and those treated with docetaxel or pemetrexed (47).

The Hellenic group trial compared erlotinib versus pemetrexed as second-line treatment of patients with advanced/metastatic NSCLC. There was no difference in terms of ORR (11.6% versus 6.8%; p=0.166), median TTP (2.9 months versus 3.6 months; p=0.434) and median OS (8.9 months versus 7.7 months; p=0.528) between the pemetrexed and erlotinib arms, respectively. The Disease Control Rate (DCR) was 34.1% in the pemetrexed arm and 24.7% in the erlotinib arm (p=0.082). The incidence of recurrences was significantly higher in the erlotinib (91.3%) than in the pemetrexed (78.9%) arm (p=0.003). These two salvage treatments had comparable efficacy in patients with advanced NSCLC (48).

TAILOR study enrolled patients who had metastatic NSCLC, had had platinum-based chemotherapy, and had wild-type EGFR as assessed by direct sequencing. Patients were randomly assigned (1:1) to receive either erlotinib orally 150 mg/day or docetaxel. The primary endpoint was overall survival in the intention-to-treat population.

Median OS was 8.2 months (95% CI 5.8-10.9) with docetaxel versus 5.4 months (95% CI 4.5-6.8) with erlotinib (adjusted HR 0.73; 95% CI 0.53-1.00; p=0.05). PFS was

significantly better with docetaxel than with erlotinib. Median PFS was 2.9 months (95% CI 2.4-3.8) with docetaxel versus 2.4 months (95% CI 2.1-2.6) with erlotinib (adjusted HR 0.71; 95% CI 0.53-0.95; p=0.02). In this study, chemotherapy was more effective than erlotinib for second-line treatment for previously treated patients with NSCLC who have wild-type EGFR tumours. This trial suggested that EGFR TKIs should not be used in patients who do not harbour EGFR mutations (49).

Sadly, despite the discovery of the relationship between activating EGFR mutations and sensitivity to TKIs in 2004, many prospective clinical trials between 2005 and 2009 were negative and failed to show the correlation. This was largely due to a lack of tissue sampling with the lung cancer diagnosis and the inability to perform mutation analysis on cytology. Therefore the statistical subset analysis was often underpowered.

1.9 First-line studies of TKI versus chemotherapy

Due to the strong suspicion concerning EGFR mutations and their correlation with the efficacy of TKIs, and the higher survival rate in Asian patients, four clinical trials evaluated the use of gefitinib in the first-line setting in Asia.

The phase III IPASS study compared previously untreated NSCLC patient to gefitinib (250mg/d) or carboplatin-paclitaxel in a selected population based on clinical characteristics (Asian, non-smoking, adenocarcinoma). The primary end point was progression free survival. The 12 month rates of PFS were 24.9% with gefitinib and 6.7% with carboplatin-paclitaxel. The study met its primary objective of showing the non-inferiority of gefitinib and also showed its superiority, as compared with carboplatin-paclitaxel, with respect to PFS in the intention-to-treat population (HR progression or

death 0.74; 95% CI 0.65-0.85; p<0.001). In the subgroup of patients who were positive for the EGFR mutation, PFS was significantly longer among those who received gefitinib than among those who received carboplatin-paclitaxel (HR for progression or death 0.48; 95% CI 0.36-0.64; p<0.001), whereas in the subgroup of patients who were negative for the mutation, PFS was significantly longer among those who received carboplatin-paclitaxel (HR for progression or death with gefitinib 2.85; 95% CI 2.05-3.98; p<0.001). Improved response rate was shown in those harboring EGFR activating mutations (43% versus 32%; p<0.001). Mok et al concluded that gefitinib was superior to carboplatin-paclitaxel as an initial treatment for pulmonary adenocarcinoma among nonsmokers or former light smokers in East Asia. The presence of an EGFR mutation was a strong predictor of a better outcome with gefitinib. Gefitinib was non-inferior to standard chemotherapy in both PFS (primary end point) and OS, but with a better tolerability profile and better quality of life. Mutation negative patients showed a better PFS when treated with chemotherapy as compared with gefitinib (21).

The Biomarker analysis and final overall survival results of the IPASS study was published by Fukuoka et al in 2011. OS was not different between treatments overall or in EGFR mutation positive or negative patients. OS results were likely confounded by the cross over effect of subsequent lines of treatments. In post hoc analyses, PFS was significantly longer for gefitinib versus chemotherapy in both the exon 19 deletions (HR 0.38; 95% CI 0.26-0.56) and the exon 21 L848R mutation (HR 0.55; 95% CI 0.35-0.87). ORR was significantly higher with gefitinib versus chemotherapy in the exon 19 deletions subgroup (84.8% versus 43.2%; OR 7.23; 95% CI 3.19-16.37) and in the L858R subgroup (60.9% versus 53.2%; OR 1.41; 95% CI 0.65-3.05). This analysis

confirmed that EGFR mutations were the strongest predictive biomarker for PFS and tumour response (50).

Similar results were obtained in the phase III Korean study (FIRST SIGNAL) which compared first-line gefitinib versus cisplatin-gemcitabine combination in never smokers with stage IIIB or IV lung adenocarcinoma. Patients were randomly assigned to receive either gefitinib (250 mg daily) or chemotherapy (cisplatin-gemcitabine). The primary objective was OS. Gefitinib did not show better OS compared with chemotherapy (22.3 months versus 22.9 months; HR 0.932; 95% CI 0.716-1.213; p=0.604, respectively). The 1 year PFS rates were 16.7% with gefitinib and 2.8% with chemotherapy (HR 1.198; 95% CI 0.944-1.520). Response rates were 55% with gefitinib and 46% with chemotherapy (p=0.101). In subgroup analysis, among patients who received gefitinib treatment, the EGFR mutation positive status, compared with mutation negative status, was significantly predictive for higher ORR (84.6% versus 25.9%, respectively; p<0.001) and longer PFS (HR 0.377; 95% CI 0.210-0.674; p<0.001), but not among patients who received chemotherapy (ORR, 37.5% versus 51.9%, respectively; p=0.36; PFS HR 0.679; 95% CI 0.343-1.345; p=0.274) (51).

These results have been confirmed further with two Japanese trials, NEJ002 and WJTOG3405 studies, comparing first-line gefitinib versus carboplatin-paclitaxel and gefitinib versus cisplatin-docetaxel, respectively. These two trials enrolled patients who harboured activating EGFR mutations only. Gefitinib once again proved to be superior over chemotherapy in patients harboring EGFR mutation in NEJ002 (PFS 10.8 months versus 5.4 months; HR 0.30; 95% CI 0.22-0.41; p<0.001) and in WJTOG3405 (PFS 9.2 months versus 6.3 months; HR 0.489; 95% CI 0.336-0.710, p<0.0001). The objective

response rate was significantly higher in the gefitinib group than the chemotherapy group in the NEJ002 (73.7% versus. 30.7%; OR 6.32; 95% CI 3.55-11.25; p<0.001), and in the WJTOG3405 (62.1% versus 32.2%; OR 3.445; 95% CI 1.609-7.376; p< 0.0001) (52, 53).

In these four Asian studies, OS were similar in both gefitinib and chemotherapy arms. This was most probably because of the cross-over effect of subsequent treatments. Almost all patients who progressed after first-line chemotherapy received a TKI as second-line treatment. A meta-analysis confirmed the results in all these first-line studies comparing gefitinib and chemotherapy (54). Higher response rate (72% versus 38%; OR 4.04; p<0.001), and increase in PFS (HR 0.45; p<0.001) were associated with gefitinib and EGFR mutation. All these studies supported the approval by the European Medicines Agency (EMA) of gefitinib in Europe for the treatment of locally advanced or metastatic NSCLC in all treatment lines limited to patients bearing EGFR activating mutations.

Two first-line trials (OPTIMAL and EURTAC) comparing erlotinib to chemotherapy on selected population with EGFR activating mutations were conducted. OPTIMAL, a phase III study in China comparing erlotinib versus carboplatin-gemcitabine chemotherapy in the first-line treatment of patients with advanced EGFR mutation positive (exon 19 deletion or exon 21 L858R point mutation) NSCLC, had reached its primary endpoint of PFS. The median PFS was 13.1 months in the arm with erlotinib versus 4.6 months in the arm with chemotherapy (HR 0.16; 95% CI 0.10-0.26; p<0.0001) (20).

The EURTAC study was conducted in European patients. The safety and efficacy of erlotinib compared with standard chemotherapy (cisplatin-docetaxel or gemcitabine or carboplatin-docetaxel or gemcitabine) for first-line treatment of patients with advanced EGFR mutation positive (exon 19 deletion or L858R mutation in exon 21) NSCLC was reported by Rosell et al. The primary endpoint was progression-free survival (PFS) in the intention-to-treat population. The median PFS was 9.7 months in the erlotinib group, compared with 5.2 months in the standard chemotherapy group (HR 0.37; 95% CI 0.25-0.54; p<0.0001). Erlotinib was the only EGFR TKI which had been tested against chemotherapy in Caucasian patients (22).

Furthermore, there was evidence that TKI was associated with longer progression free survival for those with exon 19 deletions compared to L858R mutation in both the IPASS study (HR of 0.38 for exon 19 deletion and 0.55 for L858R) and the EURTAC study (HR of 0.30 for exon 19 deletion and 0.55 for L858R). A better response associated with TKI and exon 19 deletion was also reported in IPASS (ORR 84.8% with exon 19 deletion versus 60.9% in L858R) and the Spanish screening trial (OR 3.08; 95% CI 1.63-5.81; p=0.001) (21-23).

1.10 Maintenance therapy

Two phase III trials (SATURN and ATLAS) evaluated the use of TKIs as maintenance treatment. SATURN was a phase III study evaluating erlotinib or placebo as maintenance treatment after four cycles of platinum-based chemotherapy and achieved non-progressive disease. Median PFS was significantly longer with erlotinib than with placebo (12.3 weeks for patients in the erlotinib group versus 11.1 weeks for those in

the placebo group; HR 0.71; 95% CI 0.62-0.82; p<0.0001). PFS was also significantly longer in patients with EGFR-positive immunohistochemistry who were treated with erlotinib compared with EGFR-positive patients given placebo (median PFS 12.3 weeks in the erlotinib group versus 11.1 weeks in the placebo group; HR 0.69; 95% CI 0.58-0.82; p<0.0001). Cappuzzo et al. concluded that first-line maintenance with erlotinib could be considered in patients who do not progress after four cycles of chemotherapy (55).

The ATLAS phase III study evaluated maintenance therapy with bevacizumab plus erlotinib versus bevacizumab plus placebo after four cycles of platinum-based chemotherapy and bevacizumab. There was an increase in PFS (4.8 months versus 3.7 months; HR 0.71; 95% CI 0.58–0.86; p<0.001) and a small benefit in OS (14.4 months versus 13.3 months; HR 0.92; 95% CI 0.70–1.21; p=0.5341) favoring the combination arm with bevacizumab plus erlotinib. There were more adverse events overall in the bevacizumab plus erlotinib arm, with more grade 3-4 adverse events (rash, diarrhea), and more discontinuation of therapy. Therefore, this combination maintenance regime is not recommended with the modest OS benefit and increased toxicity with the addition of erlotinib to bevacizumab (56).

In summary of TKI maintenance therapy, erlotinib is a maintenance option for patients who achieved stable disease after first-line platinum based chemotherapy in unselected patients.

1.11 Side effects of TKIs

The side effect profiles of both gefitinib and erlotinib were favorable compared to chemotherapy. Rash, diarrhea, and asymptomatic hyper-transaminasemia were common and generally mild to moderate. In a meta-analysis by Petrelli et al, a higher incidence of skin rash was associated with erlotinib compared to gefitinib, and the erlotinib skin rash seems to be associated with a statistically significant outcomes in terms of ORR, PFS and OS (57). The TKI induced skin rash were generally mild (Grade 1 and 2) and easily managed with topical steroid cream or minocycline / doxycycline prophylaxis. Clinical guidelines for the prevention and treatment of EGFR inhibitor-associated dermatologic toxicities was published by Lacouture et al (58).

Interstitial-lung-disease events such as acute respiratory distress syndrome, interstitial lung disease, pneumonitis or radiation pneumonitis occurred in 2.6% of the patients treated with gefitinib and 1.4% of the patients treated with chemotherapy in the IPASS study (21). Similar rates of pneumonitis / pulmonary fibrosis were reported in the erlotinib arm compared to the placebo arm (1.2%) in the BR21 study (24). And in the EURTAC study, 1% patients from the erlotinib arm and the chemotherapy arm had pneumonitis (22). The interstitial-lung-disease was easily managed with treatment interruption and steroids.

1.12 Acquired resistance from long term TKI therapy

Despite the efficacy of EGFR TKI therapy in NSCLC, almost all patients develop resistance to these drugs invariably. The most commonly described acquired resistance mutation was the T790M (substitution of threonine to methionine on codon

790 in exon 20) mutation. This mutation was caused by an insertion of a bulky methionine over the ATP binding pocket, blocking access to EGFR TKIs but not to ATP (59). This secondary mutation accounts for 60% of all resistance mechanisms associated with first-line EGFR TKI treatment. Other resistance mechanisms besides T790 mutation were also described, such as cMET or HER2 amplification and PIK3CA mutation as well as small cell transformation (60).

1.13 Summary of gefitinib and erlotinib

Both gefitinib and erlotinib have demonstrated their role in the treatment of EGFR mutated adenocarcinoma in the first-, second- / third-line settings and in maintenance therapy. Furthermore, erlotinib is also indicated for the second- / third-line settings for unselected patients with no molecular diagnosis as supported by the BR21 study (24). Although the SATURN trial demonstrated higher benefits of erlotinib maintenance for patients who achieved stable disease at the end of chemotherapy (55), this form of "maintenance therapy" was considered by most as early second-line therapy (87). EGFR mutation status should be used as the predictor of response of early second-line TKI as this group of patients would benefit the most from this form of treatment.

For patients who harbour an activating EGFR mutation, TKI treatment should be initiated as early as possible based on evidence on all the first-, second- and subsequent lines studies mentioned previously. TKI therapy had been consistently shown to improve response and survival compared to chemotherapy, and the favorable side effects profile had been translated to better quality of life.

Finally, re-biopsy at the time of progression from TKI treatment should be encouraged to assess for acquired mechanism of resistance, e.g. T790M mutation, activation of alternative pathways, and small cell transformation, to guide future treatment decisions.

1.14 Second generation tyrosine kinase inhibitors

Afatinib is an oral second generation pan EGFR tyrosine kinase inhibitor. It irreversibly binds and blocks EGFR (ErbB1), HER2 (ErbB2), ErbB4 and all relevant ErbB family dimers. In the global LUX-Lung 3 study, afatinib was compared to cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. Median PFS was 11.1 months for afatinib and 6.9 months for chemotherapy (HR 0.58; 95% CI 0.43-0.78; p=0.001). In those patients with exon 19 deletions and L858R EGFR mutations, median PFS improved further with 13.6 months for afatinib and 6.9 months for chemotherapy (HR 0.47; 95% CI 0.34-0.65; p=0.001). Comparing the HR of the subgroup of patients with exon 19 deletion (HR 0.28; 95% CI 0.18-0.44) versus L858R point mutation (HR 0.73; 95% CI 0.46-1.17), exon 19 deletion appeared to have better benefit from afatinib therapy than L838R point mutation. OS was not different between the two arms at the time of publication. The prevalence for EGFR mutation in the LUX-Lung 3 study was 27%. 72% of patients were East Asian, 68% were never smokers and 65% were women. EGFR mutations were predominately exon 19 deletions (49%) and L858R point mutations (40%). Side effects from afatinib were mostly grade 1 or 2 rash, diarrhea, stomatitis and paronychia, as expected for EGFR inhibition. The side effects rarely led to drug discontinuation.

Afatinib may overcome the resistance associated with the first generation TKIs (gefitinib and erlotinib) and was shown to have activity against lung cancer with T790M mutation in a phase II trial (61). It may inhibit the selective expansion of the T790M clones.

Dacomitinib is an oral, once-daily, irreversible pan-HER kinase inhibitor. Two phase III trials evaluated dacomitinib as a second- or third-line therapy in the treatment of advanced NSCLC previously treated with chemotherapy. ARCHER 1009 trial failed to meet its objective of statistical significance in improvement in PFS when compared with erlotinib in an unselected population who had been treated with at least one chemotherapy regimen in advanced NSCLC. The second- / third-line NCIC CTG BR.26 study evaluating dacomitinib in patients with locally advanced or metastatic NSCLC after prior treatment, which included at least one chemotherapy regimen and one EGFR inhibitor treatment regimen also failed to meet its objective of prolonging OS versus placebo. ARCHER 1050 is ongoing comparing dacomitinib to gefitinib as first-line therapy in locally advanced or metastatic NSCLC patients with EGFR activating mutation (62).

1.15 EGFR overexpression

EGFR overexpression however, as detected by immunohistochemistry or Fluorescence in-situ hybridization (FISH) had not been conclusively shown to be a predictive marker for response or survival to TKIs (28, 43, 50).

EGFR overexpression was common in NSCLC and EGFR monoclonal antibodies which inhibit the extracellular domain of the EGF receptors were developed. FLEX was a phase III first-line trial, comparing stage IIIB or stage IV non-small cell lung cancer

patients to chemotherapy (cisplatin and vinorelbine) plus cetuximab or chemotherapy alone. Patients who received chemotherapy plus cetuximab had improved OS compared to the chemotherapy-alone group (median OS 11.3 months versus 10.1 months; HR for death 0.87; 95% CI 0.762-0.996; p=0.044). The main cetuximab-related adverse event was acne-like rash (63).

Analysis of the FLEX study further demonstrated that patients with high EGFR expression levels, defined as immunohistochemistry (IHC) score of ≥ 200, had better OS with the addition of cetuximab to chemotherapy (12.0 months versus 9.6 months; HR 0.73; 95% CI 0.58-0.93; p=0.011). However in the low EGFR expression group, defined as IHC score < 200, the median OS was not different between the two groups (9.8 months in chemotherapy plus cetuximab versus 10.3 months in chemotherapy alone, HR 0.99; 95% CI 0.84-1.16; p=0·88). High EGFR expression was also associated with increased response rate with chemotherapy plus cetuximab (44.4%) compared to chemotherapy alone (28.1%), p=0.002 (64).

Cetuximab is currently not approved for the treatment of NSCLC by the FDA or EMA as the OS benefit was small with a HR of 0.87 from the overall population in the FLEX study.

1.16 Other molecular developments in lung cancer

1.16.1 EML4-ALK translocation

In 2007, a fusion gene formed by Anaplastic Lymphoma Kinase (ALK) and the echinoderm microtubule-associated protein-like 4 (ELM4) was identified in the tumour of a Japanese patient with adenocarcinoma of the lung (65). This rearrangement was

associated with adenocarcinoma, younger age and never or light smokers. This represented approximately 3-7% of all adenocarcinoma (66, 67). This fusion gene exists in multiple variants which encode the same intracellular TK domain of ALK but different truncations of EML4. The most common variants are variant 1 (33%), in which exon 13 of EML4 is fused to exon 20 of ALK (E13;A20), and variant 3a/b (29%), in which exon 6 of EML4 is fused to exon 20 of ALK (E6a/b;A20) (68). All of these ALK fusion proteins undergo ligand-independent dimerization mediated by the coiled-coil domain of the fusion partner, resulting in constitutive activation of the ALK tyrosine kinase, leading to phosphorylation and downstream signaling pathways (JAK-STAT, MEK-ERK, and PI3K-AKT) (69, 70). Tyrosine kinase inhibitors that target the kinase activity of ALK have been found to have pronounced anti-proliferative and pro-apoptotic effects in EML4-ALK positive lung cancer.

1.16.2 ALK tyrosine kinase inhibitors

Crizotinib was the first clinically available ALK TKI. It was initially developed as an inhibitor of MET. The phase I trial (PROFILE 1001) was a dose finding trial (71), but was expanded to enroll a total of 149 ALK rearrangement positive patients based on promising results shown in the initial 2 patients. The ORR was 61%, independent of age, sex, performance status, or number of prior treatment regimens, and the median PFS was 9.7 months (72). Based on its pronounced clinical activity, crizotinib was approved by the US FDA in August 2011.

The subsequent phase III trial (PROFILE 1007) was a study comparing ALK positive advanced NSCLC (adenocarcinoma) who had previously received platinum based

chemotherapy first-line, to standard second-line chemotherapy (pemetrexed or docetaxel) or crizotinib. Crizotinib was associated with a better ORR (65% versus 20%; p<0.001) and longer PFS (7.7 months versus 3.0 months; HR 0.49; 95% CI 0.37-0.64; p<0.001) compared to chemotherapy. OS was not different between the two treatment groups as a result of crossover to the comparator treatment arm (73).

Another phase III trial (PROFILE 1014) is ongoing, comparing first-line crizotinib to chemotherapy (cisplatin-pemetrexed or carboplatin-pemetrexed), in ALK positive NSCLC (adenocarcinoma).

Crizotinib is well tolerated, the common side effects are mild (grade 1 or 2) and include nausea and vomiting, diarrhea, constipation, peripheral oedema and visual abnormalities, characterized as flashes of light or shadows, occur in 41% of patients (71). Interstitial lung disease, hepatotoxicity and prolong QT interval can occur but very rare (1-2%).

Of note, Reactive oxygen species 1 (ROS-1) translocation is another mutation associated with adenocarcinoma, younger patient age, and never smokers, presenting in 1-2% of all NSCLC. Crizotinib has shown to be of benefit in lung cancer with ROS-1 translocation in a phase I trial where 57% showed an objective response (74).

Drug resistance invariably developed with crizotinib treatment. In comparison to EGFR TKI associated secondary mutations where T790M accounts for 60% of all resistance mechanisms, crizotinib associated secondary mutations account for only 30%. 70% of crizotinib resistance mechanisms were due to copy number gain of ALK (75, 76), expression of second oncogene, and activation of alternative signaling pathways, such

as those mediated by EGFR or KIT (77-79). Repeat biopsy and molecular analysis of relapsed tumours would be recommended and ideal for further treatment planning to overcome acquired resistance.

Other ALK TKIs in developments include alectinib, ceritinib, AP26113, and ASP-3026. The L1196M substitution occurs at the gatekeeper position of ALK (a position that controls the binding of nucleotides and TKIs), and it is similar to the T790M substitution in EGFR and the T315I substitution in the Bcr-Abl fusion protein in CML. These substitutions confer resistance to corresponding TKIs. In contrast to crizotinib, alectinib and AP26113 are active against the L1196M mutant of ALK. The phase I/II AF-001JP trial conducted in Japan shown marked activity of alectinib in ALK positive lung cancer. Of the 46 patients, 43 achieved an objective response (93.5%) and 44 achieved disease control (95.7%). Treatment-related adverse events of grade 3 were recorded in 12 (26%) of 46 patients. Serious adverse events occurred in five patients (11%). No grade 4 adverse events or deaths were reported. The most common grade 1 or 2 adverse events were dysgeusia and liver dysfunction (80). Another similar phase I/II (AF-002JG, NCT01588028) study was conducted in the US, where the ORR was 54.5% (81). The FDA granted breakthrough-therapy designation for alectinib on the basis of this trial data, with early approval being expected.

A phase III clinical trial comparing alectinib with crizotinib in ALK positive NSCLC is ongoing in Japan. A global single-arm Phase II study of alectinib in patients with ALK-rearranged NSCLC resistant to crizotinib is ongoing (NCT01801111).

AP2613 is another selective small molecule ALK inhibitor that shows activity against the L1196M mutant. A Phase I/II study (NCT01449461) is ongoing. In the phase I portion of the study, ORR was 63% overall, and 75% for those who had progressed after previous crizotinib therapy. Four out of five patients also showed objective response for metastasis in the central nervous system (82).

Ceritinib (LDK378) is a potent and selective small-molecule ALK inhibitor. In a phase I trial reported by Shaw et al, 114 patients with NSCLC who received at least 400 mg of ceritinib per day, the overall response rate was 58% (95% CI 48%-67%). Among 80 patients who had received crizotinib previously, the response rate was 56% (95% CI 45%-67%). Responses were observed in patients with various resistance mutations in ALK and in patients without detectable mutations. Among patients with NSCLC who received at least 400 mg of ceritinib per day, the median PFS was 7.0 months (95% CI 5.6-9.5). The authors concluded that ceritinib was highly active in patients with advanced, ALK-rearranged NSCLC, including those who had had disease progression during crizotinib treatment, regardless of the presence of resistance mutations in ALK (83).

A Phase III trial (NCT01828112) comparing ceritinib with chemotherapy (pemetrexed or docetaxel) for ALK-rearranged NSCLC who have progressed after prior treatment with both crizotinib and platinum based chemotherapy is ongoing. Another Phase III trial (NCT01828099) comparing ceritinib with standard platinum-based first line chemotherapy (cisplatin or carboplatin plus pemetrexed) in untreated ALK-rearranged NSCLC is ongoing.

1.16.3 Molecular diagnosis of ALK rearrangement positive NSCLC

Compared to EGFR mutation where PCR is the standard for molecular diagnosis, EML4-ALK fusion gene were detected by break-apart FISH analysis. In break-apart FISH analysis, the 5' and 3' portion of the ALK gene are separately labelled with red or green fluorescent probes. If the signals of the two probes overlap, resulting in a yellow fluorescence, then there is no translocation. If a translocation is present, the two probes are separated and each is detected as an isolated signal (red or green). Tumors are positive for ALK rearrangement if 15% or more of the tumor cells show isolated signals. This analysis detects ALK rearrangement regardless of the ALK fusion partner of the specific EML4-ALK variant. The break-apart FISH assay is a unique diagnostic approach approved for screening for ALK rearrangement in NSCLC by the FDA.

However, false-negative results do occur with the use of break-apart FISH alone. ALK is not expressed in normal lung tissue or in lung cancer negative for ALK rearrangement, any level of ALK expression is considered to be abnormal and expected to be the result of ALK rearrangement. Immunohistochemistry combining with break-apart FISH analysis resulted in marked increase in the sensitivity of detection of ALK fusion proteins.

Reverse transcription-polymerase chain reaction (RT-PCR) is a highly sensitive and specific method for the identification of ALK rearrangement. Unlike FISH and IHC, it can also determine both the fusion partner of ALK and the EML4-ALK variant. This method is pending validation (84).

1.16.4 VEGF inhibition

Bevacizumab, a recombinant human monoclonal antibody that binds to and neutralizes VEGF, thereby inhibiting angiogenesis, had been shown to improved survival in non-squamous NSCLC in two randomized phase III trials. The Eastern Cooperative Oncology Group (ECOG) trial compared patients with recurrent or advanced NSCLC (stage IIIB or IV) with chemotherapy (carboplatin and paclitaxel) alone or chemotherapy plus bevacizumab. Chemotherapy was administered for 6 cycles and bevacizumab was continued until disease progression or toxic effects were intolerable. Patients with squamous-cell tumors, brain metastases, clinically significant hemoptysis, or inadequate organ function or performance status (ECOG performance status >1) were excluded. The primary end point was overall survival. The median overall survival favored combination therapy (12.3 months versus 10.3 months; HR 0.79; 95% CI 0.37-0.92; p=0.003). The median PFS also favored combination therapy (6.2 months versus 4.5 months; HR 0.66; 95% CI 0.57-0.77; p<0.001). There were more significant grade 3-4 adverse events such as haemorrhage, hypertension, proteinuria and neutropenia in the chemotherapy plus bevacizumab arm compared to the chemotherapy alone arm (85).

AVAiL study was similar to the ECOG study, except the chemotherapy used was cisplatin and gemcitabine, and low dose (7.5mg/kg) versus high dose (15mg/kg) bevacizumab were also compared. PFS and ORR were improved with the addition of bevacizumab. The hazard ratios for PFS were 0.75 in the low dose group (6.7 months versus 6.1 months for placebo; p=0.003), and 0.82 in the high dose group compared with placebo (6.5 months versus 6.1 months for placebo; p=0.03). ORRs were 20.1%,

34.1% and 30.4% for placebo, low dose bevacizumab and high dose bevacizumab, respectively. Duration of follow up was not sufficient for OS analysis (86).

Based on these results, bevacizumab was approved by the FDA for first-line treatment of unresectable, locally advanced, recurrent or metastatic non-squamous NSCLC in combination with chemotherapy. In the 2nd ESMO Consensus Conference on Lung Cancer, the preferred chemotherapy combination with bevacizumab is carboplatin and paclitaxel (87).

1.16.5 cMet overexpression

cMet overexpression was initially thought to be an escape / override mechanism of resistance developed from the first generation TKI treatment. This was further explored and up to 50% of patients with NSCLC had cMet overexpression. A phase II second-line study by Spigel et al comparing erlotinib plus MetMab (Onartuzumab) or placebo for NSCLC patients who progressed on chemotherapy, demonstrated PFS and OS benefit of MetMab plus erlotinib for those who overexpressed cMet. In this study, “MET high” or MET positive was defined as ≥ 50% tumour cells with MET IHC staining intensity of 2+ or 3+. Co-primary end points were PFS in the intent-to-treat (ITT) and MET-positive population. There was no improvement in PFS or OS in the ITT population (PFS HR 1.09; 95% CI 0.73-1.62; p=0.69; OS HR 0.80; 95% CI 0.50-1.28; p=0.34). The median OS for cMet overexpressed or “MET high” patients treated with the combination was 12.6 months compared with 3.8 months in the placebo plus erlotinib arm (HR 0.37; 95% CI 0.19-0.72; p=0.002). The median PFS was 2.9 months versus 1.5 months, for onartuzumab plus erlotinib and placebo plus erlotinib, respectively (HR 0.53; 95% CI

0.283-0.99; p=0.04). However, worsening of PFS and OS were seen in MET-negative patients treated with onartuzumab plus erlotinib. PFS was 1.4 months for onartuzumab plus erlotinib versus 2.7 months in those treated with placebo plus erlotinib (HR 1.82; 95% CI 0.99-3.32; p=0.05). OS was 8.1 months with onartuzumab plus erlotinib and 15.3 months with placebo plus erlotinib (HR 1.78; 95% CI 0.79-3.99; p=0.16) (88).

However, the phase III MetLung study (89), which randomized patients with previously treated MET-positive advanced NSCLC to onartuzumab plus erlotinib or erlotinib and placebo was being stopped early, following an interim analysis that suggested a lack of clinically meaningful efficacy. Genentech, the company developing the drug, made the announcement in March 2014 (90).

1.16.6 Lung Cancer Mutation Consortium

The Lung Cancer Mutation Consortium (LCMC) in America generated much interested. This was presented as an abstract in the ASCO 2013 meeting by Johnson et al. The LCMC was established in 2009 to assay lung adenocarcinomas for driver genomic alterations in 10 genes and to study and treat patients by their molecular subtypes. It is a multi-center effort where fourteen cancer centers across the US were involved to make molecular testing available for their patients.

Lung cancer tissues were collected and analyzed for KRAS, EGFR, HER2, BRAF, PIK3CA, AKT1, MEK1, and NRAS mutations using multiplexed assays, and for ALK rearrangements and MET amplifications using fluorescence in situ hybridization (FISH).

1,007 underwent testing for at least one genomic alteration with 733 undergoing testing for all 10 genes. 60% of patients were women with a median age of 63; 34% were never smokers 58% were former smokers. A driver alteration was detected in 622 (62%) of the 1,007 with any genotyping, and in 465 (63%) of the 733 fully genotyped cases. Among the tumors with full genotyping, drivers mutations were found as follows: KRAS (25%), sensitizing EGFR mutation (15%), ALK rearrangements (8%), other EGFR mutations (6%), two genes mutations (4%), BRAF mutation (2%), HER2 mutation (2%), PIK3CA(1%), MET amplification (1%), NRAS mutation (1%), MEK1 (<1%), and AKT1 (0%).

Results were used to select targeted therapy or targeted trials in 279 patients with a driver alteration (28% of 1,007 total). Among 938 patients with clinical follow-up and treatment information, 264 with a driver alteration treated with a targeted agent had a median survival of 3.5 years; 313 with a driver who did not receive targeted therapy had a median survival of 2.4 years; while 361 without an identified driver had a median survival of 2.1 years ($p<0.0001$).

The authors concluded that an actionable driver alteration was detected in 62% of tumors from patients with lung adenocarcinomas, leading to the use of a targeted therapy in 28%. The patients with an identified driver treated with a targeted agent lived longer than those patients who did not receive targeted therapy. Multiplexed genomic testing can aid physicians in matching patients with targeted treatments and appropriate clinical trials.

This type of mass genotyping allowed us to understand the types of common mutations that are associated with NSCLC. From the LCMC, 96% of mutations were mutually exclusive. This creates the uniqueness of TKI treatment success in mutated lung cancer (91).

1.17 Summary and situation in South Africa

LCINS differs significantly from lung cancer in smokers in aetiology, clinical treatment response and molecular characteristics – smokers are likely to harbour KRAS mutations and p53 mutations, while LCINS harbour EGFR mutations. KRAS mutations and EGFR mutations are usually mutually exclusive. It has been reported that the frequency of EGFR mutations is inversely associated with the amount of exposure to tobacco smoke, for both passive and active smokers (11, 12, 23). The knowledge of molecular characteristics enables us to understand the cancer biology and develop targeted treatment.

In an oral abstract presented at the 2011 SASMO/SASCRO meeting, we looked at EGFR mutations in lung cancer in South Africa. The data were collected from Dr Chris Maske at Lancet Laboratories between September 2009 and May 2011. 93 samples were successfully analysed with an EGFR mutation rate of 23.6%. 55% were exon 19 deletions, 27% were EGFR L858R mutation (92).

In the 2013 SASMO/SASCRO meeting, a poster presentation by Slavik et al. from Ampath Laboratories documented its own laboratory lung cancer mutation patterns in South Africa. The period during which the mutations were analyzed was July 2011 to July 2013. 110 cases were tested for mutations and the EGFR mutation rate was

identified in 20.41%, ALK rearrangement in 14.29% and KRAS mutation in 27.78%. Of the EGFR mutations, 40% were exon 19 deletions, and 30% L858R mutation (93). There were no other EGFR mutation data to date in South Africa regarding NSCLC to my knowledge.

In black patients, there were no data on EGFR mutation in lung cancer in South Africa and only limited data in the rest of the world. There were four studies from a Pubmed search that described EGFR mutations in NSCLC in African Americans. Three out of four trials gave similar incidence to white patients, ranging from 11.9% to 31.3% (94-97).

This study will explore the factors associated with EGFR mutations, and outcomes in these patients with NSCLC. Due to limited resources in South Africa in regards to the EGFR mutation testing, the study population that were screened were selected by oncologists who were aware of the higher probability of EGFR mutations in never smokers or light smokers AND adenocarcinoma. Therefore patients with these characteristics were preferentially selected for EGFR mutation testing.

EGFR mutations in lung cancer and its associated success with TKI treatment completely changed the lung cancer treatment approach and opened up a new era of personalized treatment for NSCLC. The success of TKI treatment was largely due to the mutually exclusive nature of the mutations in lung cancer and the oncogenic addiction theory (91), and the improved response and PFS with a lower toxicity and better quality of life associated with TKI therapy. This “tailored approach” had been

adopted into the current NCCN and ESMO guidelines. Molecular testing upfront to plan for first-line treatment of stage IIIB / IV lung adenocarcinoma is the current standard.

2.0 PATIENTS AND METHODS

This is a retrospective record review of NSCLC patients who had EGFR mutation analysis in South Africa.

2.1 Methods of data collection and timeline

This study was done by tracing records of patients who had EGFR mutation analysis done on their lung cancer samples at the Molecular Pathology Department, Lancet Laboratories, Johannesburg, during the period of 1st September 2009 to 30th June 2012.

These lung cancer samples came from various oncology practices in South Africa during this period, as Lancet Laboratories was the only molecular laboratory at the time to offer this EGFR mutation analysis. The samples came from different provinces as well as private practices and government hospitals.

From the clinical records of these patients who had EGFR mutation analysis, their clinical data were entered into a data collection table (APPENDIX A). These included categorical variables such as gender, race, smoking status, as well as numerical variables such as age. Some numerical data were calculated from the date of diagnosis and the date of progression or death, for statistical analysis later on.

The entering of data into the data collection table was done by myself for patients who were in Charlotte Maxeke Johannesburg Academic Hospital, Wits Donald Gordon Medical Centre, and Sandton Oncology Centre.

Details of patients from Rosebank Oncology were entered by myself with the assistance of Dr Bernardo Rapoport.

There were doctors from other practices or provinces who assisted with collection of data by filing in the data collection table from records of patients via emails. These practices and doctors were:

a. Dr Samuel Fourie (Wilgers Oncology Centre, Pretoria)
b. Dr Gary McMichael and Dr Daleen Geldenhuys (West Rand Oncology Centre)
c. Dr Dino Chetty (Wits Donald Gordon Medical Centre)
d. Dr Sayeuri Buddu (University of Free State, Bloemfontein)
e. Dr Elre van Heerdan (GVI Oncology, George)
f. Dr Leon Gouws (GVI Oncology, Cape Town)

The patients were followed from the date of EGFR mutation request at Lancet Laboratories to the 31st December 2012.

A total of 76 patients' clinical data were collected and the data were entered into an excel spreadsheet. Some additional basic demographic data were available from the list of testing samples from Lancet Laboratories. These data were age, gender, race and histology.

2.2 Descriptive review

1. Describing the types and frequency of EGFR mutations.
2. Describing the demographics and clinical characteristics of the overall population and of those with positive and negative EGFR mutations, e.g. race, smoking status, gender, histology, stage, ECOG performance status (98) etc.

3. Describing the common treatment practices in South Africa, in patients with EGFR mutation positive and negative NSCLC, in the first- and subsequent lines of treatments.
4. To document response (RECIST 1.0) (99) after first-line and second-line treatments in EGFR mutation positive and negative NSCLC.

2.3 Statistical analysis and objectives

Statistical analysis was done with Epi Info™ version 3.5.4 with the following objectives:

1. To determine the EGFR mutation rate. All the samples across South Africa were tested at the Lancet Laboratories during this period, reflecting the EGFR mutation rate in NSCLC in South Africa.
2. To determine the characteristics which could be associated with EGFR mutations, e.g. race, smoking status, gender and histology, by chi-squared test for the categorical variables.
3. Using Kaplan-Meier survival analysis to obtain PFS and OS in EGFR mutation positive and negative NSCLC patients.
4. Cox proportional hazards were used to determine the significance of certain subgroups of patients, e.g. smokers vs non-smokers, whites vs non-whites, females vs males and those who had chronic lung disease in relation to PFS and OS within the EGFR mutation positive and negative NSCLC.

2.4 Methods of detecting EGFR mutations

EGFR mutation test was performed at the Molecular Pathology Department, Lancet Laboratories, Johannesburg.

The EGFR Mutation Screen Kit (DXS Diagnostics, Manchester) was used in the evaluation of EGFR mutations from paraffin embedded tumor tissue. This was a real-time PCR assay that was run on the Roche Lighcycler 480 v.II and has a sensitivity of 1% of mutant DNA copies in a background of wild type gene copies. The assay detects 28 sensitizing mutations in the EGFR tyrosine kinase domain.

The 28 somatic mutations detected in the EGFR gene (between exon 18 to exon 21) include:

- Exon 18 – G719X (G719S, G719C, G719A)
- Exon 19 – 19 deletions
- Exon 20 – S768I, T790M, 3 insertions
- Exon 21 – L858R, L861Q

3.0 RESULTS

3.1 EGFR mutation rate

183 tissue samples were submitted for EGFR mutation analysis between September 2009 and June 2012 at the molecular department at Lancet Laboratories in Johannesburg. The majority of samples were from Gauteng province.

13 cases were excluded:

- 3 were not lung cancer
- 10 failed PCR quantitative requirement

170 samples were evaluable for EGFR mutation analysis.

37 samples were EGFR mutation positive.

Overall EGFR mutation rate: 37 / 170 = 21.8%.

3.2 Specific EGFR mutations

Of these 37 positive EGFR mutations, the breakdown of the specific mutations between exon 18 to 21 was shown in Table 3.1. Exon 19 deletions and L858R point mutations made up of 89% of all EGFR mutations (33/37 cases).

Table 3.1 Type of EGFR mutations

EGFR mutations	**Number (%)**
Exon 19 deletions	22 (59.5%)
L858R (exon 21)	11 (29.7%)
G719X (exon 18)	2 (5.4%)
S768I (exon 20)	1 (2.7%)
Exon 20 insertions	1 (2.7%)
Total	37 (100%)

3.3 Demographics and clinical characteristics

The clinical characteristics and demographics of the EGFR mutation positive and negative NSCLC patients were shown in Table 3.2. The median age of the overall population was 63 (range 27-85). There was no difference in the median age between the EGFR mutation positive and negative groups. 169 patients were evaluable for sex and there were more females (n=94, 55.6%) than males (n=75, 44.4%) sent for EGFR mutation testing. The majority of patients who had EGFR mutation testing were whites (n=120, 71%), followed by blacks (n=31, 18.3%), and other race (n=18, 10.7%). Other race consists of Indians (n=15), Chinese (n=1) and Mixed race (n=2) (Figure 3.1).

64 out of 75 cases (85%) of all NSCLC samples sent for EGFR mutation testing were Adenocarcinoma (Figure 3.2). There were 16 adenocarcinoma, 1 adeno-squamous carcinoma and 1 large cell carcinoma which were EGFR mutation positive. All squamous cell lung cancer samples tested were negative for EGFR mutation (n=4).

73 clinical records were evaluable for smoking status. The breakdown of all patients in terms of smoking status was shown in Table 3.3.

Current smokers and former smokers were grouped together for statistical analysis as these smokers had the most smoking exposure. They were grouped as “smokers”. Never smokers and former light smokers were grouped together for the same purpose and were labelled as “non-smokers”.

In the EGFR mutation positive group of patients, 88% were never or former light smokers. In the EGFR mutation negative group of patients, the majority were current

and former smokers (68%) (Figure 3.3 and Figure 3.4). Smoking status was inversely proportional to EGFR mutation status (2-tailed chi-squared test, $p < 0.001$).

74 patients were evaluable for ECOG performance status and 64 out of 74 patients (86%) had good ECOG performance status of 0 or 1.

Unfortunately there was not sufficient documentation of the history of HIV status, past and current TB history, alcohol history, recreational drug use, history of mining, past exposure to asbestos and/or silica, and history of indoor pollution (indoor cooking with coal or organic fuel). These variables were therefore not statistically evaluable for the associations with EGFR mutational status.

Table 3.2 Clinical characteristics in EGFR mutation positive and negative patients

	EGFR mutation positive (N = 37)	**EGFR mutation negative (N = 133)**
Age		
Median	64.5	63
Range	38-82	27-85
Gender		
Females (N=94)	23 (62%)	71 (54%)
Males (N=75)	14 (38%)	61 (46%)
Race		
White (N=120)	22 (61%)	98 (74%)
Black (N=31)	7 (19%)	24 (18%)
Others (N=18)	7 (19%)	11 (8%)
Histology		
Adenocarcinoma (N=64)	16 (89%)	48 (84%)
Squamous (N=4)	0	4 (7%)
Others (N=7)	2 (11%)	5 (9%)
Smoking Status		
Current & Former (N=40)	2 (12%)	38 (68%)
Never & Former light (N=33)	15 (88%)	18 (32%)
Chronic lung disease		
Yes (N=17)	3 (17%)	14 (25%)
No (N=56)	15 (83%)	41 (75%)
Stage		
Locally Advanced (IIIA or IIIB) (N=26)	4 (22%)	22 (39%)
Metastatic (IV) (N=48)	14 (78%)	34 (61%)
ECOG Performance status		
0 or 1 (N=64)	14 (78%)	50 (89%)
>1 (N=10)	4 (22%)	6 (11%)
Positive family history of cancer		
Yes (N=19)	6 (37%)	13 (25%)
No (N=49)	10 (63%)	39 (75%)
Mine exposure		
Yes (N=5)	0	5
No (N=38)	7	31
Asbestos exposure		
Yes (N=2)	0	2
No (N=40)	7	33
Silica exposure		
Yes (N=2)	0	2
No (N=38)	7	31

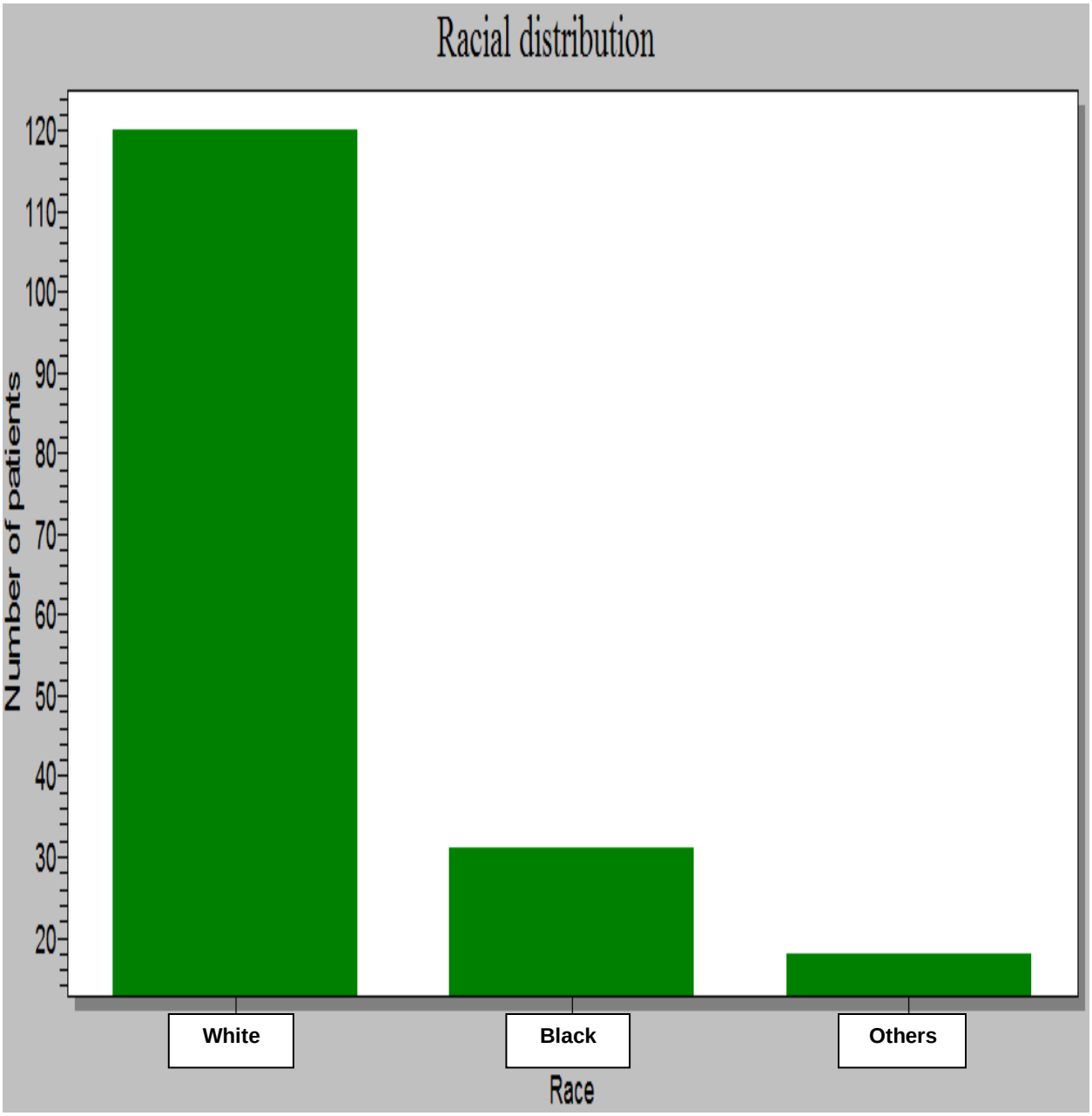

Figure 3.1 Racial distribution of patients tested for EGFR mutations

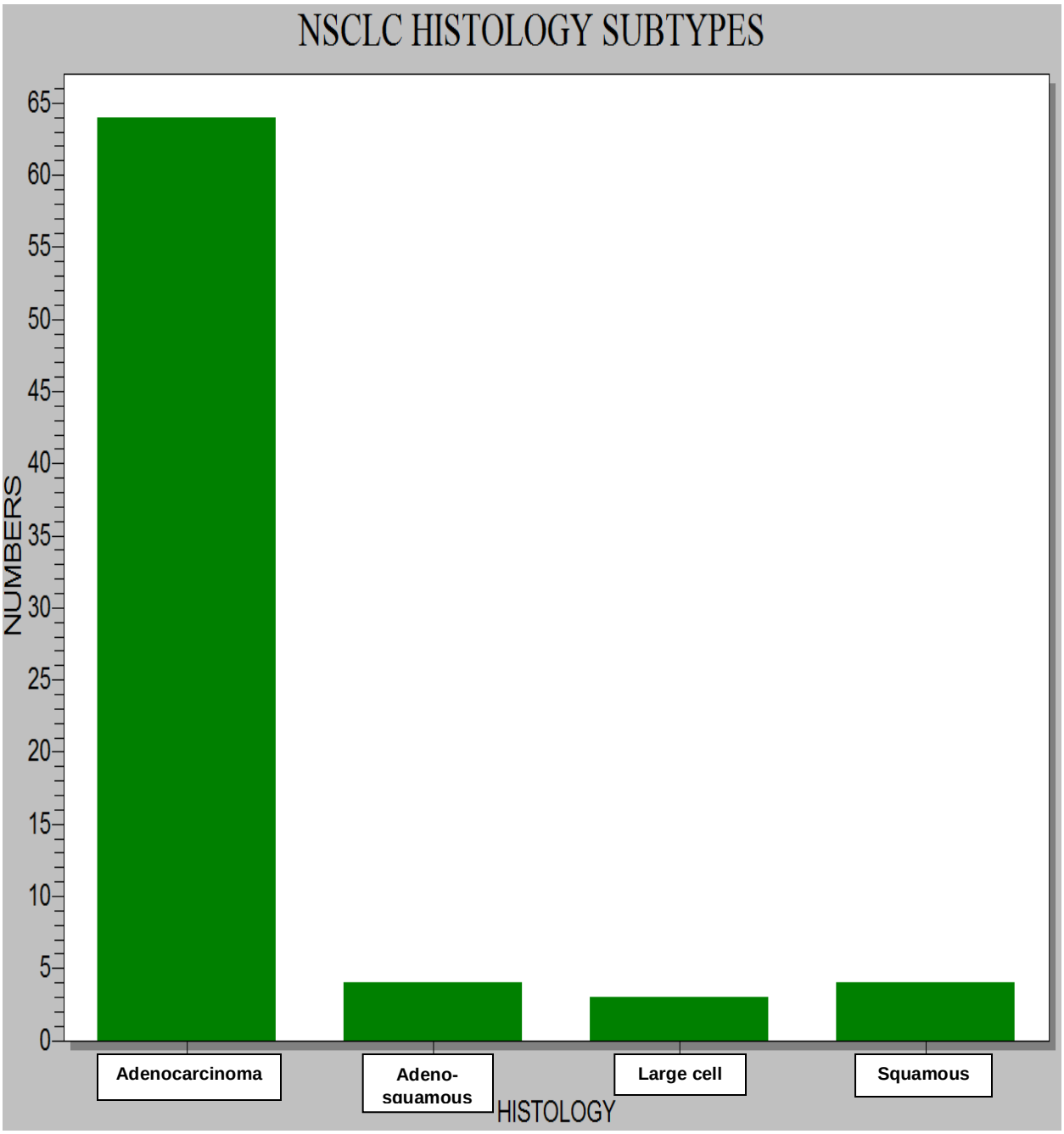

Figure 3.2 NSCLC histology submitted for EGFR mutation testing

Table 3.3 Smoking status of all patients who had EGFR mutation testing

Smoking status	Number (%)
Current smokers	22 (30%)
Former smokers	18 (25%)
Former light smokers	4 (5%)
Never smokers	29 (40%)

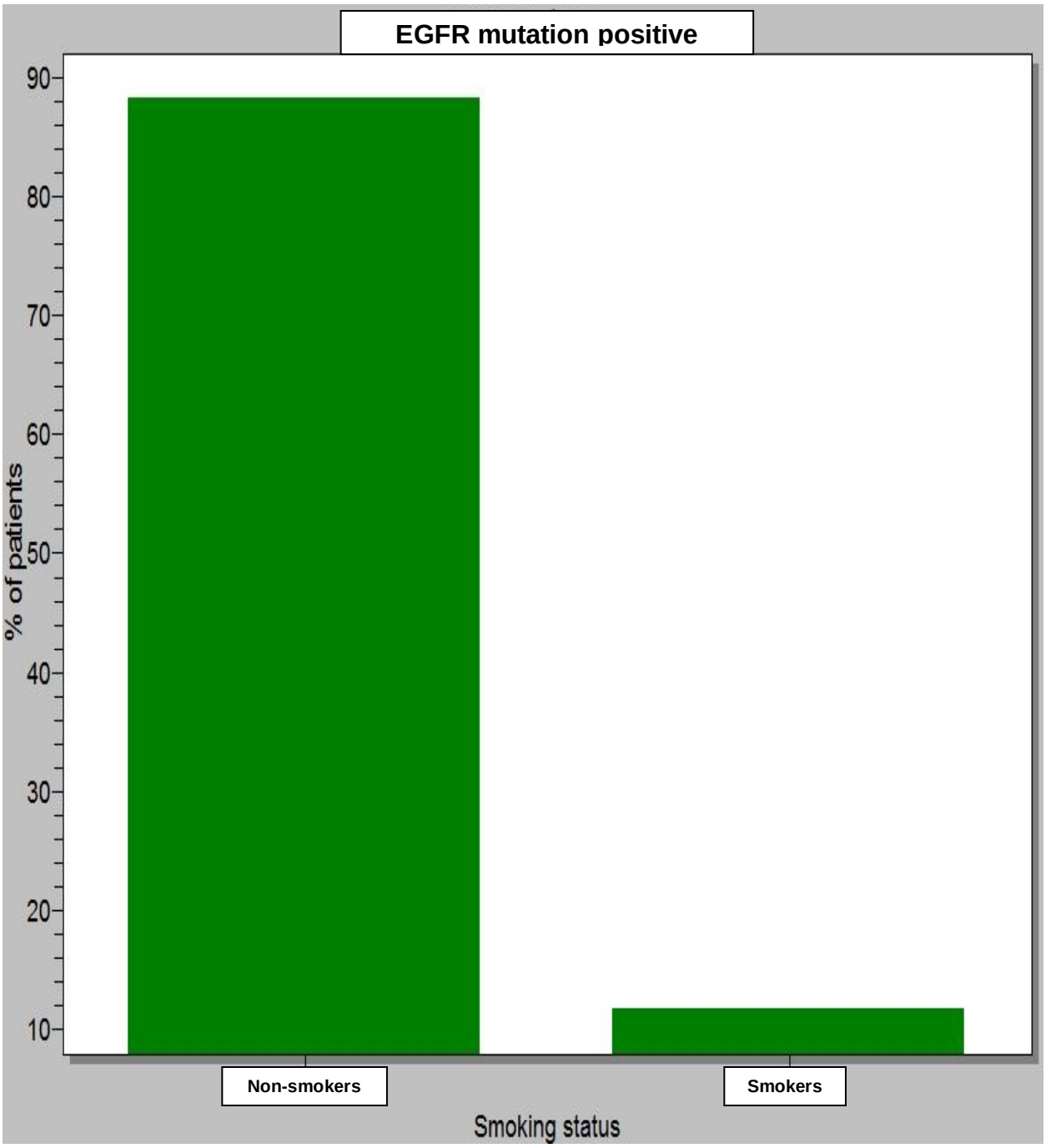

Figure 3.3 Smoking status and positive EGFR mutation status

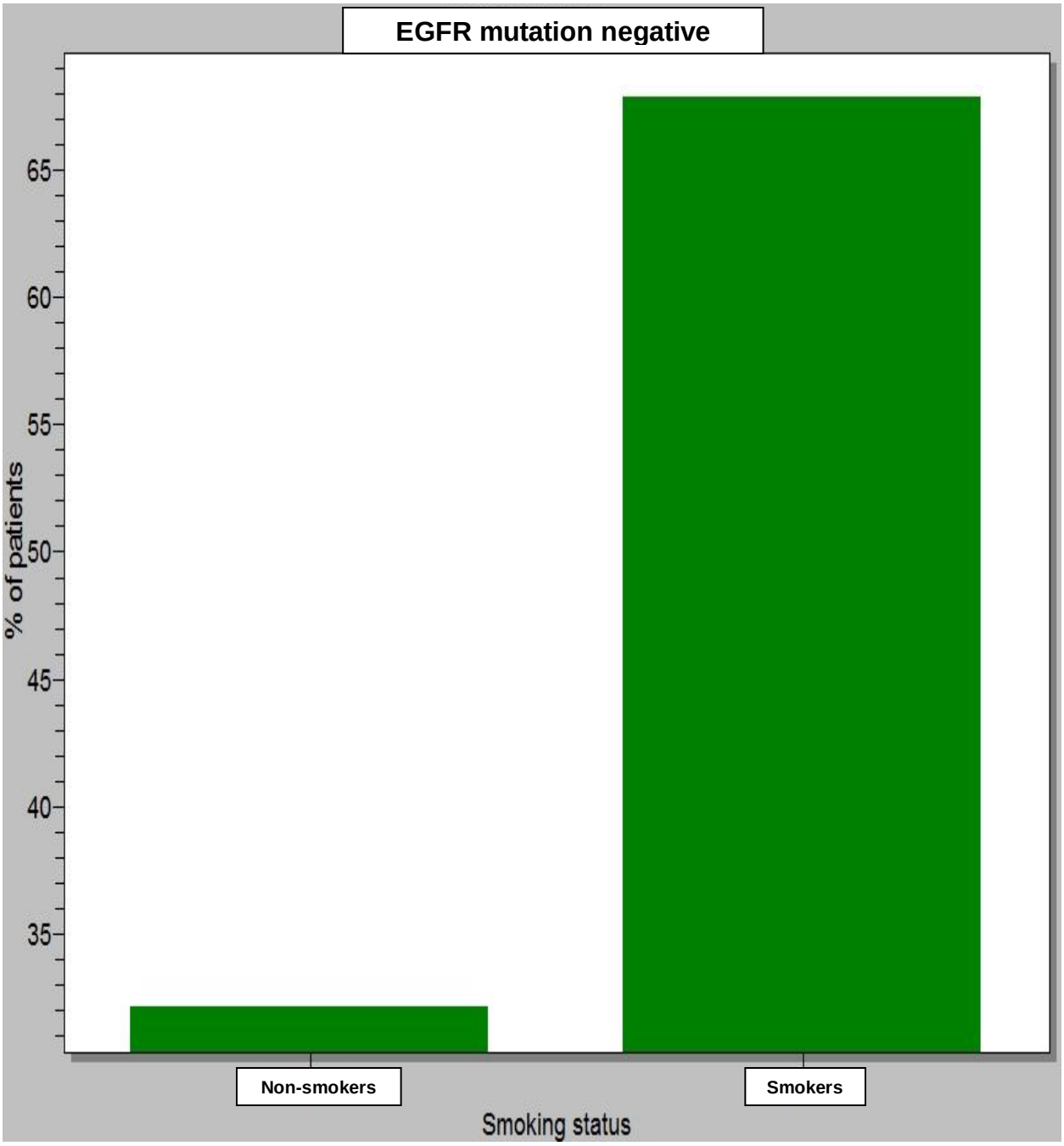

Figure 3.4 Smoking status and negative EGFR mutation status

3.4 First-line treatments received in NSCLC

68 patients were evaluable for first-line treatments. 68% of patients received systemic therapy consisting of either chemotherapy or tyrosine kinase inhibitors (Figure 3.5). This correlated well with the majority of patients (86%) with good ECOG performance status of 0 or 1, and that most patients had metastatic (stage IV) disease (48/74 patients, 65%).

The majority of patients received first-line platinum based doublet chemotherapy in the form of either cisplatin or carboplatin and one of vinorelbine, paclitaxel, gemcitabine or pemetrexed in descending order of frequency. Only 4 patients received first-line tyrosine kinase inhibitors, 2 with gefitinib and 2 with erlotinib.

10 patients had surgery for early stage disease where the lung cancers were resectable. 8 patients had radiotherapy or chemo-radiation for either palliative treatment first-line or for definitive treatment of earlier stage lung cancer where they were either irresectable on staging CT due to anatomy or the patients were medically unfit for surgery.

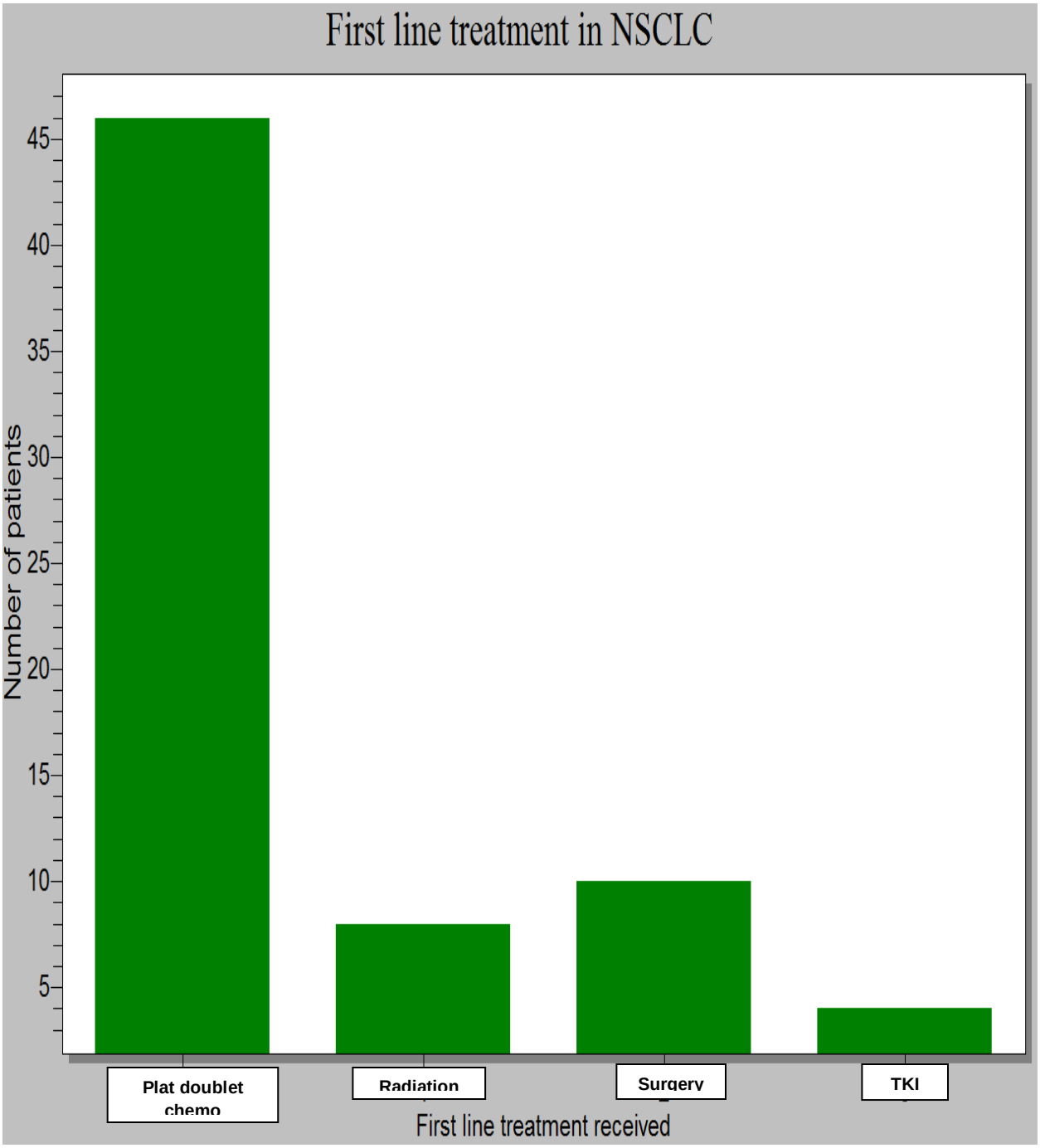

Figure 3.5 First-line treatments received in NSCLC

3.5 Response assessment post first-line systemic treatments

62 patients had RECIST 1.0 response documented post first-line treatment with systemic therapy. The response was summarized in Table 3.4.

The objective response rate (ORR) was 56% (partial response and complete response). 23% of patients had stable disease, and 21% patients had progressive disease. On chi-squared test, there were no statistically significant difference in response among various subgroups of patients (sex, race, histology, smoking status, chronic lung disease, and EGFR mutation status).

Table 3.4 RECIST 1.0 response assessment post first-line systemic therapy

RECIST 1.0 response	Number of patients (%)
Complete response (CR)	7 (11%)
Partial response (PR)	28 (45%)
Stable disease (SD)	14 (23%)
Progressive disease (PD)	13 (21%)

3.6 Second- and third-line treatments in NSCLC

Out of the 68 patients who had first-line treatments, 42 patients progressed and were fit for second-line treatments. 64% (n=27) were able to received systemic therapy (chemotherapy or TKI) second-line. The chemotherapy consisted of single agent chemotherapy (docetaxel, pemetrexed, vinorelbine or gemcitabine). 4 patients had TKI therapy (3 on erlotinib, 1 on gefitinib). 9 patients had palliative radiation therapy and 2 patients who had first-line / neoadjuvant chemotherapy and with subsequent good partial response went on to had surgery (Figure 3.6).

10 patients went on to receive third-line treatments. 5 patients had palliative single agent chemotherapy (docetaxel, gemcitabine or vinorelbine). 1 patient had palliative radiation, and 4 patients had TKI therapy (erlotinib).

3.7 Response assessment post second-line systemic treatments

Compared to post first-line treatment response, the ORR post second-line systemic (single agent chemotherapy or tyrosine kinase inhibitors) treatments had dropped to 24%. 30% patients had stable disease and 46% had progressive disease (Table 3.5).

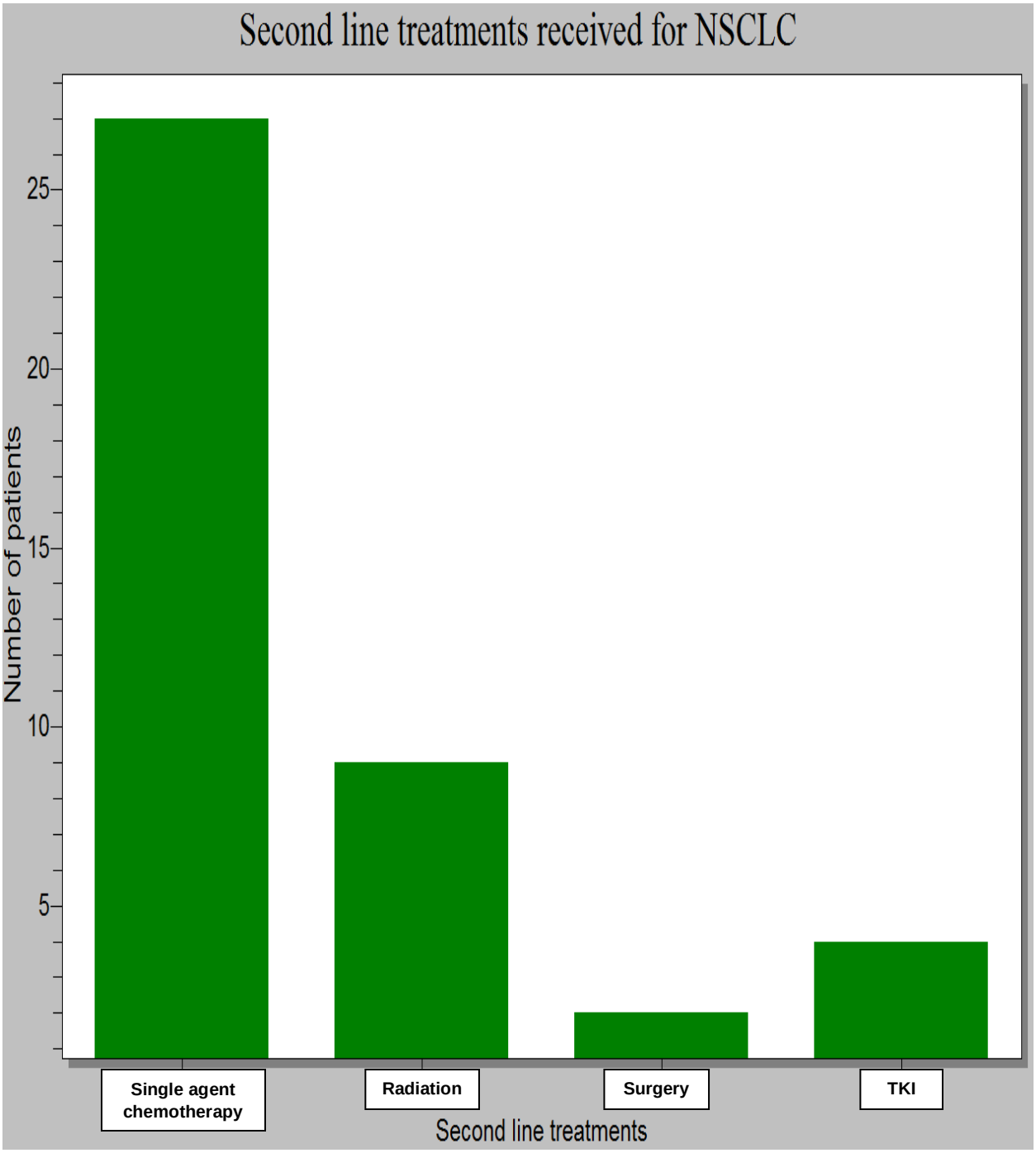

Figure 3.6 Second-line treatments received in NSCLC

Table 3.5 Response assessment post second-line systemic treatments

RECIST 1.0 response	Number of patients (%)
Complete response (CR)	1 (3%)
Partial response (PR)	8 (21%)
Stable disease (SD)	11 (30%)
Progressive disease (PD)	17 (46%)

3.8 Progression free survival

There were 57 patients in total that had progression status documented. 19 patients had not progressed (33.3%) and 38 patients had progressed (66.7%). Progression free survival between the EGFR mutation positive and negative groups of patients, for those that received systemic therapy (chemotherapy and TKI) was shown in Figure 3.7.

Median PFS was 6.85 months (range 2.2-11.1 months) for the EGFR mutation positive subgroup (n=8) and 6.8 months (range 1.9-39.1 months) for the EGFR mutation negative subgroup (n=30). There were no statistical difference in PFS between EGFR mutation positive and negative patients (HR 1.60; 95% CI 0.70-3.65; p=0.2543).

In the multivariate analysis of the subgroups of patients and PFS, there was no statistical difference demonstrated among various subgroups of patients and EGFR mutation status with progression free survival (Table 3.6).

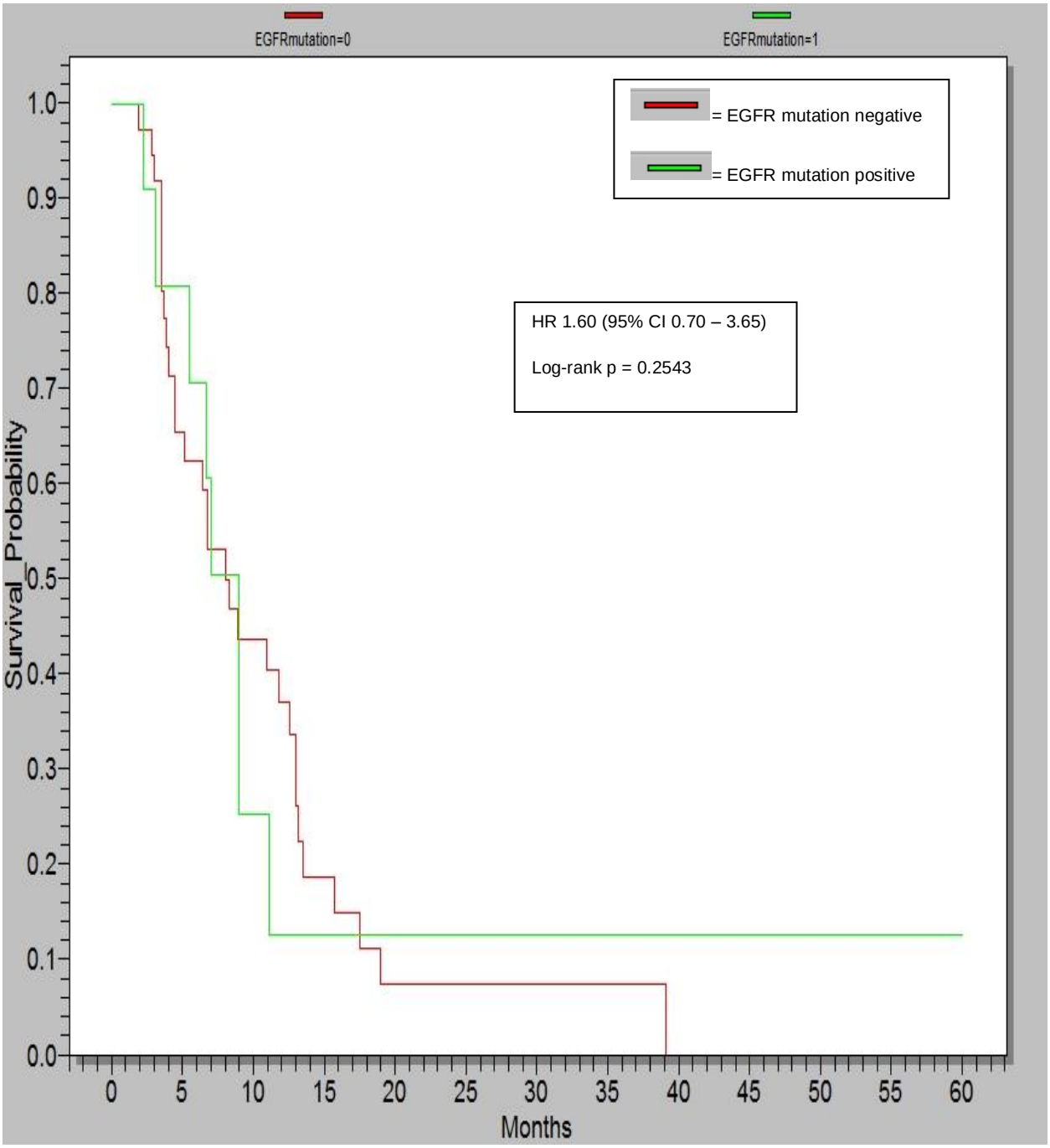

Figure 3.7 Progression Free Survival in EGFR mutation positive and negative groups

Table 3.6 Multivariate analysis of Progression Free Survival

Variable	Progression Free Survival	
	Hazard Ratio (95% CI)	p Value
Sex	0.63 (0.26 – 1.52)	0.30
Race	1.31 (0.49 – 3.45)	0.59
Smoking Status	1.03 (0.38 – 2.78)	0.95
Chronic lung disease	0.62 (0.22 – 1.71)	0.35

3.9 Overall Survival

During the period under review, 35 patients had documented deaths and were evaluable for overall survival. Overall Survival between the EGFR mutation positive and negative groups was shown in the Kaplan Meier curves (Figure 3.8). The median OS for the EGFR mutation positive subgroup (n=7) was 11.5 months (range 1.1-79.9 months), while the median OS for the EGFR mutation negative subgroup (n=28) was 12.9 months (range 1-65 months). There was no association between EGFR mutation status and OS (HR 0.70; 95% CI 0.28-1.75; p=0.44).

In the multivariate analysis of various subgroups, only race was statistically significant (Table 3.7).

In the EGFR mutation positive group of patients, the median OS of white race (n=3) was longer, 59.3 months (range 12.1-79.9 months) compared to 5.2 months (range 1.1-11.5 months) in the non-white group (n=4). The Kaplan Meier curve between race and OS in the EGFR mutation positive group was shown in Figure 3.9. Not being white was associated with marked decrease in OS in the EGFR positive group (HR 17.53; 95% CI 1.9-161.61; p=0.0115).

In the EGFR mutation negative group, the median OS of whites (n=22) was longer 13.75 months (range 1.1-65 months) compared to non-whites (n=6), 7.8 months (range 1.0-17.9 months). The Kaplan Meier curves were shown in Figure 3.10. However, a statistical significance had not been reached (HR 2.13; 95% CI 0.80-5.69; p=0.131).

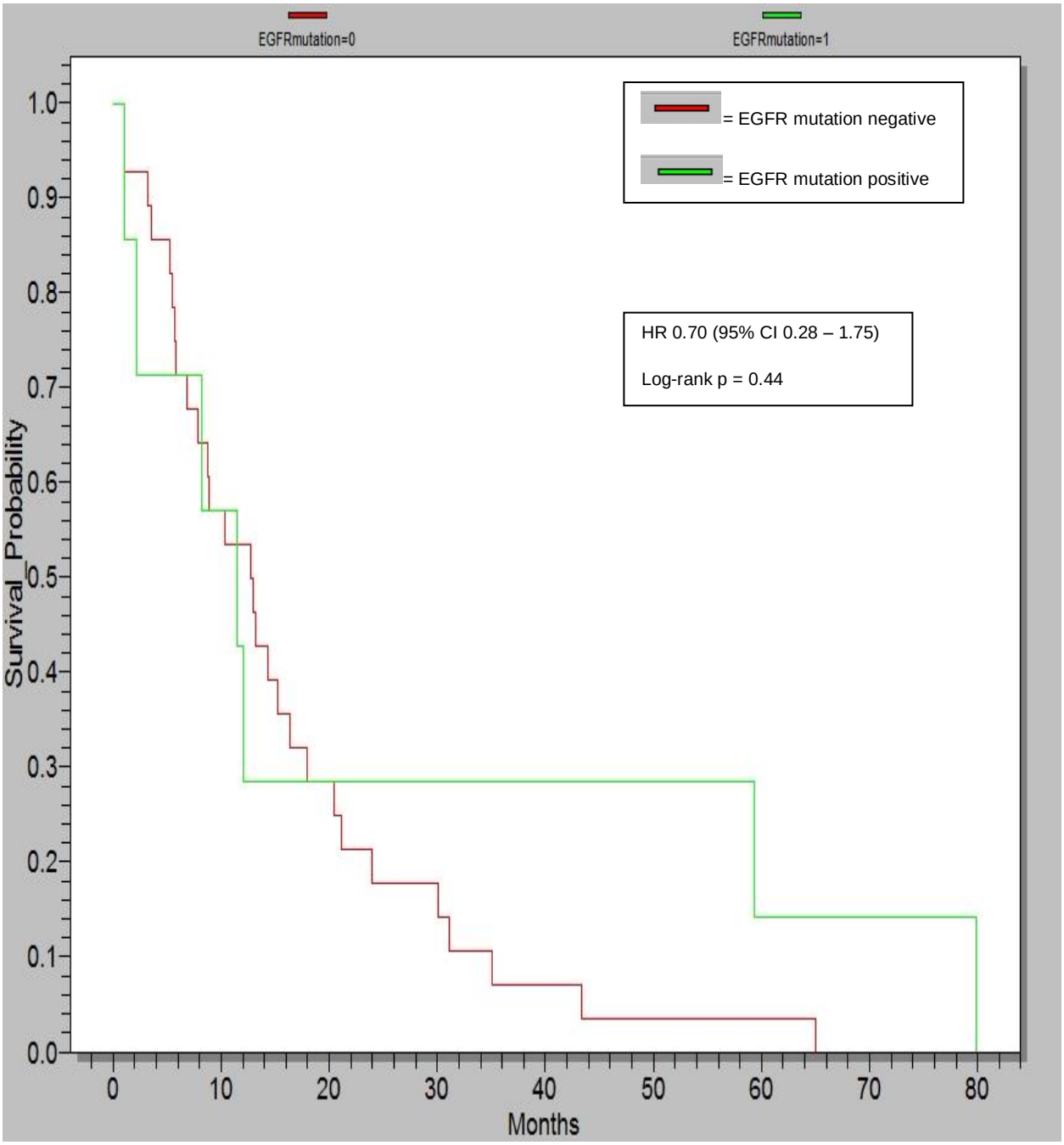

Figure 3.8 Overall survival and EGFR mutation positive and negative groups in NSCLC

Table 3.7 Multivariate analysis of Overall Survival

Variable	Overall Survival	
	Hazard Ratio (95% CI)	p Value
Sex	0.41 (0.13 – 1.25)	0.12
Race	6.66 (2.31 – 19.19)	0.0004
Smoking Status	1.57 (0.43 – 5.70)	0.50
Chronic lung disease	0.85 (0.26 – 2.84)	0.79

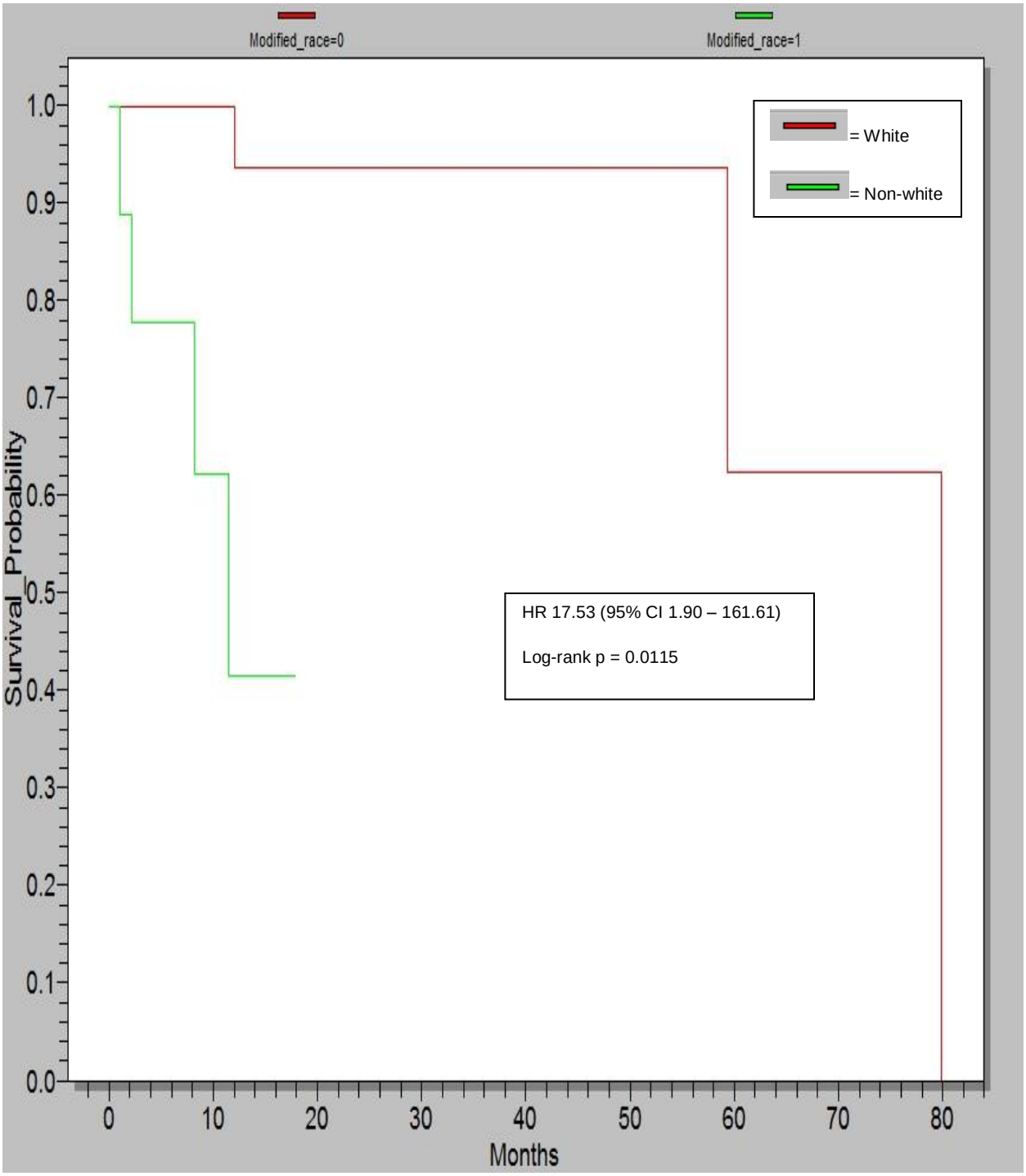

Figure 3.9 Overall Survival and Race in the EGFR mutation positive group

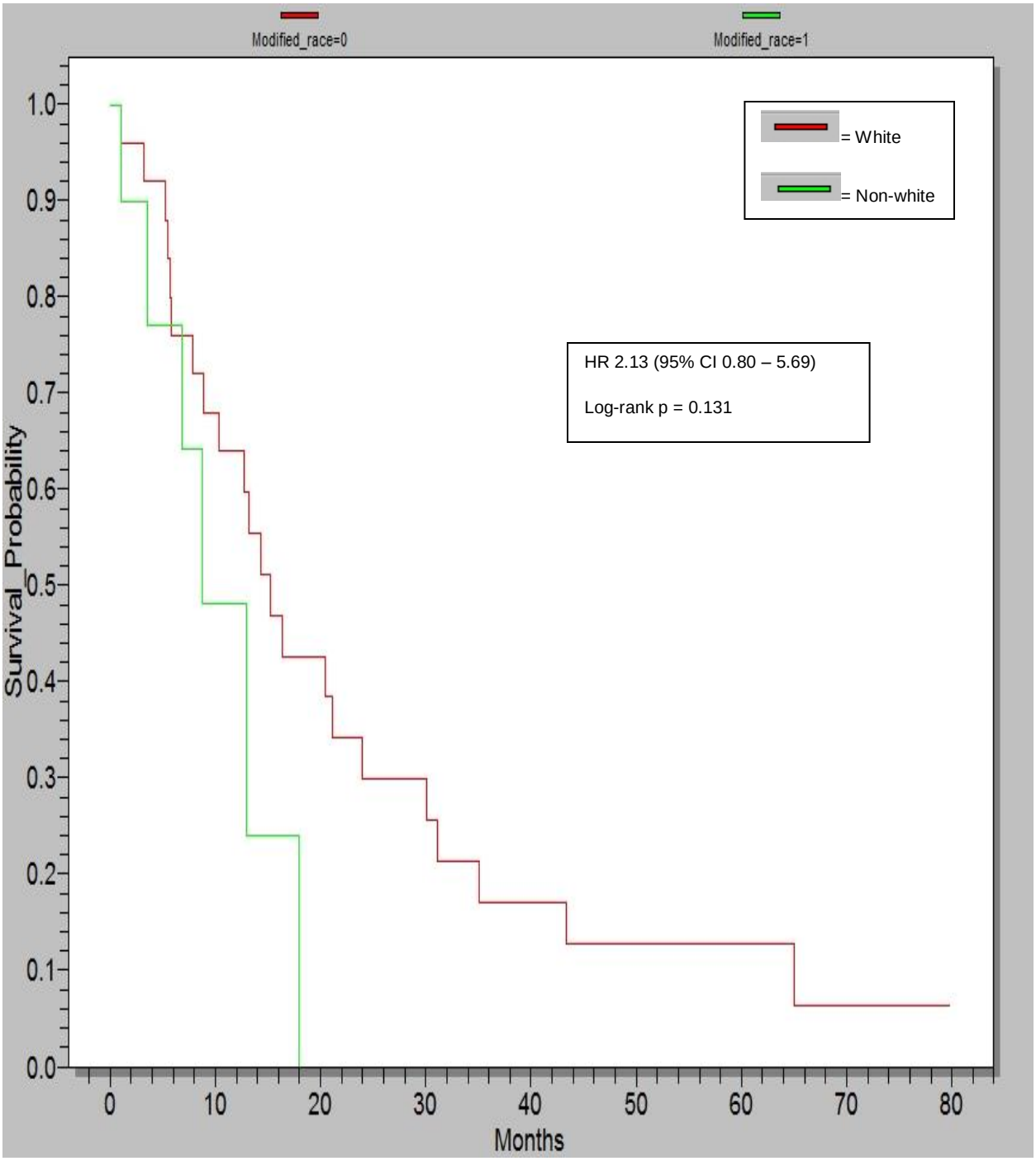

Figure 3.10 Overall Survival and Race in EGFR mutation negative group

4.0 DISCUSSION AND CONCLUSION

4.1 Comparison to current literature

The unique period under review, i.e. 1st September 2009 to 30th June 2012, when the Molecular Division of Lancet Laboratories was the only molecular laboratory offering EGFR mutation analysis in South Africa, enabled us to determine an estimated EGFR mutation rate in NSCLC of 21.8%. The selection of lung cancer samples for EGFR mutation testing was however enriched by patient pre-selection based on clinical parameters, and limited by funding and TKI treatment availability in both private and public settings. This mutation rate was similar to other western countries (22-28).

In the subgroup of black patients (n=31), the EGFR mutation rate was 22.6%. This was consistent with the other three international studies describing the EGFR mutation rate in NSCLC in African Americans, ranging from 11.9% to 31.3% (94-97).

Exon 19 deletions and L858R point mutations were the most common mutations in our study, representing 59% and 30% of all EGFR mutations, and this was similar to other international studies (17-19, 23). More than 80% of NSCLC biopsies sent for EGFR mutation testing were adenocarcinomas. This was the most common histology associated with activating EGFR mutations in NSCLC (14-15).

In 73 patients where smoking status was evaluable, 45% were never and former light smokers and 55% were current and former smokers. The higher proportion of never and former light smokers were unusual in the general lung cancer population where historically up to 80% of lung cancer patients were current or former smokers. However, practicing oncologists who were requesting EGFR mutation analysis for lung

cancer had already known that there was a higher probability of having positive EGFR mutation with never or former light smokers. Selection bias from the doctors requesting the EGFR mutation analysis was the most likely cause of a higher proportion of never and former light smokers in this study. This selection bias also influenced more females tested for EGFR mutation.

The inversely proportional relationship between smoking status and EGFR mutation status in this study was consistent with current literature (11,12, 23).

With the majority of patients (86%) having good ECOG performance status of 0 or 1, most patients were able to receive first-line platinum doublet chemotherapy (68%). Most achieved good response with 45% achieving partial response and 23% achieving stable disease. Response decreased with subsequent lines of treatments, which was expected from traditional chemotherapy treatments for NSCLC.

4.2 Challenges of lung cancer treatment in South Africa

Both PFS and OS were not influenced by EGFR mutation status. This was contrary to international studies like IPASS and EURTAC where PFS was improved with first-line TKI treatment in those harbouring activating EGFR mutations. However, few patients received TKI treatment first- and subsequent lines in our retrospective review, mostly because no first-line EGFR TKI (gefitinib or erlotinib) was registered in South Africa to date. Erlotinib for second- or third-line use, was registered in South Africa since April 2009, and was covered by most private medical schemes.

EGFR mutation testing and EGFR TKI's were not available in the government settings. The Iressa™ Donation Programme provided access to gefitinib for both the government

and private settings, but gefitinib was not registered in South Africa and access to gefitinib required approval via the Medicine Control Council under Section 21 of Act 101 of 1965. Therefore the majority of patients who had the activating EGFR mutation test came from the private medical facilities, which was represented by the majority of white patients in this study.

For the above reasons, despite a significant percentage of patients harbouring activating EGFR mutation, very few patients received EGFR TKI treatment in first- and subsequent lines. Therefore, PFS and OS were not different between the EGFR mutation positive and negative subgroups as most patients ended up receiving chemotherapy.

The only significant parameter associated with improved OS was being white. This was probably due to better supportive care as the majority of the white patients from this study came from those who were able to afford private medical care and hence reduce the delay in timely supportive treatment.

The 21.8% EGFR mutation rate should change how South African doctors approach NSCLC. Targeted therapy with EGFR TKI had been consistently shown to be of benefit in all lines of treatments for NSCLC harbouring activating EGFR mutations. Although no OS benefit was shown in international trials to date due to crossover effects of trial design, the beneficial effects associated with TKI and its marked improvement in PFS and response in NSCLC harbouring activating EGFR mutations had become important endpoints in many current lung cancer trial designs.

A targeted approach should be adopted from first-line therapy. Biopsy should be obtained for tissue and molecular diagnosis. Cytology is inadequate and often lead to inadequate quantitative requirements for molecular test. Of all the mutations associated with NSCLC, EGFR mutation remained the most important as the testing method was well established and the EGFR TKI treatments had shown benefits in all lines of treatments. The next molecular test of importance in NSCLC is EML4-ALK as the ALK inhibitor, crizotinib, was shown to have marked improvements in PFS and response in the second-line setting. As most NSCLC mutations are mutually exclusive, once a mutation is detected, the tumour is unlikely to harbour another mutation.

4.3 Limitations of this study

The collection of patient's clinical data was often incomplete due to the retrospective nature of the analysis. Assistance from some doctors throughout South Africa by telecommunication was limited due to their busy practices.

Due to the retrospective nature of the study, no controlled comparison between different treatment arms was possible in the positive and negative EGFR mutation subgroups. This can be overcome with a future prospective randomized controlled study.

The small numbers for PFS and OS in certain subgroups, i.e. specific EGFR mutation subtypes (Exon 19 Deletion vs L858R), renders the interpretation of results difficult.

Risk factors such as HIV status, past or present TB, asbestos or silica exposure were poorly documented making statistical correlation impossible. Correlation with these potential risk factors and EGFR mutational status may be explored further in future studies.

4.4 Future direction

Finally, as more patients were identified harbouring EGFR mutations and had EGFR TKI therapy, acquired resistance will emerge. T790M mutation will be the most common mechanism of resistance, accounting for approximately 60% of all resistance mechanisms, and afatinib may overcome this resistance (59, 61). The way to approach acquired resistance from TKI therapy and the treatment thereof will still need to be explored in future studies.

Lung cancer in never smokers (LCINS) is becoming more frequently diagnosed throughout the world. As outdoor air pollution is now a major health hazard, it will be interesting to explore whether air-born particulate matters are associated with EGFR mutations or other mutations in lung cancer.

APPENDIX A

DATA COLLECTION TABLE

Coding number for patients	1	2	3	4	5	6
Initials						
Age						
Gender						
Race (White / Black / Mixed Race / Indian / East Asian)						
Histology (Adeno / Squam / Large cell)						
EGFR activating mutation (+ / -)						
EGFR L858R						
EGFR exon 19 deletion						
EGFR mutation others						
Stage (TNM IASLC 7th edition)						
Performance status (ECOG)						
HIV status (+ / - / unknown)						
Current / Previous TB infection						
Chronic lung disease (Asthma, Chronic bronchitis, COPD, emphysema, idiopathic pulmonary fibrosis)						
Prior Radiation Treatment (Hodgkin's, Breast cancer etc.)						
Smoking History (Never / Former / Current / Former light)						
Alcohol History (significant = >2 units/day)						
Recreational Drugs						
Family History of Cancer						
Address (Province & Urban or Rural - Born & Grew up)						
Address (Province & Urban or Rural - Current)						
Occupation 1 (mining / farming / office / service / military / textile / blacksmith / manufacturing / car)						
Occupation 2						
Occupation 3						
Mine exposure (family vs worker / current residence or @ birth)						

Asbestos exposure (family vs worker / current residence or @ birth)						
Silica exposure (occupational)						
Indoor air pollution (coal / biomass / modern cooking fuel)						
Treatment (RT / Surg / Chemo / TKI) - 1st line						
Start date of treatment - 1st line						
End date of treatment - 1st line						
Response (CR / PR / SD / PD - RECIST) - post 1st line						
Treatment (RT / Surg / Chemo / TKI) - 2nd line						
Start date of treatment - 2nd line						
End date of treatment - 2nd line						
Response (CR / PR / SD / PD - RECIST) - post 2nd line						
Status (Alive / Dead / Lost to follow up)						
Date of death						

APPENDIX B

Permission from the Molecular Pathology Department of Lancet Laboratories, Johannesburg for the use of EGFR mutation results

JOHANNESBURG
Switchboard (011) 358 0800
Client Services (011) 358 0888
Po Box 8475, Johannesburg, 2000

PRETORIA
(012) 483 0100
(012) 483 0110

DURBAN
(031) 308 6500
(031) 308 6655

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PATHOLOGISTS
LANCET LABORATORIES
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25 May 2011

Wits Human Research Ethics Committee (Medical)

Dear Sir/ Madam

PERMISSION TO USE LABORATORY DATA IN RESEARCH REPORT FOR MMED DEGREE: DR SZE WAI CHAN

Dr Chan proposes to use laboratory data generated in the analysis of lung carcinomas for EGFR mutations. This data has been produced during diagnostic testing of patients referred from different regions of South Africa over a 20 month period, and going forward to the conclusion of her study.

Lancet Laboratories and the Molecular Pathology Department agree to release this data for the purposes of research. This data will include demographic data of the patient, the laboratory data (histology of tumour, EGFR mutation status), region from which sample was referred, where possible, treating physician.

Dr Chan proposes to contact physicians to expand on clinical, treatment and outcome data for the patients tested for EGFR mutations. For this, names of patients will be required to be released to Dr Chan. This is done on the understanding that no personal identification of patients will be possible in any research output (publication or presentation), without appropriate consent from the patient or representative.

Yours faithfully,

Dr Christopher Maske
MBChB BSc(Hons) DPhil FCPath
Head of Department, Molecular Pathology
Lancet Laboratories

APPENDIX C

Permission from the Division of Medical Oncology, Charlotte Maxeke Johannesburg Academic Hospital for record review

GAUTENG PROVINCIAL GOVERNMENT

DEPARTMENT OF HEALTH

Division of Medical Oncology
Area 495
Charlotte Maxeke Johannesburg
Academic Hospital
Private Bag X39
JOHANNESBURG
2000

Tel: (011) 488-3495/0
Fax: (011) 488-4515
E-mail: jhboncology@witshealth.co.za

30 May 2011

Wits Human Research Ethics Committee

RE: MMED RESEARCH REVIEW

Dr. SW Chan has the permission from Professor Paul Ruff to review the files of Lung Cancer patients with EGFR mutation testing performed at Lancet Laboratories.

The patient's names will be blinded for the study and for future publications.

Many thanks

PROFESSOR PAUL RUFF
PROFESSOR AND HEAD
DIVISION OF MEDICAL ONCOLOGY
DEPARTMENT OF MEDICINE

APPENDIX D

Permission from the CEO of Charlotte Maxeke Johannesburg Academic Hospital for record review

health and social development
Department: Health and Social Development
GAUTENG PROVINCE

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Office of the Chief Executive Officer
Charlotte Maxeke Johannesburg Academic Hospital
Tell: 011 488 3792
Fax: 011 488 3753
Email: lindiwe.mngomezulu@gauteng.gov.za
Date: 30th August 2011

Dr. Sze Wai Chan
Specialist Medical Oncology
CMJAH

Dear Dr. Chan

RE: "Epidermal growth factor receptor mutations in non small cell lung cancer patients in South Africa"

Please note that your above request is provisionally approved. Your study can only commence once ethics approval is obtained.

Yours sincerely

Dr. Barney Selebano
Chief Executive Officer

APPENDIX E

Permission from Wits Donald Gordon Medical Centre for patient's files review

Medical Oncology Unit,

Wits DGMC.

Ph: 011-3566900
Fax: 011-3566526

6th September 2011

Dr Sze Wai Chan,
Division of Medical Oncology,
University of Witwatersrand.

Dear Dr Chan,

We are happy to allow you to have access to patients' files with NSCLC for purposes of evaluation for your MMed Dissertation on EGFR-1 mutations in NSCLC.

Sincerely yours,

PROF PAUL RUFF
MEDICAL ONCOLOGY
WITS DONALD GORDON MEDICAL CENTRE
17 ETON ROAD
PARKTOWN 2193

APPENDIX F

University of Witwatersrand Human Research Ethics Committee (HREC) (Medical) Approval

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Sze Wai Chan

CLEARANCE CERTIFICATE	**M110651**
PROJECT	Epidermal Growth Factor Receptor Mutations in Non Small Cell Lung Cancer Patients in South Africa
INVESTIGATORS	Dr Sze Wai Chan.
DEPARTMENT	Department of Internal Medicine/Medical Oncology
DATE CONSIDERED	24/06/2011
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 24/06/2011 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof Paul Ruff

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

APPENDIX G

Presentations and abstract from 2011 SASCRO/SASMO Congress

Chan SW, Darby J, Maske C, Ruff P. EGFR mutations in NSCLC patients in South Africa [Abstract]. In: SASCRO SASMO Congress; 2011 August 24-27; Sun City, South Africa.

EGFR MUTATIONS IN NSCLC PATIENTS IN SOUTH AFRICA

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INTRODUCTION: Limited data is available in South Africa regarding molecular pathology in lung cancer patients. EGFR activating mutations are associated with improved outcomes in NSCLC patients being treated with EGFR tyrosine kinase inhibitors such as gefitinib and erlotinib. Exon 19 deletion and exon 21 point mutation (L858R) are the two most common mutations in the Far East and in Europe. This is a collection of data to date reporting on the pattern of EGFR mutations in South African NSCLC patients.

PATIENTS AND METHODS: The dataset comprises 100 consecutive samples submitted for EGFR mutation analysis in patients with non-small cell lung carcinoma. Samples included formalin-fixed paraffin embedded tissue from biopsy or resection of tumour tissue (96%) and cytology material (4%). DNA was extracted from the tissue using optimised protocols. EGFR mutation analysis was performed with the EGFR Mutation Screen kit (DXS Diagnostics, UK) by real-time PCR detection of mutations.

RESULTS: 100 samples were received over a 20 month period. Seven of these failed the quantitative parameters of the assay due to insufficient tumour tissue. 93 samples were successfully analysed with an EGFR mutation positive rate of 23.6%. The most common mutations were EGFR exon 19 deletions (54%) and L858R point mutation (27%). Other mutations were detected at lower frequency (G917X 9%; S768I 4.5%; exon 20 insertion 4.5%). Tumours analysed consisted of adenocarcinoma and large cell carcinoma (97.8%) and squamous cell carcinoma (2.2%). All mutations detected have been in adenocarcinoma/ large cell carcinoma. No squamous cell carcinomas to date have been positive for EGFR mutations (n=2). Average mean analysis of the mutation rate shows a plateau at 23%.

DISCUSSION AND CONCLUSIONS: Our data show that EGFR mutations occur at a rate of 23% in patients selected for EGFR mutation screening in NSCLC. The most frequent mutations are the exon 19 deletions and L858R point mutation, consistent with international data. Current methodology does not include T790M resistance mutation. Our data confirm the value of EGFR mutation screening in selected NSCLC patients for selection for TKI therapy.

APPENDIX H

Letter from Sanofi

27-01-2014
Dr SW Chan
Specialist Oncologist
JHB

Prestigious Top Medical Oncology Fellow Award 2011

To whom it may concern,

Sanofi sponsors the Medical Oncology Fellow top presentation award at the SASMO/SASCRO conference.

In 2011, Dr SW. Chan was awarded the best presentation by the organizing committee of SASMO.

Dr SW. Chan had the opportunity to choose an international oncology meeting and attended the ESMO conference in 2012, held in Vienna.

Sanofi takes this opportunity to wish Dr Chan all of the success in her career.

Best Regards,

Karuna Thord-Gray
Sanofi Oncology

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