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Research Article

Survival prediction of tuberous sclerosis complex gene variant in patients with advanced non-small-cell lung cancer treated with platinum doublet

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Tuberous sclerosis complex (TSC) 1 and 2 function as tumor suppressors by inactivating the mammalian target of rapamycin (mTOR) pathway. Although the effect of platinum on TSC function has been studied, associations between *TSC* gene variants and survival of cancer patients treated with platinum-based chemotherapy were not evaluated. Genetic variants of *TSC1* and *TSC2* were identified by next-generation sequencing and selected for further clinical evaluation based on predetermined criteria. Associations of the gene variants with treatment outcomes (progression-free survival, PFS; overall survival, OS) were evaluated in testing and validation sets of patients with advanced non-small-cell lung cancer (NSCLC). Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated with the multivariable Cox model. The *TSC1* Met322Thr (rs1073123) variant met the criteria for further analysis in testing and validation sets each containing 183 patients. The median PFS for the 366 patients was 4.9 months. Fifty-three patients (14.5%) had the *TSC1* (Met322Thr or Thr322Thr) variant. *TSC1* Met322Thr associated with longer PFS in the testing set (HR adjusted for age, gender, smoking habits, Eastern Cooperative Oncology Group performance status, histology, and stage [aHR] and 95% CI: 0.63 and 0.45–0.87, Cox P=0.009), and this was confirmed in the validation set (aHR and 95% CI: 0.58 and 0.36–0.93, Cox P=0.004). However, no association was found between the *TSC1* gene variant and OS. These findings suggest that the *TSC1* gene variant is an important predictive marker for platinum doublet chemotherapy outcomes in NSCLC patients.

Introduction

Lung cancer is the most common cause of cancer-related mortality worldwide. The 5-year survival of patients with advanced stage non-small-cell lung cancer (NSCLC) remains disappointingly low at 16%. Despite recent advances in targeted immunological therapies, platinum doublet chemotherapy still plays a pivotal role in the treatment of advanced NSCLC.

It is not fully understood how cisplatin acts on cancer cells, but accumulating evidence indicates that cisplatin forms DNA adducts; thereby, inhibiting replication and transcription, which results in cell cycle arrest and apoptosis. Therefore, the development of biomarkers to predict the effects of platinum on NSCLC has focused on DNA damage responses, including homologous recombination, nucleotide excision repair, and mismatch repair [1]. In addition, the phosphoinositide-3-kinase catalytic subunit-α (PI3KCA)/AKT/mammalian target of rapamycin (mTOR) signaling pathway has also been investigated to identify platinum resistance mechanisms and to predict platinum effects [2–5]; however, the clinical relevance of this pathway in NSCLC has not yet been validated. Further, pyruvate dehydrogenase kinase

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(PDK) and AMP-activated protein kinase activities were enhanced in stomach, liver, and colon cancer cell lines that were treated with cisplatin, and these two kinases contributed to cell survival via interactions with tuberous sclerosis complex (TSC) 1 and TSC 2 [6–8].

The *TSC1* and *TSC2* genes are located at chromosomes 9q34 and 16p13.3, respectively, and according to Knudson's tumor suppressor model, it has been established that *TSC1* and *TSC2* are involved in the development of TSC syndrome [9]. *TSC1* and *TSC2* encode for hamartin and tuberin, respectively. The hamartin and tuberin heterodimer has been shown to function as a tumor suppressor by inactivating mTOR through suppression of the small GTPase Rheb (Ras-homolog enriched in brain). However, the clinical implications of genetic variations in *TSC1* or *TSC2* in cancer patients have not yet been elucidated.

In this study, we screened for genetic variants of *TSC1* and *TSC2* and associated genes to determine whether genetic variants associated with platinum doublet chemotherapy outcomes in NSCLC patients.

Methods

Selection of study population and acquisition of clinical information

From over 500 NSCLC patients with stage III or IV disease who were diagnosed between March 2000 and December 2005 as part of the Lung Cancer Cohort of Inha University Hospital (Incheon, South Korea) [10], we selected 368 patients who were treated with more than two cycles of platinum-based chemotherapy as a first-line treatment (Supplementary Figure S1). Patients who were evaluated after every two or three chemotherapy cycles, who had complete follow-ups at Inha University Hospital, and whose peripheral blood lymphocytes were available for analysis were included in this study. Information regarding treatment, tumor response, follow-up, survival, smoking habits, and performance status according to the Eastern Cooperative Oncology Group (ECOG) were collected. The patients' clinical stages were reassessed according to the 7th edition of the Tumor Node Metastasis classification system [11]. Patient response to platinum doublet treatment, which is a secondary endpoint, was updated according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [12]. A total of 366 patients were randomly assigned to two groups for testing and validation using the Zelen permuted block randomization method [13]. This study was approved by the Institutional Review Board of Inha University Hospital.

Selection of genetic variants of candidate genes and genetic analysis of TSC1

DNA was isolated from the buffy coat and quality control was performed (Supplementary Method). Next-generation sequencing was performed on an Illumina Hiseq2000 platform, and a custom panel composed of 150 cancer-related genes was used for the initial screening of 24 patients with advanced stage NSCLC. Among the 150 genes, *TSC1*, *TSC2*, and the related genes *PI3KCA*, *AKT1*, *mTOR*, and *PDK1* were selected for this study (Supplementary Table S1). Thirty-three genetic variants of *TSC1*, 22 variants of *TSC2*, 9 variants of *PI3KCA*, 44 variants of *AKT1*, 52 variants of *mTOR*, and 21 variants of *PDK1* were identified. A genetic variant was determined to have a clinical association if it met the following criteria: minor allele frequency >5%, call rate >90%, Hardy–Weinberg equilibrium *P*-value >0.001, and a high or moderate effect impact according to the SnpEff variant prediction program [14]. The *TSC1* Met322Thr (rs1073123) variant met the criteria and was chosen for further analysis. Genotyping for the *TSC1* Met322Thr variant was performed using the TaqMan assay (Applied Biosystems).

Clinical endpoint analysis

The primary endpoint in this study was progression-free survival (PFS) from the start date of chemotherapy to recurrence. Patients who were still alive and progression-free at the end of the follow-up were treated as censored at the date of follow-up. The secondary endpoint was overall survival (OS), which was calculated from the time of diagnosis to the time of the last follow-up or death due to any cause.

Statistical analysis

The characteristics of the two groups within the study population were compared using the χ^2 test. The effect of an individual clinical variable or genetic variant of *TSC1* on survival was estimated using the Kaplan–Meier method and log-rank testing. Observations were censored at survival, loss to follow-up or death from other causes. The hazard ratios (HRs) and 95% confidence intervals (CIs) for all of the clinical variables were estimated using the Cox proportional hazards model. Significance was determined using a two-tailed test and *P*-values <0.05 were considered significant. Analyses were performed using the IBM SPSS statistical software package (version 19.0; SPSS Inc.; Chicago, IL, U.S.A.) and Stata (version 12.1; StataCorp, Ltd.; College Station, TX, U.S.A.).

Table 1 Clinical characteristics of the patients in testing, validation, and combined sets

		Combined, %		Testing, %		Validation, %		χ^2 *P*
Age	Median (min–max)	65.0 (32–86)		63.5 (34–80)		65.1 (32–86)		0.375
Gender	Women	101	27.6	57	56.4	44	43.6	0.168
	Men	265	72.4	126	47.5	139	52.5	
Smoking habit	Never	102	27.9	55	53.9	47	46.1	0.352
	Ever	263	72.1	127	48.3	136	51.7	
Histology	ADC	200	54.6	101	50.5	99	49.5	0.943
	SQC	133	36.3	65	48.9	68	51.1	
	Others	33	9.0	17	51.5	16	48.5	
ECOG PS	0–1	298	82.1	151	50.7	147	49.3	0.413
	2 or more	65	17.9	29	44.6	36	55.4	
Stages	IIIA	36	9.8	17	47.2	19	52.8	0.923
	IIIB	91	24.9	45	49.5	46	50.5	
	IV	239	65.3	121	50.6	118	49.4	
First-line regimens	Platinum plus							
	Gemcitabine	124	33.9	62	50.0	62	50.0	0.972
	Taxane	112	30.6	58	51.8	54	48.2	
	Irinotecan	95	26.0	45	47.4	50	52.6	
	Pemetrexed	26	7.1	13	50.0	13	50.0	
	Others	9	2.4	5	55.6	4	44.4	
No. cycles	2	92	25.2	46	50.0	46	50.0	0.769
	3–4	159	43.6	81	50.9	78	49.1	
	4–6	113	31.0	55	48.7	58	51.3	
	7	1	0.2	0	0.0	1	100.0	
Response	CR or PR	146	39.9	66	45.2	80	54.8	0.252
	SD	97	26.5	55	56.7	42	43.3	
	PD	122	33.3	61	50.0	61	50.0	
	Not evaluated	1	0.2	1	100.0	0	0.0	
TSC1 Met322Thr	Met/Met	313	85.5	156	49.8	157	50.2	0.989
	Met/Thr	51	14.0	26	51.0	25	49.0	
	Thr/Thr	2	0.5	1	50.0	1	50.0	
PFS	Median, months (95% CIs)	4.9 (4.57–5.29)		4.7 (4.21–5.18)		5.1 (4.47–5.59)		0.272[a]
	Event	344		176		168		

P-values indicates χ^2 testing between testing and validation except [a]log-rank test.
Abbreviations: ADC, adenocarcinoma; CR, complete remission; ECOG PS, ECOG performance status; PD, progressive disease; PFS, progression free survival; PR, partial remission; SD, stable disease; SQC, squamous cell carcinoma.

Results

Patient characteristics

The clinical variables and the *TSC1* variants for all of the patients in the cohort are shown in Table 1. For the first-line regimen, the gemcitabine doublet was given to 124 patients (34%) and the taxane doublet was given to 112 patients (31%). Three hundred and thirteen of the patients (85%) had the wild-type *TSC1* genotype and 53 patients (14.5%) had a variant *TSC1* genotype (52 had the Met322Thr variant and two had the Thr322Thr variant). Progression after chemotherapy was observed in 344 patients (94%). There were no differences in the clinical variables, responses to chemotherapy, and *TSC1* gene variants between the testing and validation sets.

Effect of the TSC1 gene variant on survival of patients in the testing set

Histology and response to platinum doublet associated with PFS (log-rank P=0.001 and <0.001, respectively). Disease stage also associated with PFS (log-rank P=0.056). The median PFS for patients with the *TSC1* Met322Thr variant was 5.9 months and was longer than the median PFS for patients with the *TSC1* Met322Met variant (log-rank P<0.001; Figure 1 and Supplementary Table S2). After adjusting for confounding variables, including age, gender, ECOG performance status, smoking habits, histology, and disease stage, the Cox model showed that patients with

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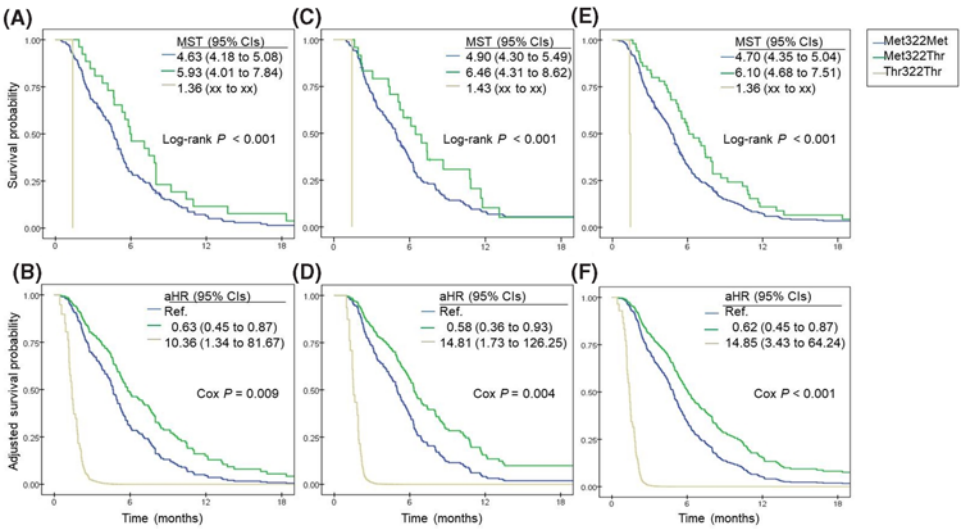

Figure 1. PFS of patients by the *TSC1* genetic variant

PFS of patients by *TSC1* genetic variant in the testing (**A,B**), validation (**C,D**), and combined (**E,F**) sets by Kaplan–Meier plot and the Cox proportional hazard model.

the *TSC1* Met322Thr variant had longer PFS than patients with the *TSC1* Met322Met variant (HR adjusted for age, gender, smoking habits, ECOG performance status, histology, and stage [aHR] and 95% CI: 0.63 and 0.45–0.87, Cox P=0.009). However, the *TSC1* gene variants did not affect OS (median survival time [MST] in months and 95% CIs: 14.1 and 10.8–17.4 for Met322Met; 16.4 and 11.1–21.8 for Met322Thr; log-rank P=0.461; Supplementary Figure S2).

Effect of the TSC1 gene variant on survival of patients in the validation set

Gender, histology, stage, first-line regimen, and the response to platinum doublet associated with PFS (log-rank P=0.031, 0.004, <0.001, 0.002, and <0.001, respectively). The median PFS for patients with the *TSC1* Met322Thr variant was 6.4 months and was longer than the median PFS for patients with the *TSC1* Met322Met variant (log-rank P<0.001). After adjusting for confounding variables, the Cox model showed that patients with the *TSC1* Met322Thr variant had longer PFS than patients with the *TSC1* Met322Met variant (aHR and 95% CI: 0.58 and 0.36–0.93, Cox P=0.004). However, the *TSC1* gene variants did not affect OS (MST, months and 95% CIs: 13.8 and 11.4–16.1 for Met322Met; 17.1 and 14.7–19.5 for Met322Thr; log-rank P=0.641).

Effect of the TSC1 gene variant on survival of patients in the combined set

When associations between clinical variables and PFS were analyzed in the entire cohort using the log-rank test, we found that smoking habits, histology, stage, and first-line regimen associated with PFS. The multivariate Cox proportional hazard model was performed to further assess the effects of these variables. Histology and stage affected PFS (Cox P=0.008 and 0.003, respectively). Age and gender associated marginally with shorter PFS (aHR and 95% CIs: 1.01 and 1.00–1.03 for age; 1.43 and 0.96–2.13 for gender) (Table 2). The median PFS for patients with the *TSC1* Met322Thr variant was 6.1 months and was longer than the median PFS for patients with the *TSC1* Met322Met variant (log-rank P<0.001). The Cox proportional hazard model also showed that patients with the *TSC1* Met322Thr variant had longer PFS than patients with the *TSC1* Met322Met variant (aHR and 95% CIs: 0.63 and 0.45–0.87, Cox P<0.001). However, the *TSC1* gene variants did not affect OS (MST, months and 95% CIs: 13.9 and 12.0–15.7 for Met322Met, 16.7 and 13.9–19.5 for Met322Thr; log-rank P=0.359).

Discussion

This study found that a genetic variant of the *TSC1* gene is a robust predictor of the effects of platinum doublet therapy in patients with advanced stage NSCLC. This finding supports the model in which platinum acts on mTOR signaling through TSC1. TSC1 is composed of 1164 amino acids and the region that is responsible for interaction

Table 2 Effects of clinical variables or genetic variation of *TSC1* on PFS in combined set

		aHR	95% CI	Cox *P*
Age	Increasing	1.01	1.00–1.03	0.085
Gender	Women	Ref.		0.077
	Men	1.43	0.96–2.13	
ECOG PS	0–1	Ref.		0.930
	2 or more	1.01	0.75–1.36	
Smoking habit	Never	Ref.		0.278
	Ever	0.80	0.54–1.19	
Histology	ADC	Ref.		0.008
	SQC	0.66	0.50–0.85	
	Others	0.76	0.56–1.32	
Stages	IIIA	Ref.		0.003
	IIIB	1.49	0.95–2.32	
	IV	2.02	1.30–3.14	
TSC1 Met322Thr	Met/Met	Ref.		<0.001
	Met/Thr	0.62	0.45–0.87	
	Thr/Thr	14.86	3.43–64.24	

Abbreviations: ADC, adenocarcinoma; ECOG PS, ECOG performance status; SQC, squamous cell carcinoma.

with TSC2 is amino acid 302–430 [9], which is also the region where the TSC1 variants exist. Therefore, we suggest that the *TSC1* missense variant Met322Thr affects the stability of TSC2, which results in inhibition of mTOR and cellular growth and proliferation. Our data that patients with *TSC1* Met322Thr had longer PFS in the testing and validation sets support this hypothesis.

We have not provided evidence for the biological basis of the predictive value of the *TSC1* gene variant. However, previous experimental studies have demonstrated the effects of platinum on TSC1. It has been shown that susceptibility to cell death increased upon DNA damage by an alkylating agent in a *TSC1*-deficient cell line [15]. In addition, the mTOR survival pathway was activated in lung and ovarian cancer cell lines that were treated with cisplatin, and sensitivity to cisplatin was enhanced by inhibiting the mTOR pathway [2, 3]. Further, preclinical and clinical data has shown a synergistic effect with cisplatin and an mTOR inhibitor [16]. In summary, DNA-damaging agents, including cisplatin, can activate PDK1, which results in inhibition of the mTOR survival pathway through TSC1 [6].

The results of this study should be interpreted with some caution. First, PFS was evaluated as an endpoint, but the PFS measurements may be not precise due to evaluation or measurement bias in the retrospective study design [17]. Nevertheless, responses were evaluated after every two to three cycles of platinum doublet using criteria from RECIST version 1.1. Second, druggable mutations, including *EGFR* activating mutations, and their effects on survival were not evaluated in this study because more than half of the patients were included before this testing was available in Korea. In the testing, validation, and combined data sets, the *TSC1* gene variant did not affect the use of targeted agents post-platinum doublet chemotherapy (data not shown). Regardless of these limitations, we believe that this study contributes translationally relevant information on *TSC1* gene variants. In conclusion, we found that the *TSC1* Met322Thr variant plays a predictive role in NSCLC patients treated with platinum doublet.

Perspectives

- Platinum doublet chemotherapy still play a pivotal role in treatment of advanced NSCLC, its predictive biomarker remains unknown.
- Genes related to mTOR pathway were analyzed with next-generation sequencing. We found that TSC1 Met322Thr conferred longer PFS in both testing and validation set (aHR and 95% CIs: 0.63 and 0.45–0.87, Cox $P<0.001$).
- TSC1 gene variant is an important predictive marker for platinum-based chemotherapy outcomes in NSCLC patients.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

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Author contribution

J.S.R. conceived and designed the present study. J.S.R., J.H.L., and H.J.K. performed the experiment. J.H.L., H.J.K., M.J.K., M.H.P., and J.S.K. collected, analyzed, and explained the experiment. J.S.R., M.J.K., M.H.P., and J.S.K drafted and critically revised the article. All authors approved the final submission.

Abbreviations

aHR, HR adjusted for age, gender, smoking habits, ECOG performance status, histology, and stage; AKT, protein kinase B; AMP, adenosine monophosphate; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; HR, hazard ratio; mTOR, mammalian target of rapamycin; NSCLC, non-small-cell lung cancer; OS, overall survival; PDK, pyruvate dehydrogenase kinase; PFS, progression-free survival; PI3KCA, phosphoinositide-3-kinase catalytic subunit-α; RECIST, Response Evaluation Criteria in Solid Tumor; TSC, tuberous sclerosis complex.

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Supplementary Information

Content:

Targeted next-generation sequencing

Target regions were captured from 1 μg of genomic DNA using the Agilent SureSelect Custom kit following the manufacturer's protocols (Agilent, Santa Clara, CA, USA). Briefly, DNA was sheared using the Covaris system (Covaris, Woburn, MA, USA) and purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA). The ends of the fragments were repaired and adaptors were ligated to the fragments. The resulting DNA library was purified using Agencourt AMPure XP beads and amplified by PCR. The quality and quantity of the DNA library was assessed with the Agilent 2100 Bioanalyzer. The DNA library was captured by hybridization to biotinylated RNA library baits. Bound genomic DNA was purified with streptavidin-coated magnetic Dynabeads (Invitrogen, Carlsbad, CA, USA) and then re-amplified. The targeted DNA library was sequenced on an Illumina Hiseq2000 with 100 base pair paired-end reads using protocols recommended by the manufacturer (Illumina, San Diego, CA, USA).

DNA preparation and quality control

DNA was isolated from buffy coat with QuickGene mini-310 (KURABO Industries, Osaka, Japan) and QuickGene DNA whole blood kit S (KURABO Industries, Osaka, Japan). The quantity and quality of the samples were determined using the Nano-drop® 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) and by gel electrophoresis using 1% Agarose E-gels (Invitrogen, Carlsbad, CA, USA).

The requirement for the genomic DNA source for the BioMark platform (Fluidigm) is high purity without degradation. DNA purity is indicated by OD_{260}/OD_{280} and OD_{260}/OD_{230} ratios. The OD_{260}/OD_{280} ratio should be 1.8–2.0, and the OD_{260}/OD_{230} ratio should be > 1.5. The BioMark platform requires a DNA concentration of 60 ng/μl.

Genotyping Using the TaqMan assay

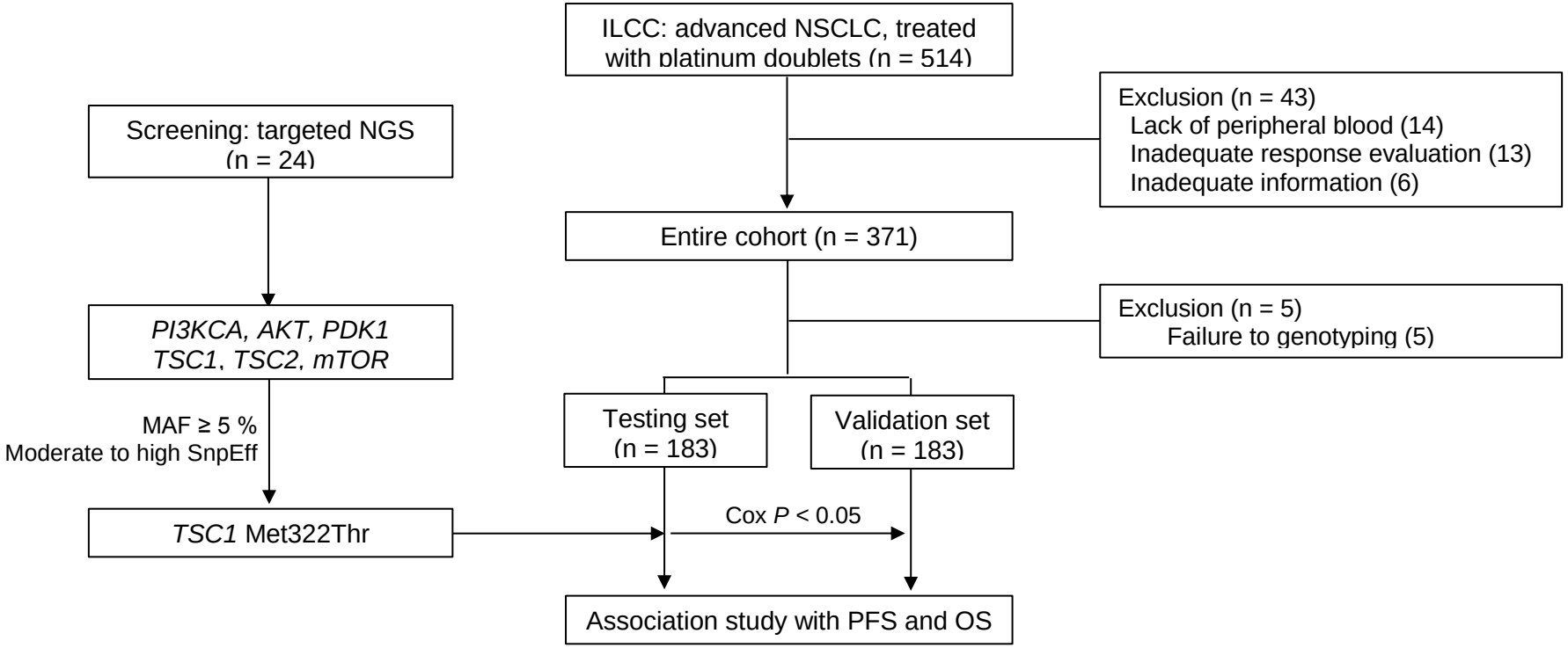
The TaqMan assay (Applied Biosystems) was performed according to the manufacturer's instructions. Genotyping of *TSC1* and *TSC2* was performed using the TaqMan fluorogenic 5’ nuclease assay (ABI, Foster City, CA, USA). The final polymerase chain reaction (PCR) volume was 5 μl and contained 10 ng of genomic DNA, 2.5 μl of TaqMan Universal PCR Master Mix, and 0.13 μl of 40X Assay Mix. The thermal cycle conditions were 50°C for 2 min to activate the uracil N-glycosylase and to prevent carry-over contamination, 95°C for 10 min to activate the DNA polymerase, and then 45 cycles of 95°C for 15 s and 60°C for 1 min. All of the reactions were performed using 384-well plates and a Dual 384-Well GeneAmp PCR System 9700 (ABI, Foster City, CA, USA). The endpoint fluorescent readings were

measured with an ABI PRISM 7900 HT Sequence Detection System (ABI, Foster City, CA, USA). Duplicate samples and negative controls were included to ensure the accuracy of the genotyping.

Supplementary Information

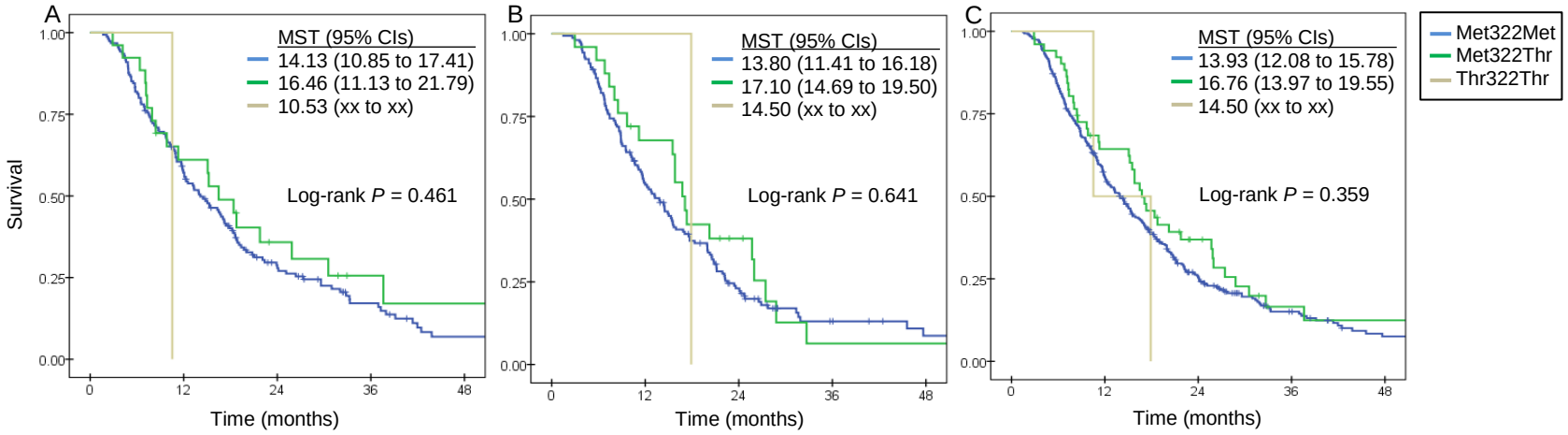
Content:

Supplementary Fig S1. Patients' enrollment and study scheme



Abbreviations: ILCC, Inha Lung Cancer Cohort; NSCLC, non-small-cell lung cancer; MAF. minor allele frequency; PFS, progression-free survival; OS, overall survival

Supplementary Fig S2. Overall survival of the patients by genetic variation of *TSC1* gene in testing (A), validation (B), and combined (C) sets: Kaplan-Meier plot



Abbreviations: MST, median survival time, months; CI, confidence interval

Supplementary Information

Content:

Supplementary Table S1. Information on genetic variations of *TSC1* or *TSC2* gene and their related genes identified by next generation sequencing

GENE	Chromosome	Position	SNPID	Minor allele frequencies	Call rate	HWE Permutation test	Effect	Impact
	9	135786904	rs1073123	0.0833	1	1	non-synonymous	moderate
	9	135776612	rs77262230	0.0208	1	1	intron	low
	9	135773164	rs12350315	0.0833	1	1	intron	low
	9	135776341	rs7020438	0.0833	1	1	intron	low
	9	135776845	rs7020175	0.0833	1	1	intron	low
	9	135777121	rs11243931	0.0833	1	1	intron	low
	9	135788227	rs7875422	0.0833	1	1	intron	low
	9	135788272	rs7875558	0.0833	1	1	intron	low
	9	135788287	rs7865232	0.0833	1	1	intron	low
	9	135776925	rs75802666	0.25	1	0.5964	intron	low
	9	135776034	rs1076160	0.4583	1	0.6797	intron	low
	9	135781239	rs118203567	0.0625	1	1	synonymous	low
	9	135772717	rs4962081	0.0833	1	1	synonymous	low
	9	135782221	rs7862221	0.0833	1	1	synonymous	low
	9	135804139	rs80258442	0.0625	1	1	upstream	low
	9	135777793	chr9_135777793	0.0208	1	1	downstream	modifier
TSC1	9	135782769	rs118203508	0.0208	1	1	downstream	modifier
	9	135796621	chr9_135796621	0.0208	1	1	downstream	modifier
	9	135797655	chr9_135797655	0.0208	1	1	downstream	modifier
	9	135780223	chr9_135780223	0.0417	1	1	downstream	modifier
	9	135777451	rs7044064	0.0833	1	1	downstream	modifier
	9	135779530	rs12345576	0.0833	1	1	downstream	modifier
	9	135782277	rs7872606	0.0833	1	1	downstream	modifier
	9	135782479	rs7872860	0.0833	1	1	downstream	modifier
	9	135767185	rs11553763	0.125	1	1	UTR_3_prime	modifier
	9	135767488	chr9_135767488	0.0208	1	1	UTR_3_prime	modifier
	9	135767517	chr9_135767517	0.0208	1	1	UTR_3_prime	modifier
	9	135770816	chr9_135770816	0.0208	1	1	UTR_3_prime	modifier
	9	135771567	chr9_135771567	0.0208	1	1	UTR_3_prime	modifier
	9	135769688	rs10491534	0.0417	1	1	UTR_3_prime	modifier
	9	135767943	rs1050700	0.2292	1	0.2889	UTR_3_prime	modifier
	9	135770347	rs2809244	0.375	1	0.6666	UTR_3_prime	modifier
	9	135770300	rs2809243	0.4167	1	1	UTR_3_prime	modifier
TSC2	16	2129027	chr16_2129027	0.0208	1	1	non-synonymous	moderate
	16	2130243	rs45517295	0.0208	1	1	non-synonymous	moderate

	16	2111779	rs17135764	0.25	1	1	intron	low
	16	2103408	chr16_2103408	0.0208	1	1	synonymous	low
	16	2113009	chr16_2113009	0.0208	1	1	synonymous	low
	16	2097110	rs2516740	0.0208	1	1	upstream	low
	16	2097158	rs2516739	0.0208	1	1	upstream	low
	16	2105115	chr16_2105115	0.0208	1	1	upstream	low
	16	2108740	chr16_2108740	0.0208	1	1	upstream	low
	16	2112481	chr16_2112481	0.0208	1	1	upstream	low
	16	2114033	chr16_2114033	0.0208	1	1	upstream	low
	16	2123248	chr16_2123248	0.0208	1	1	upstream	low
	16	2124148	chr16_2124148	0.0208	1	1	upstream	low
	16	2107489	rs2072314	0.0417	1	1	upstream	low
	16	2107288	chr16_2107288	0.0625	1	1	upstream	low
	16	2111571	chr16_2111571	0.0625	1	1	upstream	low
	16	2110571	rs2074968	0.3125	1	1	upstream	low
	16	2120402	rs7187438	0.3125	1	0.6353	upstream	low
	16	2106475	chr16_2106475	0.0208	1	1	downstream	modifier
	16	2106534	chr16_2106534	0.0208	1	1	downstream	modifier
	16	2131993	rs117153104	0.0417	1	1	downstream	modifier
	16	2115819	rs8063461	0.2083	1	1	downstream	modifier
	3	178916519	rs11709323	0.0833	1	1	intron	low
	3	178918696	chr3_178918696	0.0208	1	1	downstream	modifier
	3	178921838	chr3_178921838	0.0625	1	1	downstream	modifier
	3	178927111	rs56143971	0.1042	1	1	intron	low
PIK3CA	3	178927149	rs55685804	0.1042	1	1	intron	low
	3	178927345	rs3729682	0.1042	1	1	intron	low
	3	178935799	rs1568205	0.1042	1	1	upstream	low
	3	178942176	rs17849074	0.0417	1	1	intron	low
	3	178943947	chr3_178943947	0.0208	1	1	downstream	modifier
	14	105235558	rs2498801	0.2708	1	1	downstream	modifier
	14	105235824	rs58565216	0.0208	1	1	UTR_3_PRIME	modifier
	14	105235825	rs17846828	0.0208	1	1	UTR_3_PRIME	modifier
	14	105235860	rs3803305	0.0208	1	1	UTR_3_PRIME	modifier
	14	105236287	rs35416681	0.0208	1	1	UTR_3_PRIME	modifier
AKT1	14	105236557	rs17846826	0.0208	1	1	UTR_3_PRIME	modifier
	14	105237401	rs7140735	0.0417	1	1	INTRON	low
	14	105238670	rs8192700	0.0208	1	1	INTRON	low
	14	105238954	rs61761200	0.0208	1	1	INTRON	low
	14	105239146	rs3803304	0.1042	1	1	INTRON	low

	14	105239192	rs2494732	0.1667	1	1	intron	low
	14	105239894	rs1130233	0.3750	1	0.1938	synonymous_coding	low
	14	105240111	rs3730362	0.0208	1	1	intron	low
	14	105240450	rs78470418	0.0208	1	1	intron	low
	14	105240784	rs2494733	0.2917	1	1	intron	low
	14	105240885	rs2494734	0.2708	1	1	intron	low
	14	105241576	rs3730344	0.0208	1	1	intron	low
	14	105241660	rs17846812	0.0208	1	1	intron	low
	14	105242228	rs2498797	0.2917	1	1	intron	low
	14	105242831	rs3001371	0.3125	1	1	intron	low
	14	105242926	rs17846822	0.0208	1	1	intron	low
	14	105242966	rs2494735	0.2708	1	1	intron	low
	14	105243435	rs2498795	0.4375	1	0.2415	intron	low
	14	105246325	rs2494737	0.2708	1	0.6357	upstream	low
	14	105246384	rs17846818	0.0208	1	1	upstream	low
	14	105246407	rs3730358	0.0625	1	1	upstream	low
	14	105246565	rs17846816	0.0208	1	1	upstream	low
	14	105246681	rs35635404	0.0208	1	1	upstream	low
	14	105246686	rs2494738	0.4583	1	0.6797	upstream	low
	14	105246692	rs12588965	0.0208	1	1	upstream	low
	14	105246989	rs2494739	0.4583	1	0.6797	upstream	low
	14	105259706	rs10138227	0.0833	1	1	UTR_5_prime	modifier
	14	105259734	rs1130214	0.0833	1	1	UTR_5_prime	modifier
	14	105260301	rs28634999	0.0208	1	1	UTR_5_prime	modifier
	14	105261123	rs117096287	0.0625	1	1	upstream	low
	14	105261419	rs10144641	0.0208	1	1	upstream	low
	14	105262368	rs2498786	0.1458	1	1	upstream	low
	14	105262718	chr14_105262718	0.0208	1	1	upstream	low
	14	105262781	rs74090038	0.0833	1	1	upstream	low
	14	105262912	rs2494750	0.3125	1	1	upstream	low
	14	105262961	rs2494751	0.3125	1	1	upstream	low
	14	105263143	chr14_105263143	0.0625	1	1	upstream	low
	14	105263227	rs117871152	0.0208	1	1	upstream	low
	14	105263608	rs2494752	0.2917	1	0.6211	upstream	low
	1	11166480	chr1_11166480	0.0208	1	1	downstream	modifier
	1	11166713	rs2536	0.2083	1	1	UTR_3_prime	modifier
mTOR	1	11167146	rs12139042	0.2083	1	1	stop_gained	high
	1	11167760	rs12117235	0.2083	1	1	downstream	modifier
	1	11167829	rs12117241	0.2083	1	1	downstream	modifier

1	11169676	rs2275525	0.2083	1	1	intron	low
1	11175176	rs2000393	0.1458	1	1	upstream	low
1	11175270	rs2275522	0.2083	1	1	upstream	low
1	11181147	rs17848567	0.0208	1	1	intron	low
1	11181327	rs11121691	0.0417	1	1	synonymous_coding	low
1	11181457	rs17235633	0.2083	1	1	intron	low
1	11181630	rs12143194	0.2083	1	1	intron	low
1	11184489	rs868080	0.2083	1	1	intron	low
1	11186897	rs3737611	0.2083	1	1	downstream	modifier
1	11187342	chr1_11187342	0.0208	1	1	downstream	modifier
1	11187477	chr1_11187477	0.0208	1	1	downstream	modifier
1	11187662	chr1_11187662	0.0208	1	1	downstream	modifier
1	11189191	rs3730379	0.2083	1	1	downstream	modifier
1	11190546	rs17848555	0.0208	1	1	downstream	modifier
1	11192956	rs2275526	0.2083	1	1	synonymous_coding	low
1	11194591	rs3730381	0.2083	1	1	upstream	low
1	11194667	chr1_11194667	0.0208	1	1	upstream	low
1	11194836	rs12142442	0.2083	1	1	upstream	low
1	11199149	rs2275528	0.2083	1	1	non_synonymous_coding	moderate
1	11205058	rs1057079	0.2500	1	1	non_synonymous_coding	moderate
1	11205340	rs74630019	0.0208	1	1	upstream	low
1	11206690	rs2275942	0.2083	1	1	upstream	low
1	11209899	rs17036411	0.2083	1	1	upstream	low
1	11217665	rs72871466	0.2083	1	1	intron	low
1	11264828	rs2273127	0.2083	1	1	intron	low
1	11269796	rs1010447	0.0833	1	1	intron	low
1	11272529	rs28730685	0.0208	1	1	non_synonymous_coding	moderate
1	11273418	rs12116957	0.2083	1	1	intron	low
1	11276053	chr1_11276053	0.0417	1	1	intron	low
1	11288758	rs1064261	0.0833	1	1	synonymous_coding	low
1	11298362	chr1_11298362	0.0208	1	1	intron	low
1	11301714	rs1135172	0.0833	1	1	synonymous_coding	low
1	11301841	rs2076656	0.2083	1	1	intron	low
1	11302065	rs11121706	0.0833	1	1	intron	low
1	11303153	rs12141961	0.2083	1	1	intron	low
1	11303383	rs28730693	0.0417	1	1	intron	low
1	11307412	rs78613694	0.1042	1	0.2057	intron	low
1	11308509	rs56797473	0.2083	1	1	intron	low
1	11317310	rs12121319	0.2083	1	1	intron	low

	1	11318236	rs7525957	0.0833	1	1	intron	low
	1	11318763	rs2076657	0.2917	1	0.6211	intron	low
	1	11318885	rs17848582	0.0625	1	1	intron	low
	1	11318983	rs12142905	0.2083	1	1	intron	low
	1	11322156	rs1883965	0.0833	1	1	intron	low
	1	11322565	rs2295079	0.3125	1	0.3567	UTR_5_prime	modifier
	1	11322620	rs17027474	0.0208	1	1	upstream	low
	1	11322628	rs2295080	0.2917	1	0.6211	upstream	low
	2	173418939	rs10178654	0.1250	1	0.298	upstream	low
	2	173418990	rs10202550	0.1250	1	0.298	upstream	low
	2	173419009	rs74807097	0.0208	1	1	upstream	low
	2	173419115	rs77953241	0.0208	1	1	upstream	low
	2	173419805	rs10181851	0.2500	1	0.1131	upstream	low
	2	173420609	rs114945843	0.0417	1	1	upstream	low
	2	173435757	rs13394924	0.1250	1	0.298	downstream	modifier
	2	173460169	chr2_173460169	0.0208	1	1	intron	low
	2	173460195	rs13392808	0.1250	1	0.298	intron	low
	2	173460336	rs79485922	0.0208	1	1	intron	low
PDK1	2	173460640	rs12693005	0.1250	1	0.298	synonymous_coding	low
	2	173460803	rs1530864	0.1250	1	0.298	UTR_3_prime	modifier
	2	173461090	rs1530865	0.1250	1	0.298	UTR_3_prime	modifier
	2	173461799	rs70937083	0.1250	1	0.298	downstream	modifier
	2	173461820	rs11904158	0.2500	1	0.1131	downstream	modifier
	2	173462230	rs11904366	0.1250	1	0.298	downstream	modifier
	2	173462593	rs70937084	0.1250	1	0.298	UTR_3_prime	modifier
	2	173463138	rs2357637	0.1042	1	0.2057	UTR_3_prime	modifier
	2	173463175	chr2_173463175	0.0208	1	1	UTR_3_prime	modifier
	2	173463646	rs70937086	0.0208	1	1	UTR_3_prime	modifier
	2	173464187	rs13388338	0.1250	1	0.298	downstream	modifier

Supplementary Table S2. Progression-free survival of the patients by clinical characteristics and genetic variation of *TSC1* gene in testing, validation, and combined sets

Variables		Testing			Validation			Combined		
		mPFS	95% CI	log-rank P	mPFS	95% CI	log-rank P	mPFS	95% CI	log-rank P
Gender	Women	4.83	4.181 to 5.485	0.761	4.43	2.763 to 6.104	0.031	4.70	4.227 to 5.173	0.173
	Men	4.66	4.168 to 5.165		5.30	4.653 to 5.947		5.00	4.588 to 5.412	
Smoking habit	Never	4.63	3.768 to 5.498	0.613	5.10	4.510 to 5.690	0.626	4.40	3.564 to 5.236	0.009
	Ever	4.93	4.407 to 5.460		4.16	2.816 to 5.517		5.10	4.763 to 5.437	
ECOG PS	0 to 1	4.90	4.327 to 5.473	0.303	5.10	4.510 to 5.690	0.626	5.00	4.620 to 5.380	0.351
	2 or more	4.56	3.992 to 5.142		4.16	2.816 to 5.517		4.43	3.775 to 5.091	
Histology	ADC	4.00	3.020 to 4.980	0.001	4.60	3.900 to 5.300	0.004	4.46	3.834 to 5.099	<0.001
	SQC	5.20	4.656 to 5.743		6.26	5.653 to 6.880		5.76	4.911 to 6.622	
	Others	5.80	3.380 to 8.220		4.86	2.468 to 7.265		4.86	3.041 to 6.692	
Stage	IIIA	5.33	2.608 to 8.059	0.056	7.10	4.259 to 9.941	<0.001	7.06	4.367 to 9.767	<0.001
	IIIB	5.20	4.422 to 6.978		6.00	5.036 to 6.964		5.00	5.020 to 6.180	
	IV	4.56	3.829 to 5.305		4.86	4.306 to 5.427		4.63	4.236 to 5.031	
First line regimens	Platinum Plus									0.002
	Gemcitabine	5.00	4.329 to 5.671	0.341	5.10	4.356 to 5.844	0.002	5.06	4.659 to 5.474	
	Taxane	4.30	3.139 to 5.461		4.70	3.817 to 5.583		4.60	3.938 to 5.262	
	Irinotecan	4.66	4.496 to 4.838		5.93	4.360 to 7.506		4.86	4.326 to 5.407	
	Pemetrexed	4.00	0.594 to 7.406		4.43	2.398 to 6.469		4.00	2.268 to 5.732	
	Others	9.16	4.515 to 13.819		26.90	x to x		10.40	6.796 to 14.004	
Response	CR or PR	5.76	4.405 to 7.128	<0.001	6.56	5.937 to 7.196	<0.001	6.33	5.666 to 7.000	<0.001
	SD	5.56	4.749 to 6.384		5.30	4.883 to 5.717		5.30	4.788 to 5.812	
	PD	2.26	1.803 to 2.731		2.33	2.114 to 2.553		2.30	2.081 to 2.519	
	Not evaluated	5.33	xx to xx		-	-		5.33	xx to xx	
TSC1 Met322Thr	Met/Met	4.63	4.186 to 5.081	<0.001	4.90	4.309 to 5.491	<0.001	4.70	4.356 to 5.044	<0.001
	Met/Thr	5.93	4.018 to 7.849		6.46	4.312 to 8.621		6.10	4.684 to 7.516	
	Thr/Thr	1.36	xx to xx		1.43	x to x		1.36	xx to xx	

Abbreviations: mPFS, median progression free survival, months; ECOG PS, Eastern Cooperative Oncology Group performance status; SQC, squamous cell carcinoma; ADC, adenocarcinoma; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease