Original Research Paper

Volume-8 | Issue-6 | June-2018 | PRINT ISSN No 2249-555X

Oncology

ESTIMATION OF PLASMA EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) MUTATION STATUS AND ITS CORRELATION WITH TUMOR TISSUE EGFR MUTATION STATUS IN ADVANCED NON-SMALL CELL LUNG CANCER PATIENTS

Praveena Voonna*	Department Of Medical Oncology, Sri Venkateswara Institute Of Medical Sciences (svims), TIRUPATHI – 517507 *Corresponding Author
T. Kannan	Department Of Medical Oncology, Svims, TIRUPATHI – 517507.
Bhargavi D	Department of Medical Oncology, SVIMS, TIRUPATHI – 517507.
P. V. G. K. Sarma	Department of Biotechnology, SVIMS, TIRUPATHI – 517507.
Shilpa Kandipalli	Department of Medical Oncology, SVIMS, TIRUPATHI – 517507.
P. Santosh	Department of Biotechnology, SVIMS, TIRUPATHI – 517507.

ABSTRACT

Background: Patients with non-small cell lung cancer (NSCLC) harboring activating EGFR mutations respond to treatment with EGFR tyrosine kinase inhibitors (TKIs). However, obtaining adequate tumor tissue from NSCLC patients can be difficult. The aim of this study was to determine whether tumor tissue EGFR analysis can be replaced with plasma EGFR analysis to assess mutation status and to evaluate the association of mutation status with survival outcomes.

Methods: We prospectively evaluated EGFR gene mutation status (exons 18,19,20 and 21) in paired tissue and plasma from 68 advanced NSCLC patients (before starting anticancer treatment) during the period, May 2016 to May 2017 using Real Time based Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) assay and Allele specific PCR techniques respectively. Concordance and discordance rates were assessed and survival analysis was done.

Results: Among 68 NSCLC patients, biopsy tissue was not sufficient for analysis in 6 patients, hence 62 patients were included in final analysis. EGFR mutations were identified in 14/62 (23%) biopsy samples and 25/62 (40%) plasma samples. Most common mutation identified in both tissue and plasma was exon 19 deletion. The overall concordance of EGFR mutation status between tissue and plasma reached 66% (41/62) (Kappa coefficient: 0.24; P=0.038). The sensitivity, specificity, positive and negative predictive values of plasma EGFR detection were 64%, 67%, 36% and 87% respectively. Among biopsy EGFR mutant patients, progression free survival (PFS) of plasma EGFR mutation detected patients was less compared to that of undetected patients.

Conclusion: EGFR gene mutation analysis of plasma is feasible with allele specific PCR assays with a high negative predictive value. It can be considered in frail patients not suitable for biopsy. But further investigation is required to determine whether plasma sample can be considered for determining EGFR mutation status in future.

KEYWORDS : EGFR (Epidermal growth factor receptor), non-small cell lung cancer, PCR.

INTRODUCTION

Non-small cell lung cancer (NSCLC) has a poor prognosis with one of the highest mortality rates among all cancers (1). Patients with NSCLC having epidermal growth factor receptor (EGFR) mutations like exon 19 deletion or exon 21 L858R mutation, respond to treatment with EGFR tyrosine kinase inhibitors (TKIs) (2,3). Large randomized phase III trials comparing EGFR TKIs like gefitinib, erlotinib or afatinib with cytotoxic chemotherapy have shown higher response rates and prolonged progression-free survival (PFS) with TKIs in EGFR mutant NSCLC patients (4-7). Thereafter, EGFR TKIs have become first-line therapy in such patients.

Obtaining adequate tumor tissue from NSCLC patients for molecular analysis can be difficult. First, tumor tissue obtained from biopsy procedure is often small, and the tumor cells in that may be too low to allow analysis. Second, the biopsy tissue may not be representative of the total burden of mutated cells, especially in patients with metastases. A third problem is that genetic changes may take place during the period between removal of the biopsy and start of TKI therapy, especially in patients receiving radiotherapy or chemotherapy (8). Fourth, getting tumor tissue is an invasive procedure, some patients may require a repeat procedure to identify resistance mutations like T790M, which may not be tolerable because of comorbid conditions like chronic obstructive pulmonary disease.

Because of these problems in getting tumor tissue, a lot of research has been going on to identify alternate sources of DNA to detect EGFR mutations. Plasma DNA might provide a noninvasive source of detecting these mutations. Allele-specific polymerase chain reaction (PCR) with Scorpion-amplification(9,10), High performance liquid chromatography (11), peptide nucleic acid (PNA)-mediated PCR clamping methods(12), BEAMing (13), digital droplet PCR(14,15), mass spectrophotometry genotyping(16), and next-generation sequencing(17) have been established methods for the detection of EGFR mutations in DNA isolated from plasma with comparable performance (18) and have shown correlation between mutation status in tumor tissue and plasma.

With this background, the present study is aimed to determine whether tumor tissue EGFR analysis can be replaced with plasma EGFR analysis to assess mutation status.

OBJECTIVES OF THE STUDY

Primary objectives:

1. To assess concordance and discordance rates between the tumor tissue and plasma EGFR mutation analysis.
2. To calculate sensitivity, specificity, positive and negative predictive values (PPV& NPV) of plasma EGFR mutation analysis.

Secondary objective:

1. To compare progression-free survival of tumor tissue EGFR mutant patients with or without EGFR mutations in plasma.

METHODS

Patients of advanced NSCLC attending Sri Venkateswara Institute of Medical Sciences, a tertiary care center in Andhra Pradesh from South India between May 2016 to May 2017 were included in the study.

Inclusion criteria:

- Stage III and IV NSCLC patients
- Biopsy or cell block proven adenocarcinoma, squamous cell carcinoma, large cell carcinoma and NOS
- Patients having ECOG PS ≤ 2

Exclusion criteria:

- Cytologically confirmed malignancy (in whom cell block has not been prepared)

- Small cell carcinoma lung
- Patients having ECOG PS ≥ 3
- Patients not willing to participate in this study

Study design:

A prospective study to evaluate the utility of plasma EGFR analysis in NSCLC patients. Newly diagnosed biopsy or cell block proven stage III and IV non-small cell lung cancer patients (Adeno carcinoma, Squamous cell carcinoma, Large cell carcinoma and NOS) were recruited to assess EGFR mutation status in tumor tissue and plasma samples using with Real-Time based Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS PCR) assay and allele-specific PCR respectively. Concordance and discordance rates between those two and sensitivity, specificity, positive predictive value and negative predictive values of plasma EGFR detection were calculated. Patients were treated according to standard treatment guidelines based on stage. All recruited subjects were followed up at every subsequent 3 – 4 weeks for a minimum period of 6 months. Chest x-ray was performed at every visit to check for obvious clinical progression and detail imaging with CT or PET-CT was done only when clinical progression was in doubt. Disease progression was assessed using RECIST criteria version 1.1 with appropriate imaging measures (CT chest/Abdomen, PET-CT). PFS (Progression free survival) was defined as the duration from the commencement of first-line anticancer therapy to the day with clinical disease progression (noted on chest imaging or physical examination) or death. PFS rates for patients with detectable and undetectable plasma EGFR mutations were compared.

Tissue EGFR analysis has been taken as the **gold standard** in calculating the sensitivity of plasma EGFR analysis.

- **True-positives** were patients with positive tissue and plasma EGFR mutation.
- **True negatives** were patients with negative tissue and plasma EGFR mutation.
- **False positive cases** were those with positive plasma and negative tissue EGFR mutation.
- **False negative cases** were those with positive tissue and negative plasma EGFR mutation.

Methods of analyzing EGFR gene:

- Mutation analyses of EGFR gene on tumor tissues were done with ARMS PCR assay on FFPE (Formalin Fixed Paraffin Embedded Blocks)
- **Mutational analysis of EGFR gene extracted from patient's blood:**

Four to five ml of patient's blood was collected in EDTA bottle. Genomic DNA was extracted from human blood. Extracted DNA was analyzed using PCR. PCR was used to amplify the exons 18, 19, 20 and 21 comprising the EGFR gene using DNA.

Figure 1: Primer pairs used for analysis

The 50 µl PCR reaction mixture contains 100 pM of each primer, 100 µM of dNTPS mix, 10 mM Tris-HCl (pH 8.8), 1.5 mM MgCl2, 1 U of hot start Taq DNA Polymerase (Merck Biosciences pvt Ltd) and 0.5 µg of chromosomal DNA.

Amplification parameters include an initial denaturation step for 5 min at 94°C; 35 cycles of 94°C for 40 seconds of denaturation, 60 seconds of annealing at 62°C and 50 seconds of amplification at 72°C which will be followed by a final extension step at 72°C for 5 min in a Mastercycler gradient Thermocycler (Eppendorf). The amplified products were analyzed on 1.8% agarose gel electrophoresis.

Statistical Analysis:

Data were recorded on a pre-designed proforma using Microsoft excel spread sheet. Statistical analysis was done using Statistical Package for Social Sciences (SPSS) software version 20. For continuous variables mean± SD was calculated. Categorical data were expressed in percentages. The relationship between EGFR mutation and various parameters like sex, age, stage, histologic type and smoking was assessed using chi-square test or Fisher exact test. Concordance rates were assessed with concordance coefficient test. Sensitivity, specificity, and positive and negative predictive values of plasma EGFR detection were calculated using Medcalc online software. PFS was calculated and compared using Kaplan – Meier survival analysis. P value <0.05 was considered significant.

RESULTS

A total of 80 NSCLC patients attended department of medical oncology during the study period, out of which 68 patients met the inclusion criteria. Biopsy tissue was not sufficient for mutation analysis in 6 patients, hence 62 patients were included in final analysis (Figure 2).

Figure 2: Consort Flow Diagram

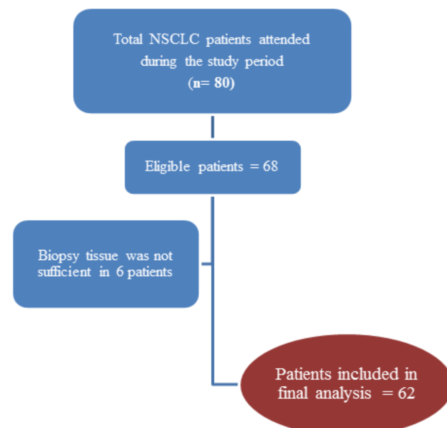
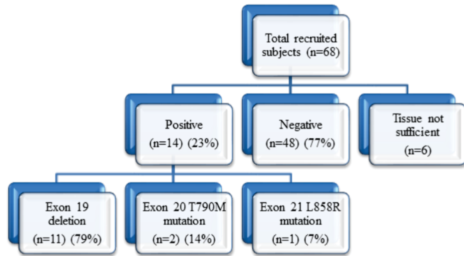


Table 1: Summary of clinical characteristics of all subjects

Characteristic	Value (Percentage)
Total number of patients, n	62
Median Age, years ± SD	56.5 ± 11.3
Age distribution	
31-50	20 (32%)
51-70	34 (55%)
71-90	8 (13%)
Sex	
M	46 (74%)
F	16 (26%)
Smoking History	
Current smoker	33 (53%)
Former smoker	5 (8%)
Never smoker	24 (39%)
Among smokers - No of pack years	
10-50	29(76%)
51-100	7 (18%)
100-150	2 (5%)
Stage	
IIIA	3 (5%)
IIIB	3 (5%)
IV	56 (90%)
Histology	
Adeno carcinoma	50 (81%)
Squamous Cell carcinoma	7 (11%)
NSCLC (NOS)	5 (8%)
Site of biopsy	
Primary Tumour	49 (79%)
Node	6 (10%)
Pleural fluid & nodule	3 (4.8%)
Paravertebral mass	1(1.6%)
Brain mets	2 (3%)
Bone mets	1 (1.6%)

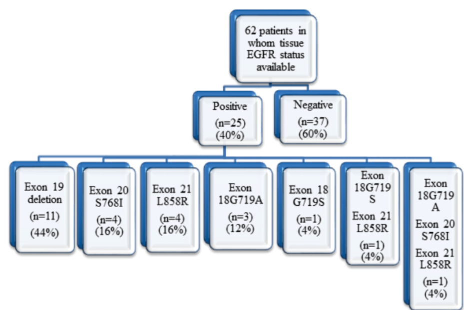
Majority of patients (55%) were between 51-70 years of age. Median age at presentation was 56.5 ± 11.3 years (Median ± SD). Majority (74%) were males; 26% were females. Male: Female ratio was 2.9:1. Thirty eight (61%) were smokers (53% current and 8% former smokers), 24(39%) were never smokers. Among smokers, majority were between 10-50 pack years. Primary tumor (79%) was the most common site of biopsy followed by node (10%). Stage IV (90%) constitutes predominant stage. Predominant histology was Adeno carcinoma (81%). (Table 1)

Figure 3: Results of tumor tissue EGFR mutation analysis



PCR was done on blocks of 62 patients in which 23% (14/62) showed positive result for EGFR mutation. Most common type of mutation identified was Exon 19 deletion (79%) (11/14).

Figure 4: Results of plasma EGFR mutation analysis

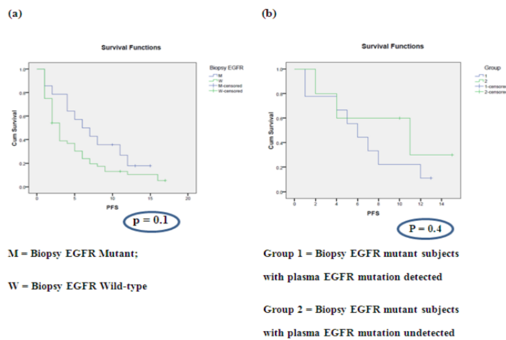


Forty percent (25/62) were positive for EGFR mutation. Most common type of mutation identified was Exon 19 deletion (44%).

Survival Analysis:

PFS of tumor tissue EGFR mutant subjects was 6 months (95% CI; 2.3 to 9.6 months) compared to 3 months (95% CI; 2.2 to 3.7 months) for tissue EGFR wild-type patients, but the difference was not statistically significant (P = 0.1). Among tumor tissue EGFR mutant patients, PFS of plasma mutation detected versus undetected was 6 (95% CI; 3 to 9 months) and 11 months (95% CI; 0.08 to 22 months) respectively, but the difference was not statistically significant (P=0.4).

Figure 5: Kaplan-Meier curves showing (a) PFS of Biopsy EGFR mutant versus wild-type subjects and (b) PFS of Biopsy EGFR mutant subjects with plasma EGFR mutation detected versus undetected



DISCUSSION

This prospective observational study was aimed to evaluate plasma as a surrogate sample for EGFR mutational analysis. It is one of the very few Indian studies of this type.

In the present study, total 62 advanced NSCLC patients were included for final analysis. Median age at presentation was 56.5 years which is comparable to other Indian studies which reported median ages of 58,56 and 60 years in the studies done by Stalin et al (19), Krishna Murthy et al (20) and Dey et al (21) respectively. It is lower compared to studies done by David et al and Kimura et al which reported median age of 64 years at presentation. Nine percent (6/68) patients belong to young age (≤ 40 years) in the present study. Majority (74%) were males. Male to female ratio was 2.9:1 which is lower, compared to the study by Dey et al (21) in which ratio was 4.14:1.

Sixty one percent (38/62) patients were smokers, whereas 39% were never-smokers. Smoker to never-smoker ratio was 1.6:1. Out of 38 smokers, 33(53%) and 5(8%) were current and former smokers respectively. Number of pack-years was between 10 to 50 for the majority of smokers (76%). Adenocarcinoma was the most common histology accounting for 81% of all cases, which is higher, compared to the studies by Krishnamurthy et al (20) and Dey et al. (21) in which adenocarcinoma was seen in 49% and 31% patients, respectively. Squamous cell carcinoma was seen in only 11% of patients which is lower compared to reports by Stalin et al (19), Krishna Murthy et al (20) and Dey et al (21). In 8% of patients, NSCLC subtype could not be established.

Source of tumor tissue was primary tumor in 49 (79%) patients followed by nodal mass in 10% and other metastatic sites in remaining cases. In the present study, majority (90%) were stage IV followed by IIIA (5%) and IIIB (5%). Comparison of clinical characteristics with other Indian studies is tabulated in Table 2.

Table 2: Comparison of clinicopathologic characteristics with other Indian studies

	Stalin et al[19]	Krishna Murthy et al[20]	Dey et al[21]	Present study
Median age (years)	58	56	60	56.5
Male:Female	2.53:1	3.5:1	4.14:1	2.9:1
Smokers:Non-smokers	1:1	1.52:1	2.7:1	1.6:1
Adeno carcinoma (%)	70.9	49	30.81	81
Squamous cell carcinoma (%)	18.7	18.3	35.09	11

Among 62 patients, tissue EGFR mutations were identified in 14 (23%) patients which is consistent with Indian data (22) and higher than that of Caucasians whereas plasma EGFR mutations were detected in 25 (40%) patients.

Most common mutation identified in both tissue (11/14) (79%) and plasma (11/25) (44%) was exon19 deletion. T790M mutations are commonly observed in cases of acquired resistance to TKIs (23-25), but in the present study upfront T790M mutations were identified in tissue analysis of 2 patients. One patient had double mutation (Exon18 G719S and Exon 21L858R) and 1 had triple mutation (Exon18 G719S, Exon20 S768I and Exon21L858R) in plasma. The reason for this might be tumor heterogeneity in which different clones of tumor cells bearing different types of mutations might exist in a single tumor or in the primary tumor and metastatic sites. This might have caused leakage of tumor cells containing different mutations into circulation.(26)

In the present study, EGFR mutation was more frequent in patients with age ≤ 60 years, females and never-smokers, and the correlation between mutation status and smoking was statistically significant (P=0.01; P=0.03 for tissue and plasma respectively).Tissue EGFR mutation frequency was 10% and 42% among smokers and nonsmokers respectively, whereas plasma EGFR mutation frequency was 29% versus 58%. Among patients with adeno carcinoma, 26% were positive for tissue EGFR mutation, whereas all squamous cell carcinoma patients were negative. (Table 3)

Table 3: Characteristics of EGFR mutant subjects compared with EGFR wild-type subjects

Characteristic	Tissue EGFR		P	Plasma EGFR		P
	Mutant	Wild		Mutant	Wild	
Total, n	14	48		25	37	
Age			0.75			0.6

≤ 60	10 (24%)	31 (76%)		18 (44%)	23 (56%)	
> 60	4 (19%)	17 (81%)		7 (33%)	14 (67%)	
Sex			0.16			0.7
M:F ratio	1.3:1	3.8:1		2.6:1	3.1:1	
M	8 (17%)	38 (83%)		18 (39%)	28 (61%)	
F	6 (38%)	10 (62%)		7 (44%)	9 (56%)	
Smoking History						
SM:NS			0.01			0.03
Smokers	4 (10%)	34 (90%)		11(29%)	27(71%)	
Nonsmokers	10 (42%)	14 (58%)		14(58%)	10(42%)	
Stage			0.38			0.6
IIIA	0 (0%)	3 (100%)		1 (33%)	2 (67%)	
IIIB	0 (0%)	3 (100%)		2 (67%)	1 (33%)	
IV	14 (25%)	42 (75%)		22 (39%)	34 (61%)	
Histology			0.3			0.9
Adeno carcinoma	13 (26%)	37 (74%)		20 (40%)	30 (60%)	
Squamous cell carcinoma	0 (0%)	7 (100%)		3 (43%)	4 (57%)	
NSCLC (NOS)	1 (20%)	4 (80%)		2 (40%)	3 (60%)	

Table 4: Rate of EGFR mutations detected in Plasma and Tissue samplesTable 4: Rate of EGFR mutations detected in Plasma and Tissue samples

	Tissue EGFR Negative	Tissue EGFR Positive	Total
Plasma EGFR Negative	32	5	37
Plasma EGFR Positive	16	9	25
Total	48	14	62

Overall Concordance rate = 66%
Cohen's Kappa Coefficient = 0.24; P=0.038

Table 5: Diagnostic Sensitivity, Specificity, PPV and NPV of Plasma EGFR analysis

	Estimated value	95% CI
Sensitivity	64%	35% to 87%
Specificity	67%	52% to 80%
Positive Predictive value(PPV)	36%	24% to 50%
Negative Predictive value(NPV)	87%	75% to 93%

Results of tissue and plasma EGFR mutation analyses were concordant in 41 (66%) patients, discordant in 21 (34%). Depending on the technique, the concordance rates varied between 64% to 92% in various studies. (27 – 36) (Table 6)

Table 6: Comparison of concordance rates of present study with other studies

	Assess study[33] (n=1162)	IPASS study [28] (n = 86)	Hu C et al[29] (n=47)	Wang y et al[34] (n = 287)	Weber et al[30] (n = 199)	Chai X et al[35] (n = 61)	Present study (n = 62)
Concordance rates	89%	66%	92%	64%	91%	75%	66%

Nine patients had mutations in both tumor tissue and plasma, whereas 32 patients did not have mutations in both. Five patients had mutations in tumor tissue, but not in plasma. EGFR mutations were identified in plasma of 16 patients without a corresponding mutation in tumor tissue reflecting high false positive rate in the present study. Hence, PPV is low (36%) compared to other studies (Table 7). Because of low false negative rate, NPV is high i.e. 87%. Sensitivity and specificity are 64% and 67% respectively.

Table 7: Comparison of sensitivity, specificity, PPV, and NPV of present study with other studies

	IPASS study[28] (n = 86)	Duan H et al[32] (n = 94)	David C.L.et al[31] (n = 74)	IGNITE trial Asian Pacific patients[36](n=1,687)	IGNITE trial Russian patients[36] (n=894)	Present study (n = 62)
Sensitivity	43%	50%	79%	50%	30%	64%
Specificity	100%	100%	97%	97%	93%	67%
PPV	100%	100%	97%	92%	39%	36%
NPV	55%	75%	77%	73%	91%	87%

A high number of false positives in the present study might be explained by technical errors or tumor heterogeneity resulting in leakage of tumor cells containing EGFR mutations into circulation, while the area from which biopsy was obtained might not have contained mutations.(26)

Median PFS of the total 62 patients was 3 months (95% CI ;1.5 – 4.5). Median follow-up duration ± SD (range) was 3 ± 4.9 (1 to 17) months. PFS of biopsy mutant patients was 6 months which is longer compared to PFS of 3 months for biopsy wild-type patients, but the difference was not statistically significant (P = 0.1). Among biopsy EGFR mutant patients, we compared PFS of plasma mutation detected patients with plasma undetected patients and Kaplan-Meier analysis showed better PFS for plasma undetected patients (11 versus 6 months), but the difference was not statistically significant (P =0.4).This reflects that plasma mutation detection might be an indirect signal of high tumor burden with high activity of tumor causing leakage of tumor DNA into systemic circulation thus conferring a worse prognosis.(37) It is possible that the better PFS observed in plasma undetected patients might indicate less active tumor causing less leakage of tumor cells into circulation, but this needs to be validated in large prospective randomized studies. Similar survival results have been observed in the study done by David C.L.Lam et al. (31)

CONCLUSIONS

- EGFR gene mutation analysis of plasma is feasible with allele-specific PCR assays with a high negative predictive value.
- It can be considered in frail patients not suitable for biopsy. But further studies are required to determine whether plasma sample can be considered for determining EGFR mutation status in future.

Limitations of the study:

- The drawback of the present study is small sample size. Further studies with a larger patient population might be necessary to validate these findings.
- Samples are not well matched.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61: 69- 90.
2. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004; 350: 2129-2139.
3. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science. 2004; 304: 1497-1500.
4. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang JJ, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009; 361: 947-957.
5. Han JY, Park K, Kim SW, Lee DH, Kim HY, Kim HT, Ahn MJ, Yun T, Ahn JS, Suh C, Lee JS, Yoon SJ, Han JH, et al. First-SIGNAL: first-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. J Clin Oncol. 2012; 30: 1122- 1128.
6. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, Zhang S, Wang J, Zhou S, Ren S, Lu S, Zhang L, Hu C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol. 2011; 12: 735-742.
7. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, Li W, Hou M, Shi JH, Lee KY, Xu CR, Massey D, Kim M, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. Lancet Oncol. 2014; 15: 213-222.
8. Bai H, Wang Z, Chen K, Zhao J, Lee JJ, Wang S, Zhou Q, Zhuo M, Mao L, An T, Duan J, Yang L, Wu M, Liang Z, Wang Y, Kang X, Wang J: Influence of chemotherapy on EGFR mutation status among patients with non-small-cell lung cancer. J Clin Oncol. 2012, 30 (25): 3077-3083. 10.1200/JCO.2011.39.3744.
9. Abedi-Ardekani B, Dar NA, Mir MM, et al. Epidermal growth factor receptor (EGFR) mutations and expression in squamous cell carcinoma of the esophagus in central Asia.

BMC Cancer 2012; 12:602.

10. Kuang Y,RogersA,YeapBY, et al.Noninvasive detection ofEGFRT790Min gefitinib or erlotinib resistant nonesmall-cell lung cancer. Clin Cancer Res 2009; 15:2630-6.
11. Bai H, Mao L, Wang HS, et al. Epidermal growth factor receptor mutations in plasma DNA samples predict tumor response in Chinese patients with stages IIIB to IV nonesmall-cell lung cancer. J Clin Oncol 2009; 27:2653-9. .
12. Kim HR, Lee SY, Hyun DS, et al. Detection of EGFR mutations in circulating free DNA by PNA-mediated PCR clamping. J Exp Clin Cancer Res 2013; 32:50.
13. Taniguchi K, Uchida J, Nishino K, et al. Quantitative detection of EGFR mutations in circulating tumor DNA derived from lung adenocarcinomas. Clin Cancer Res 2011; 17:7808-15.
14. Oxnard GR, Paweletz CP, Kuang Y, et al. Noninvasive detection of response and resistance in EGFR-mutant lung cancer using quantitative next-generation genotyping of cell-free plasma DNA. Clin Cancer Res 2014; 20:1698-705.
15. Yung TK, Chan KC, Mok TS, Tong J, To KF, Lo YM. Single-molecule detection of epidermal growth factor receptor mutations in plasma by microfluidics digital PCR in nonesmall-cell lung cancer patients. Clin Cancer Res 2009; 15:2076-84.
16. Brevet M, Johnson ML, Azzoli CG, Ladanyi M. Detection of EGFR mutations in plasma DNA from lung cancer patients by mass spectrometry genotyping is predictive of tumor EGFR status and response to EGFR inhibitors. Lung Cancer 2011; 73:96-102.
17. Kukita Y, Uchida J, Oba S, et al. Quantitative identification of mutant alleles derived from lung cancer in plasma cell-free DNA via anomaly detection using deep sequencing data. PLoS One 2013; 8:e81468.
18. Liu X, Lu Y, Zhu G, et al. The diagnostic accuracy of pleural effusion and plasma samples versus tumour tissue for detection of EGFR mutation in patients with advanced nonsmall-cell lung cancer: comparison of methodologies. J Clin Pathol 2013; 66:1065-9.
19. Stalin B, Sadashivudu G et al. Clinicopathological features and outcomes in advanced nonsmall cell lung cancer with tailored therapy. IJMPO 2016 ;37: 4; 242-250.
20. Krishnamurthy A, Vijayalakshmi R, Gadigi V, Ranganathan R, Sagar TG. The relevance of "Nonsmoking- associated lung cancer" in India: A single-centre experience. Indian J Cancer 2012;49:82-8.
21. Dey A, Biswas D, Saha SK, Kundu S, Sengupta A. A comparison study of clinicoradiological profile of primary lung cancer cases: An Eastern India experience. Indian J Cancer 2012; 49: 89-95
22. Choughule A, Noronha V, Joshi A, et al. Epidermal growth factor receptor mutation subtypes and geographical distribution among Indian non–small cell lung cancer patients. Indian J Cancer2013 Apr-Jun;50(2):107-11. doi: 10.4103/0019-509X.117023.
23. Riely GJ, Yu HA. EGFR: the paradigm of an oncogene-driven lung cancer. Clin Cancer Res 2015;21:2221-2226. Available at:http://www.ncbi.nlm. nih.gov/ pubmed/ 25979928.
24. Yu HA, Arcila ME, Rekhtman N, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. Clin Cancer Res 2013;19:2240-2247. Available at: http://www.ncbi.nlm. nih.gov/pubmed/23470965.
25. Finlay MR, Anderton M, Ashton S, et al. Discovery of a potent and selective EGFR inhibitor (AZD9291) of both sensitizing and T790M resistance mutations that spares the wild type form of the receptor. J Med Chem 2014;57:8249-8267. Available at:http://www.ncbi.nlm.nih.gov/pubmed/25271963.
26. Andriy Marusyk and Kornelia Polyak ; Tumor heterogeneity: causes and consequences. Biochim Biophys Acta. 2010 jan; 1805(1): 105
27. Kimura H, Kasahara K, Kawaishi M, et al. Detection of epidermal growth factor receptor mutations in serum as a predictor of the response to gefitinib in patients with non-small-cell lung cancer. Clin Cancer Res 2006;12:3915-21.
28. Goto K, Ichinose Y, Ohe Y, Yamamoto N, Negoro S, Nishio K et al: Epidermal growth factor receptor mutation status in circulating free DNA in serum: from IPASS, a phase III study of gefitinib or carboplatin/paclitaxel in non-small cell lung cancer. J Thorac Oncol. 2012, 7 (1): 115-121.
29. Hu C,Liu X, Chen Y et al; Direct serum and tissue assay for EGFR mutation in non-small cell lung cancer by high-resolution melting analysis. Oncol Rep 2012 Nov;28(5):1815-21
30. Weber B, Peter M et al, Detection of EGFR mutations in plasma and biopsies from non-small cell lung cancer patients by allele-specific PCR assays. BMC Cancer 2014 Apr 28;14:294
31. David C.L.,Lam, et al. Plasma EGFR mutation detection associated with survival outcomes in Advanced – Stage Lung Cancer. Clinical Lung Cancer,Vol. 16, No. 6, 507-13; 2015.
32. Huanli Duan, Junliang Lu et al. Comparison of EGFR mutation status between plasma and tumor tissue in non-small cell lung cancer using the Scorpion ARMS method and the possible prognostic significance of plasma EGFR mutation status. Int J Clin Exp Pathol 2015;8(10):13136-45
33. Martin Reck, Koichi Hagiwara, et al. ctDNA Determination of EGFR Mutation Status in European and Japanese Patients with Advanced NSCLC: The ASSESS Study. Journal of Thoracic Oncology Vol. 11 No. 10: 1682-89
34. Wang Y, Duan J :Analysis of EGFR mutation status in tissue and plasma for predicting response to EGFR-TKIs in advanced non-small-cell lung cancer. Oncol lett 2017 Apr; 13(4): 2425–31
35. Chai X, Ren P et al A comparative study of EGFR oncogenic mutations in matching tissue and plasma samples from patients with advanced non-small cell lung carcinoma. Clin Chim Acta 2016 Jun 1;457:106-11.
36. Han B,Tjulandin S, Hagiwara K et al. *EGFR* mutation prevalence in Asia-Pacific and Russian patients with advanced NSCLC of adenocarcinoma and non-adenocarcinoma histology: The IGNITE study. Lung Cancer. 2017 Nov;113:37-44.
37. Daniel A. Haber and and Victor E. Velculescu; Blood-Based Analyses of Cancer: Circulating Tumor Cells and Circulating Tumor DNA. Cancer Discov. 2014 Jun; 4(6): 650–661.