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PI3K/PTEN/AKT/mTOR Pathway Genetic Variation Predicts Toxicity and Distant Progression in Lung Cancer Patients Receiving Platinum-based Chemotherapy

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Summary

Non-small cell lung cancer (NSCLC) is still the leading cause of cancer-related deaths. The effect of the PI3K/PTEN/AKT/mTOR signaling pathway on cancer treatment, including NSCLC, has been well documented. In this study, we analyzed associations between genetic variations within this pathway and clinical outcomes following platinum-based chemotherapy in 168 patients with stage IIIB (wet) or stage IV NSCLC. Sixteen tagging SNPs in five core genes (*PIK3CA*, *PTEN*, *AKT1*, *AKT2*, and *FRAP1*) of this pathway and identified SNPs associated with development of toxicity and disease progression. We observed significantly increased toxicity for patients with PIK3CA:rs2699887 (OR: 3.86, 95% CI: 1.08 – 13.82). In contrast, a SNP in *PTEN* was associated with significantly reduced risk for chemotherapeutic toxicity (OR: 0.44, 95% CI: 0.20 - 0.95). We identified three SNPs in *AKT1* resulting in significantly decreased risks of distant progression in patients carrying at least one variant allele with HRs of 0.66 (95% CI: 0.45 - 0.97), 0.52 (95% CI: 0.35 - 0.77), and 0.62 (95% CI: 0.42 - 0.91) for rs3803304, rs2498804, and rs1130214, respectively. Furthermore, these same variants conferred nearly two-fold increased progression-free survival times. The current study provides evidence that genetic variations within the PI3K/PTEN/AKT/mTOR signaling pathway are associated with variation in clinical outcomes of NSCLC patients. With further validation, our findings may provide additional biomarkers for customized treatment of platinum-based chemotherapy for NSCLC.

Keywords

lung cancer; chemotherapy; platinum-agents; AKT; clinical outcomes

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Introduction

Lung cancer is the leading cause of cancer mortality in the United States with over 160,000 deaths estimated in 2009 [1]. Approximately 80% of lung cancer cases are non-small cell lung cancer (NSCLC) and of these, a majority present with advanced stage [2]. The prognosis for these patients is poor with few options for treatment – including chemotherapy and radiation [3]. Because of this, there is a need for better, and more individualized treatment options for advanced NSCLC.

Platinum-based chemotherapeutic agents, such as cisplatin and carboplatin, are used to treat various types of cancers including lung cancer. Unfortunately, although platinum-based combination chemotherapy has enhanced overall survival and quality of life for lung cancer patients, the 1-year survival rate is still only 29% [4]. The major hurdles in the use of platinum agents are the development of chemoresistance and severe side effects [5,6]. The most common side effects include ototoxicity, neuropathy, nephrotoxicity, and myelosuppression. These effects are thought to be caused by increased production of reactive oxygen species and apoptosis in sensitive tissues. Several factors are known to influence a patient's response to therapy, including age, ethnicity, stage of disease, performance status, and co-morbidities. However, a patient's genetic background may also play an important role in modulating response to therapy. Therefore, one strategy to enhance the effectiveness of platinum-based treatment of NSCLC while avoiding adverse events is to gain a better understanding of the influence of genetic variations on the clinical outcome of patients.

Platinum-containing agents are cytotoxic through the creation of platinum-DNA crosslinks and the induction of cell cycle arrest and ultimately apoptosis if not properly repaired [7]. Several pathways are involved in this process, including the PI3K/PTEN/AKT/mTOR pathway that is responsible for balancing cell survival and apoptosis [8,9]. This pathway is activated in various cancer types and plays a role in the development of chemoresistance to platinum-based chemotherapy [10-14]. This pathway is complex; however, the core components include PI3K (phosphoinositide-3-kinase), PTEN (phosphatase and tensin homolog), AKT (v-akt murine thymoma viral oncogene homolog) and mTOR (mammalian target of rapamycin). Genetic variations in the genes encoding these important molecules may modulate signaling through this pathway and result in variation in the development of toxicity or clinical outcomes following platinum-based therapy.

Genetic variations within the PI3K/PTEN/AKT/mTOR have recently been reported to modulate clinical outcomes in esophageal cancer [15]. Because of the variation in response to platinum-based chemotherapy in NSCLC patients, there is a need for efficient biomarkers to predict who will benefit from the chemotherapy while avoiding the development of unnecessary adverse events. In the current study, we set out to determine the association between genetic variations in *AKT1*, *AKT2*, *PIK3CA* (catalytic subunit of PI3K), *PTEN*, and *FRAP1* (encoding for mTOR) with development of toxicity and disease progression in NSCLC patients treated with platinum-compounds.

Materials and Methods

Patient Population

All of the patients were selected from an ongoing epidemiology lung cancer study. The patients included in this analysis were enrolled from 1995 to 2004 and were newly diagnosed, histological confirmed NSCLC cases treated with primary platinum-based (carboplatin or cisplatin) combination chemotherapy at the University of Texas M. D. Anderson Cancer Center. We further restricted the analysis to non-Hispanic Caucasian

patients with stage IIIB (wet) or IV NSCLC. All the subjects signed a consent form and the study was approved by the Institutional Review Board of The University of Texas M. D. Anderson Cancer Center. Peripheral blood specimens for genetic analysis were collected from each patient at the time of diagnosis prior to chemotherapy or radiotherapy treatment.

Epidemiological and Clinical Data Collection

Epidemiological data was collected using a structured questionnaire including demographic characteristics, family history of cancer, smoking history, and alcohol consumption. We defined an individual who had never smoked or had smoked no more than 100 cigarettes in his or her lifetime as never smoker; an individual who had quit smoking at least one year before diagnosis was defined as former smoker; a person who currently smoking or had quit smoking less than one year prior to diagnosis was defined as current & recent quitter. Clinical and follow-up information were abstracted from medical records. Performance status was determined based on the ECOG scale prior to treatment [16]. Complete blood counts were performed prior to each treatment based on M. D. Anderson's practice guidelines. Toxicities included in this study were neutropenia, neutropenic fever, anemia, thromobocytopenia, leukocytopenia, and nephrotoxicity that occurred during any of the primary chemotherapy treatment cycles [17]. Time to progression was measured from date of first treatment to date of progression of disease, last follow-up or death. Local progression was limited to primary tumor site and regional lymph nodes while distant progression was defined as a metastasis located outside of the thoracic cavity or in the other lung.

SNP Selection and Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes using the Human Whole Blood Genomic DNA Extraction Kit (Qiagen, Valencia, CA). We selected tagging SNPs from 5-kb flanking and within the gene regions of five genes: *AKT1*, *AKT2*, *PIK3CA*, *PTEN* and *FRAP1* (mTOR). Sixteen tagging SNPs were identified by the tagger algorithm with a cut-off value of $r^2 = 0.8$ and a MAF (minor allele frequency) = 0.1-0.35, based on the allele frequencies from CEPH samples that were genotyped by the International HapMap Project. For each SNP, genotyping was performed using the TaqMan Pre-Designed SNP Genotyping Assays (Applied Biosystems, Foster City, CA) following manufacturer's instructions. End-point fluorescence was read by ABI Prism 7900HT Sequence Detection System with genotype calls being made with SDS software (SDS 2.1, Applied Biosystems, Foster City, CA).

Statistical Analysis

For toxicity risk, unconditional multivariate logistic regression analysis was performed to estimate adjusted odds ratios (ORs) along with the corresponding 95% confident intervals (95% CIs) for each SNP. The Cox proportional hazard model was used to assess the effect of individual SNPs on progression (local and distant)-free survival. Hazard ratios (HRs) and 95% CIs were estimated by fitting the Cox model while adjusting for age, gender, clinical stage, performance status, and smoking status. Kaplan-Meier curves and log-rank tests were used to assess progression-free survival time. All statistical analyses were performed using STATA software (version 10, STATA Corporation, College Station, TX) with $P < 0.05$ being considered statistically significant. The Benjamini-Hochberg method was used to correct for multiple comparisons based on an false discovery rate (FDR) of 10% [18].

Results

Patient Characteristics

Our patient population consisted of 168 non-Hispanic Caucasian patients with advanced stage NSCLC who received primary platinum-based chemotherapy (Table 1). A majority were treated with carboplatin-based treatment (88.7%) compared to cisplatin-based (11.3%) with an average number of treatment cycles of 4.5. Seventeen (10.1%) presented with stage IIIB (wet) and 151 (89.9%) with stage IV disease. The mean age was 58.1 years (SD: 11.08, range: 28-81 years). There were 94 men (56%) and 74 women (44%). There were 48 (28.6%) never smokers, 58 (34.5%) former smokers, and 62 (36.9%) current smokers or recent quitters. The median time enrolled in the study was 10.94 months with an overall median survival time of 10.92 months.

Associations between SNPs and Risk of Toxicity

We analyzed the 16 SNPs for associations with toxicity due to platinum-based chemotherapy. Two SNPs were found to be significantly associated with toxicity (Table 2). PTEN:rs2299939 showed a negative association with patients carrying at least one variant allele having a 56% reduced risk of developing a severe side effect (OR: 0.44, 95% CI: 0.20 - 0.95, $P = 0.036$). In contrast, patients who were homozygous for the PIK3CA:rs2699887 variant exhibited a significantly increased risk of toxicity (OR: 3.86, 95% CI: 1.08 - 13.82, $P = 0.038$). Both of these associations remained significant after correct for multiple comparisons at an FDR of 10%. No other SNPs were significantly associated with toxicity risk. Because cisplatin and carboplatin-based treatment regimens differ slightly in toxicity profiles, we stratified our analysis by platinum agent. The results in the carboplatin treatment group were similar to the full population (data not shown). Due to small sample size, we were not able to perform stratified analysis in the cisplatin group.

Associations between SNPs and Progression Risk and Progression-free Survival

We next analyzed the association between SNPs and distant progression. None of the variants were associated with local progression (Table 3), but three of the 16 SNPs were associated with distant progression risk. These SNPs (rs3803304, rs2498804, rs1130214) all tagged genetic variation in *AKT1*. Patients carrying at least one variant allele exhibited similarly reduced risks with HRs of 0.66 (95% CI: 0.45 - 0.97, $P = 0.035$), 0.52 (95% CI: 0.35 - 0.77, $P = 0.001$) and 0.62 (95% CI: 0.42 - 0.91, $P = 0.016$), respectively. Although only AKT1:rs2498804 remained at an FDR of 10%, as shown in Figure 1, all three of these variants conferred a nearly two-fold prolonged progression-free time, from 4.84 to 7.30 months for rs3803304 ($P = 0.022$), 4.11 to 8.29 months for rs2498804 ($P = 0.0005$), and 5.3 to 8.42 months for rs1130214 ($P = 0.028$). Table 4 shows the linkage disequilibrium (LD) between the four *AKT1* SNPs included in this analysis. Of the three identified as significantly associated with risk, only rs3803304 and rs2498804 exhibited modest LD ($r^2=0.75$), with rs1130214 not sharing any LD with these two SNPs ($r^2=0.00$ and 0.16, respectively). Similar to the toxicity analysis, the results in the carboplatin treatment group were comparable to the full population (data not shown).

Discussion

Lung cancer has remained the leading cause for cancer-related mortality in the United States [1]. A growing body of evidence suggests that lung tumors activate certain cellular signaling pathways to become invasive and resistant to platinum-based chemotherapy [19]. The deregulation of the PI3K/PTEN/AKT/mTOR pathway in human cancers has been extensively studied over the past few years [20-23]. Furthermore, this pathway has been reported to be associated with response to platinum-based chemotherapy treatment in lung

cancer cell lines [13,24]. In this study, we determined whether common variations in genes in this pathway (*PIK3CA*, *PTEN*, *AKT1*, *AKT2*, and *FRAP1*) were able to modulate the development of toxicity and clinical outcomes of NSCLC patients receiving platinum-based chemotherapy.

Although platinum-based agents are successful in treating several types of cancer, treatment is often associated with adverse side effects, including myleosuppression, ototoxicity, nephrotoxicity, and peripheral neurotoxicity due to increased apoptosis in cells with platinum-related DNA damage [5,6]. Cisplatin and carboplatin are the most commonly used platinum-containing chemotherapeutic agents in NSCLC. Cisplatin-based therapy has been found to provide a better survival benefit for NSCLC patients, but it is associated with more severe toxicity compared to carboplatin-based therapy. However, treatment with either of these agents is hindered by development of severe toxicities and chemoresistance [25].

Since the PI3K/PTEN/AKT/mTOR pathway is involved in the balance between cell survival and death, genetic variation in the core components of this pathway may shift this balance, resulting in altered toxicity risk. In the current study, a genetic variation (rs2299939) in the negative regulator of this pathway, PTEN, was associated with a 54% decreased risk of toxicity. PTEN protein expression is often lost in NSCLC, but this loss is rarely due to inactivating mutations, loss of heterozygosity, or hypermethlyation of the gene [26-30]. Our results suggest that genetic variations in *PTEN* may modulate PTEN activity. Specifically, since rs2299939 is associated with a decrease in toxicity, we speculate that this SNP, or another functional SNP that is tagged by this variant, may decrease the expression of PTEN and hence the inhibitory effect of PTEN on signaling through this pathway. Further investigation will be needed to understand the effect of this SNP on PTEN function. In contrast, patients carrying at least one variant rs2699887 allele in *PIK3CA*, the catalytic domain for PI3K, had a nearly 4-fold increased risk of toxicity. *PIK3CA* is a known oncogene and is responsible for initiating signaling through this pathway activating cell survival signals [31]. Decreased PI3K activity would result increased apoptosis in sensitive, non-cancer cells causing an increase in toxic side-effects. Therefore, the genetic variation tagged by the PIK3CA:rs2699887 SNP would likely cause an decrease in PI3K signaling. The contrasting results of *PTEN* and *PIK3CA* genetic variation have biological plausibility based on their function in regulating signaling through this pathway.

The serine-threonine kinase AKT is a central node in cell signaling that regulates several processes, including cell survival, proliferation, and protein synthesis [8]. AKT activation is a common molecular alteration during carcinogenesis, and it has been reported that AKT is constitutively activated in NSCLC resulting in cell survival by blocking induction of apoptosis [32]. In addition, forced expression of *AKT1* was found to be sufficient to regulate cisplatin resistance in cultured lung cancer cells [13]. We found that three *AKT1* tagging SNPs decreased risk for distant disease progression. These three SNPs do not share a high degree of linkage disequilibrium, suggesting the presence of at least two independent causal variants. The directionality of the effect indicates that the functional variants all diminish AKT1 activity causing decreased signaling through this pathway, and thus a reduction in cell survival signals. However, since the variants genotyped in this study were tagging SNPs, we are unable to identify the causative SNP and mechanism responsible. Future studies are clearly warranted in this regard.

Platinum-based chemotherapy is still the core treatment for NSCLC patients. Although knowledge and chemotherapeutic methods for treating NSCLC keep evolving, the survival rate has not improved notably with chemotherapy. New biologic insight and biomarkers are desired to find new approaches for treating patients with advanced disease. In the current study, although based on a small sample size, the homogenous nature of the treatment

regimens the patients received allowed us to identify genetic variations within the PI3K/PTEN/AKT/mTOR signaling pathway that are associated with variation in development of toxicity and clinical outcomes for NSCLC patients. With validation, our findings may provide additional biomarkers for individualized treatment in order to enhance the efficiency and reduce toxicity during chemotherapy with platinum-based agents for NSCLC.

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References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin 2009;59:225–249. [PubMed: 19474385]
2. Ries, LAG.; Melbert, D.; Krapcho, M.; Stinchcomb, DG.; Howlader, N.; Horner, MJ.; Mariotto, A.; Miller, BA.; Feuer, EJ.; Altekruse, SF.; Lewis, DR.; Clegg, L.; Eisner, MP.; Reichman, M.; Edwards, BK. SEER Cancer Statistics Review, 1975-2005. National Cancer Institute; Bethesda, MD: 2008.
3. Spira A, Ettinger DS. Multidisciplinary management of lung cancer. N Engl J Med 2004;350:379–392. [PubMed: 14736930]
4. Burdett S, Stephens R, Stewart L, Tierney J, Auperin A, Le Chevalier T, Le Pechoux C, Pignon J, Arriagada R, Higgins J, Johnson D, van Meerbeeck J, Parmar M, Souhami R, Bell D, Cartei G, Cormier Y, Cullen M, Ganz P, Gridelli C, Kaasa S, Quoix E, Rapp E, Seymour L, Spiro S, Thatcher N, Tummarello D, Williams C, Williamson I. Chemotherapy in addition to supportive care improves survival in advanced non-small-cell lung cancer: a systematic review and meta-analysis of individual patient data from 16 randomized controlled trials. J Clin Oncol 2008;26:4617–4625. [PubMed: 18678835]
5. Kartalou M, Essigmann JM. Mechanisms of resistance to cisplatin. Mutat Res 2001;478:23–43. [PubMed: 11406167]
6. Rabik CA, Dolan ME. Molecular mechanisms of resistance and toxicity associated with platinating agents. Cancer Treat Rev 2007;33:9–23. [PubMed: 17084534]
7. Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 2003;22:7265–7279. [PubMed: 14576837]
8. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell 2007;129:1261–1274. [PubMed: 17604717]
9. Nicholson KM, Anderson NG. The protein kinase B/Akt signalling pathway in human malignancy. Cell Signal 2002;14:381–395. [PubMed: 11882383]
10. Gagnon V, Van Themsche C, Turner S, Leblanc V, Asselin E. Akt and XIAP regulate the sensitivity of human uterine cancer cells to cisplatin, doxorubicin and taxol. Apoptosis 2008;13:259–271. [PubMed: 18071906]
11. Kim SH, Juhnn YS, Song YS. Akt involvement in paclitaxel chemoresistance of human ovarian cancer cells. Ann N Y Acad Sci 2007;1095:82–89. [PubMed: 17404021]
12. Lee S, Choi EJ, Jin C, Kim DH. Activation of PI3K/Akt pathway by PTEN reduction and PIK3CA mRNA amplification contributes to cisplatin resistance in an ovarian cancer cell line. Gynecol Oncol 2005;97:26–34. [PubMed: 15790433]
13. Liu LZ, Zhou XD, Qian G, Shi X, Fang J, Jiang BH. AKT1 amplification regulates cisplatin resistance in human lung cancer cells through the mammalian target of rapamycin/p70S6K1 pathway. Cancer Res 2007;67:6325–6332. [PubMed: 17616691]
14. Yang X, Fraser M, Moll UM, Basak A, Tsang BK. Akt-mediated cisplatin resistance in ovarian cancer: modulation of p53 action on caspase-dependent mitochondrial death pathway. Cancer Res 2006;66:3126–3136. [PubMed: 16540663]

15. Hildebrandt MA, Yang H, Hung MC, Izzo JG, Huang M, Lin J, Ajani JA, Wu X. Genetic variations in the PI3K/PTEN/AKT/mTOR pathway are associated with clinical outcomes in esophageal cancer patients treated with chemoradiotherapy. J Clin Oncol 2009;27:857–871. [PubMed: 19164214]
16. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649–655. [PubMed: 7165009]
17. Trotti A, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, Langer C, Murphy B, Cumberlin R, Coleman CN, Rubin P. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. Semin Radiat Oncol 2003;13:176–181. [PubMed: 12903007]
18. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 1995;57:289–300.
19. Pisick E, Jagadeesh S, Salgia R. Receptor tyrosine kinases and inhibitors in lung cancer. Scientific World Journal 2004;4:589–604. [PubMed: 15349502]
20. Bader AG, Kang S, Zhao L, Vogt PK. Oncogenic PI3K deregulates transcription and translation. Nat Rev Cancer 2005;5:921–929. [PubMed: 16341083]
21. Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB. Exploiting the PI3K/AKT pathway for cancer drug discovery. Nat Rev Drug Discov 2005;4:988–1004. [PubMed: 16341064]
22. Shaw RJ, Cantley LC. Ras, PI(3)K and mTOR signalling controls tumour cell growth. Nature 2006;441:424–430. [PubMed: 16724053]
23. Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. Oncogene 2008;27:5497–5510. [PubMed: 18794884]
24. Lee MW, Kim DS, Min NY, Kim HT. Akt1 inhibition by RNA interference sensitizes human non-small cell lung cancer cells to cisplatin. Int J Cancer 2008;122:2380–2384. [PubMed: 18224693]
25. Sanborn RE. Cisplatin versus carboplatin in NSCLC: is there one “best” answer? Curr Treat Options Oncol 2008;9:326–342. [PubMed: 19225891]
26. Forgacs E, Biesterveld EJ, Sekido Y, Fong K, Muneer S, Wistuba II, Milchgrub S, Brezinschek R, Virmani A, Gazdar AF, Minna JD. Mutation analysis of the PTEN/MMAC1 gene in lung cancer. Oncogene 1998;17:1557–1565. [PubMed: 9794233]
27. Marsit CJ, Zheng S, Aldape K, Hinds PW, Nelson HH, Wiencke JK, Kelsey KT. PTEN expression in non-small-cell lung cancer: evaluating its relation to tumor characteristics, allelic loss, and epigenetic alteration. Hum Pathol 2005;36:768–776. [PubMed: 16084946]
28. Petersen S, Rudolf J, Bockmuhl U, Gellert K, Wolf G, Dietel M, Petersen I. Distinct regions of allelic imbalance on chromosome 10q22-q26 in squamous cell carcinomas of the lung. Oncogene 1998;17:449–454. [PubMed: 9696038]
29. Soria JC, Lee HY, Lee JI, Wang L, Issa JP, Kemp BL, Liu DD, Kurie JM, Mao L, Khuri FR. Lack of PTEN expression in non-small cell lung cancer could be related to promoter methylation. Clin Cancer Res 2002;8:1178–1184. [PubMed: 12006535]
30. Yokomizo A, Tindall DJ, Drabkin H, Gemmill R, Franklin W, Yang P, Sugio K, Smith DI, Liu W. PTEN/MMAC1 mutations identified in small cell, but not in non-small cell lung cancers. Oncogene 1998;17:475–479. [PubMed: 9696041]
31. Shayesteh L, Lu Y, Kuo WL, Baldocchi R, Godfrey T, Collins C, Pinkel D, Powell B, Mills GB, Gray JW. PIK3CA is implicated as an oncogene in ovarian cancer. Nat Genet 1999;21:99–102. [PubMed: 9916799]
32. Brognard J, Clark AS, Ni Y, Dennis PA. Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. Cancer Res 2001;61:3986–3997. [PubMed: 11358816]

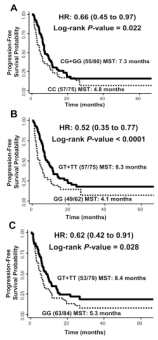

Fig 1.

Kaplan-Meier curves of distant progression-free survival times in lung cancer patients by *AKT1* SNPs A) rs3803304, B) rs2498804, and C) rs1130214. The numbers in parentheses are the numbers of patients with progression over the total number of patients by genotype. MST = median time to progression in months.

Table 1

Patient Characteristics

Characteristic	# of Patients	%
Total	168	
Age		
Mean	58.1	
SD	11.08	
Range	28-81	
Sex		
Male	94	56
Female	74	44
Clinical Stage		
Stage IIIB (wet)	17	10.1
Stage IV	151	89.9
Smoking Status		
Never	48	28.6
Former	58	34.5
Current & Recent Quitter	62	36.9
Performance Status		
0	38	22.6
1	109	64.9
2-4	21	12.5
Treatment Regimen		
Carboplatin-based	149	88.7
Cisplatin-based	19	11.3
Local Progression		
No	102	60.7
Yes	66	39.3
Distant Progression		
No	51	30.4
Yes	117	69.6+
Toxicity		
No	100	59.5
Yes	68	40.5
Histology		
Adenocarcinoma	102	60.7
Non-small cell carcinoma	23	13.7
Squamous cell cacinoma	24	14.3
Other NSCLC	19	11.3

Table 2

PI3K/PTEN/AKT/mTOR pathway genotypes and toxicity

	Toxicity				
SNP and Genotype	**Yes**	**No**	**OR***	**95% CI**	***P* value**
AKT1:rs3803304	62	93			
CC	26	49	1.00		
CG	30	37	1.43	0.71 to 2.86	0.317
GG	6	7	1.67	0.50 to 5.60	0.408
CG + GG			1.46	0.75 to 2.84	0.261
AKT1:rs2494738	65	99			
AA	53	81	1.00		
AG	11	18			
GG	1	0			
AG+GG			1.04	0.45 to 2.42	0.921
AKT1:rs2498804	66	97			
GG	21	41	1.00		
GT	37	46	1.45	0.71 to 2.95	0.305
TT	8	10	1.65	0.55 to 4.96	0.373
GT + TT			1.48	0.75 to 2.94	0.257
AKT1:rs1130214	66	97			
GG	30	54	1.00		
GT	29	38	1.38	0.69 to 2.77	0.364
TT	7	5	2.92	0.80 to 10.67	0.105
GT + TT			1.55	0.79 to 3.01	0.200
AKT2:rs892119	65	97			
AA	40	72	1.00		
AG	24	23	2.06	1.00 to 4.27	0.051
GG	1	2	1.00	0.08 to 12.26	1.000
AG + GG			1.97	0.97 to 4.02	0.061

SNP and Genotype	Toxicity				
	Yes	No	OR*	95% CI	*P* value
AKT2:rs8100018	63	95			
CC	39	46	1.00		
CG	20	39	0.54	0.26 to 1.12	0.096
GG	4	10	0.42	0.12 to 1.52	0.186
CG + GG			0.51	0.26 to 1.02	0.056
FRAP1:rs11121704	66	97			
CC	32	56	1.00		
CT	28	36	1.31	0.65 to 2.62	0.448
TT	6	5	2.79	0.73 to 10.61	0.133
CT + TT			1.47	0.76 to 2.84	0.253
FRAP1:rs2295080	66	98			
GG	27	49	1.00		
GT	32	42	1.40	0.71 to 2.76	0.333
TT	7	7	2.18	0.66 to 7.20	0.202
GT + TT			1.50	0.78 to 2.88	0.224
PIK3CA:rs7651265	66	98			
AA	58	76	1.00		
AG	8	21			
GG	0	1			
AG + GG			0.43	0.17 to 1.06	0.065
PIK3CA:rs7640662	65	99			
CC	45	74	1.00		
CG	17	24	1.05	0.49 to 2.25	0.909
GG	3	1	4.52	0.44 to 46.59	0.205
CG + GG			1.19	0.57 to 2.48	0.649
PIK3CA:rs7621329	65	99			
CC	47	63	1.00		

	Toxicity				
SNP and Genotype	**Yes**	**No**	**OR***	**95% CI**	***P* value**
CT	18	30			
TT	0	6			
CT+TT			0.63	0.31 to 1.28	0.202
PIK3CA:rs6443624	66	99			
AA	42	55	1.00		
AC	24	36			
CC	0	8			
AC + CC			0.71	0.37 to 1.37	0.310
PIK3CA:r52699887	65	98			
AA	34	56	1.00		
AG	21	38	0.93	0.46 to 1.89	0.845
GG	**10**	**4**	**3.86**	**1.08 to 13.82**	**0.038**
AG + GG			1.21	0.62 to 2.34	0.576
PTEN:rs2299939	64	93			
AA	51	58	1.00		
AC	13	29			
CC	0	6			
AC + CC			**0.44**	**0.20 to 0.95**	**0.036**
PTEN:rs12569998	66	99			
GG	46	75	1.00		
GT	19	23	1.40	0.66 to 2.97	0.386
TT	1	1	4.48	0.20 to 100.80	0.346
GT + TT			1.45	0.69 to 3.06	0.323
PTEN:rs12357281	66	99			
CC	61	86	1.00		
CG	5	12			
GG	0	1			

SNP and Genotype	Toxicity				
	Yes	No	OR*	95% CI	*P* value
CG + GG			0.54	0.17 to 1.66	0.281

* adjusted for age, gender, clinical stage, performance status, and smoking status

Table 3

PI3K/PTEN/AK17mTOR pathway genotypes and disease progression

	Local Progression					Distant Progression				
SNP and Genotype	**Yes**	**No**	**HR***	**95% CI**	***P* value**	**Yes**	**No**	**HR***	**95% CI**	***P* value**
AKT1:rs3803304	58	97				112	43			
CC	28	47	1.00			57	18	1.00		
CG	22	45	0.80	0.45 to 1.42	0.448	**44**	**23**	**0.61**	**0.41 to 0.92**	**0.017**
GG	8	5	1.59	0.71 to 3.54	0.261	11	2	0.97	0.50 to 1.86	0.921
CG + GG			0.93	0.55 to 1.57	0.790			**0.66**	**045 to 0.97**	**0.035**
AKT1:rs2494738	65	99				117	47			
AA	52	82	1.00			96	38	1.00		
AG	12	17	0.94	0.49 to 1.79	0.847	20	9			
GG	1	0				1	0			
AG+GG			0.99	0.53 to 1.86	0.985			0.80	0.49 to 1.29	0.357
AKT1:rs2498804	64	99				116	47			
GG	24	38	1.00			49	13	1.00		
GT	30	53	0.80	0.46 to 1.40	0.432	**52**	**31**	**0.47**	**0.31 to 0.72**	**< 0.001**
TT	10	8	1.26	0.60 to 2.68	0.540	15	3	0.76	0.42 to 1.37	0.357
GT + TT			0.89	0.53 to 1.50	0.661			**0.52**	**0.35 to 0.77**	**0.001**
AKT1:rs1130214	64	99				116	47			
GG	35	49	1.00			63	21	1.00		
GT	24	43	0.74	0.43 to 1.27	0.275	**45**	**22**	**0.60**	**0.40 to 0.90**	**0.013**
TT	5	7	0.91	0.35 to 2.36	0.840	8	4	0.77	0.36 to 1.63	0.492
GT+TT			0.76	0.45 to 1.28	0.305			**0.62**	**0.42 to 0.91**	**0.016**
AKT2:rs892119	65	97				114	48			
AA	46	66	1.00			80	32	1.00		
AG	18	29	0.93	0.53 to 1.63	0.788	32	15	0.83	0.54 to 1.25	0.370
GG	1	2	0.97	0.13 to 7.26	0.973	2	1	0.68	0.16 to 2.84	0.595
AG + GG			0.93	0.53 to 1.61	0.790			0.82	0.54 to 1.23	0.328

	Local Progression					Distant Progression				
SNP and Genotype	**Yes**	**No**	**HR***	**95% CI**	***P* value**	**Yes**	**No**	**HR***	**95% CI**	***P* value**
AKT2:rs8100018	60	98				110	48			
CC	30	55	1.00			57	28	1.00		
CG	24	35	1.17	0.67 to 2.02	0.588	43	16	1.31	0.86 to 1.93	0.208
GG	6	8	1.09	0.43 to 2.76	0.856	10	4	1.30	0.66 to 2.56	0.456
CG + GG			1.15	0.68 to 1.93	0.599			1.30	0.88 to 1.92	0.181
FRAP1:rs11121704	65	98				115	48			
CC	34	54	1.00			64	24	1.00		
CT	26	38	1.14	0.67 to 1.94	0.622	43	21	0.73	0.49 to 1.10	0.130
TT	5	6	1.34	0.51 to 3.57	0.553	8	3	0.81	0.38 to 1.74	0.593
CT + TT			1.17	0.71 to 1.94	0.528			0.75	0.51 to 1.09	0.129
FRAP1:rs2295080	63	101				117	47			
GG	26	50	1.00			55	21	1.00		
GT	30	44	1.21	0.71 to 2.06	0.486	51	23	0.77	0.52 to 1.13	0.186
TT	7	7	1.70	0.72 to 4.03	0.224	11	3	0.91	0.47 to 1.77	0.784
GT + TT			1.28	0.77 to 2.12	0.343			0.79	0.55 to1.14	0.215
PIK3CA:rs7651265	65	99				116	48			
AA	53	81	1.00			94	40	1.00		
AG	11	18	0.96	0.50 to 1.85	0.901	21	8	0.98	0.60 to 1.58	0.919
GG	1	0				1	0			
AG + GG			1.04	0.55 to 1.96	0.902			1.02	0.64 to 1.64	0.921
PIK3CA:rs7640662	65	99				116	48			
CC	46	73	1.00			83	36	1.00		
CG	16	25	1.1	0.60 to 2.02	0.753	31	10	1.06	0.69 to 1.64	0.791
GG	3	1	2.29	0.67 to 7.81	0.186	2	2	0.48	0.11 to 2.01	0.312
CG + GG			1.20	0.68 to 2.12	0.538			0.99	0.65 to 1.52	0.974
PIK3CA:rs7621329	65	99				117	47			
CC	44	66	1.00			79	31	1.00		

	Local Progression					Distant Progression				
SNP and Genotype	**Yes**	**No**	**HR***	**95% CI**	***P* value**	**Yes**	**No**	**HR***	**95% CI**	***P* value**
CT	17	31	0.71	0.40 to 1.26	0.238	34	14	0.76	0.51 to 1.15	0.197
TT	4	2	1.95	0.68 to 5.61	0.213	4	2	0.77	0.27 to 2.21	0.629
CT + TT			0.82	0.48 to 1.39	0.460			0.76	0.51 to 1.14	0.183
PIK3CA:rs6443624	65	100				117	48			
AA	38	59	1.00			69	28	1.00		
AC	22	38	0.81	0.47 to 1.39	0.439	44	16	0.88	0.59 to 1.30	0.516
CC	5	3	1.66	0.63 to 4.39	0.307	4	4	0.58	0.20 to 1.65	0.307
AC + CC			0.91	0.55 to 1.51	0.705			0.84	0.58 to 1.22	0.360
PIK3CA:rs2699887	65	98				115	48			
AA	34	56	1.00			67	23	1.00		
AG	25	34	1.28	0.76 to 2.18	0.353	37	22	0.77	0.51 to 1.17	0.225
GG	6	8	1.17	0.47 to 2.89	0.733	11	3	0.92	0.47 to 1.80	0.800
AG + GG			1.26	0.77 to 2.08	0.362			0.80	0.54 to 1.18	0.258
PTEN:rs2299939	61	96				110	47			
AA	45	64	1.00			77	32	1.00		
AC	15	27	0.78	0.42 to 1.43	0.416	31	11	0.94	0.61 to 1.44	0.770
CC	1	5	1.54	0.20 to 11.82	0.681	2	4	1.16	0.28 to 4.80	0.839
AC + CC			0.80	0.44 to 1.45	0.466			0.95	0.62 to 1.45	0.806
PTEN:rs12569998	65	100				117	48			
GG	48	73	1.00			83	38	1.00		
GT	15	27	1.10	0.60 to 2.02	0.761	32	10	1.34	0.86 to 2.07	0.195
TT	2	0				2	0			
GT + TT			1.23	0.69 to 2.19	0.492			1.40	0.91 to 2.14	0.127
PTEN:rs12357281	65	100				117	48			
CC	56	91	1.00			105	42	1.00		
CG	8	9	0.94	0.42 to 2.07	0.876	11	6	0.98	0.51 to 1.88	0.945
GG	1	0				1	0	1.10	0.14 to 8.41	0.929

SNP and Genotype	Local Progression					Distant Progression				
	Yes	No	HR*	95% CI	*P* value	Yes	No	HR*	95% CI	*P* value
CG + GG			1.08	0.51 to 2.29	0.848			0.99	0.53 to 1.84	0.969

* adjusted for age, gender, clinical stage, performance status, and smoking status

Table 4

Linkage disequilibrium (r^2) between *AKT1* SNPs

	AKT1:rs3803304	AKT1:rs2498804	AKT1:rs1130214
AKT1:rs2498804	0.75		
AKT1:rs1130214	0.00	0.16	
AKT1: rs2494738	0.11	0.12	0.00