Association of Genetic Polymorphisms With Afatinib-induced Diarrhoea

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Abstract. *Background/Aim: Afatinib, a 2nd generation epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) used in treatment of non-small cell lung cancer (NSCLC), causes diarrhoea in over 90% of patients. The association of genetic background with diarrhoea is poorly understood. Patients and Methods: We evaluated the roles of four single nucleotide polymorphisms (SNPs) in ATP binding cassette subfamily B member 1 (ABCB1) and ATP binding cassette subfamily G member 2 (ABCG2) genes–ABCB1 1236 C>T, 2677 G>T/A, and 3435 C>T, and ABCG2 421 C>A–on treatment-induced diarrhoea in 38 patients with NSCLC treated with afatinib. Results: Diarrhoea occurred more frequently in patients with ABCB1 2677 T(A)/T(A) (14/16, 87.5%) than in patients with non-T(A)/T(A) alleles (8/22, 36.4%) (p=0.003). ABCB1 2677 T(A)/T(A) was significantly predictive of diarrhoea (p=0.002) by multivariable regression analysis. Conclusion: Afatinib-induced diarrhoea is associated with the SNP ABCB1 2677 T(A)/T(A).*

Afatinib, a second-generation epidermal growth factor-tyrosine kinase inhibitor (EGFR-TKI), is an irreversible ErbB family blocker that is approved for the treatment of EGFR-mutated non-small cell lung cancer (NSCLC) (1). Although treatment with this drug has substantial clinical benefits, the frequency of adverse effects (AEs), such as diarrhoea (95.2% of patients) and rash or acne (89.1%), is high (2). Rash and diarrhoea of grade ≥3 are significantly more frequent with afatinib therapy than with erlotinib or

gefitinib (3). The management of AEs is critically important and occasionally requires reduced dosage or discontinuation of afatinib. Genetic background plays a role in patients' susceptibility to AEs, but its contribution to afatinib-induced AEs is unknown.

Afatinib is a substrate and an inhibitor of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), which are efflux adenosine triphosphate-binding cassette (ABC) transporters expressed on apical membranes of enterocytes (4). These transporters are involved in the absorption and excretion of various drugs. In combination with ritonavir, a P-gp inhibitor, plasma levels of afatinib increase; in contrast, they decrease with rifampicin, a P-gp inducer (5). There are several genetic polymorphisms in the *ATP binding cassette subfamily B member 1* (*ABCB1*) gene, which encodes P-gp, and the *ATP binding cassette subfamily G member 2* (*ABCG2*) gene, which encodes BCRP. The most common functional single nucleotide polymorphisms (SNPs) are 1236 C>T, 2677 G>T/A, and 3435 C>T in *ABCB1* (6), and 421 C>A in *ABCG2* (7). These SNPs can alter plasma levels of oral molecular targeted drugs. For example, patients with NSCLC possessing all T alleles in the *ABCB1* haplotype (1236C>T, 2677G>T/A, 3435C>T) had higher plasma concentrations of erlotinib than patients possessing the other haplotypes (8).

Polymorphisms in *ABCB1* and *ABCG2* might affect afatinib clearance, which might lead to an excessively high plasma concentration and an increase in AEs. To evaluate this point, we analysed the association between AEs induced by afatinib and SNPs in *ABCB1* and *ABCG2*.

Patients and Methods

Patient selection. Thirty-eight NSCLC patients administered afatinib at Saga University Hospital between April 2014 and December 2017 were enrolled. Patients whose treatment with afatinib was initiated at doses other than 40 mg/day and who had received thoracic radiotherapy were excluded from the study. A medical record survey was performed for each patient to record sex, age, body surface area (BSA), Eastern Cooperative Oncology Group performance status

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Table I. *Patient characteristics.*

Characteristic	Number or mean and SD
N	38
Gender	16/22
Age	65.4±10.2
BSA	1.59±0.19
ECOG PS	
0	14
1	21
2	2
3	1
AST	24.9±9.3
ALT	22.4±19.3
T-Bil	0.8±0.5
SCr	0.75±0.19
ALB	3.65±0.44
EGFR gene mutation	
Exon19 del	21 (55.3%)
L858R	12 (31.6%)
Other	5 (13.2%)
Number of other EGFR TKI used	
0	20 (52.6%)
1	11 (28.9%)
2	7 (18.4%)
Final dose of afatinib	
40 mg/day	4 (10.5%)
30 mg/day	19 (50.0%)
20 mg/day	14 (36.8%)
10 mg/day	1 (2.6%)
Dosing period (days)	241.0±197.8 (n=32)

(ECOG PS), aspartate aminotransferase, alanine aminotransferase, total bilirubin, serum creatinine, serum albumin, afatinib dosage and administration period, history of dosage reduction, AEs (diarrhoea, rash, other), concomitant drugs, and previous treatment history. The records were evaluated by the attending physician and other doctors to establish reliability for the detection of AEs. All data were treated anonymously, and private information of all patients was protected. All patients gave informed consent for collection of peripheral blood and genomic testing according to the Declaration of Helsinki. This study was approved by the ethics committee of the Saga University Hospital Institutional Review Board (approval No 2017-06-04).

Genotyping ABCB1 and ABCG2. Genotyping was performed for the following SNPs: *ABCB1* 1236C>T (rs1128503), *ABCB1* 2677G>T/A (rs2032582), *ABCB1* 3435C>T (rs1045642), and *ABCG2* 421C>A (rs2231142). Genomic DNA was prepared from nucleated peripheral blood cells collected in 3.8% citric acid using the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). Purified genomic DNA was stored at –20˚C. Allelic variations were determined by real-time PCR using the TaqMan® SNP genotyping assay (Applied Biosystems, Foster City, CA, USA) and the StepOne™ Real-Time PCR System (Applied Biosystems). Amplification conditions consisted of an initial denaturation step at 95˚C for 10 min, followed by 50 cycles of denaturation at 95˚C for 15 s, and finally annealing and extension at 60˚C for 90 s.

Statistical analyses. First, the categorical variables–gender and adverse effects–were compared among genotype groups with Fisher's exact test. Second, the continuous parameters-age, BSA, the elapsed time until 1st dose reduction, and the length of the dosing period–were compared with the Mann-Whitney *U*-test. Third, multivariable logistic regression analysis was performed to identify risk factors for diarrhoea among several independent factors identified by the aforementioned tests. Finally, the incidence of diarrhoea was estimated using Kaplan-Meier analysis, and genotype groups were compared with the log-rank test. All statistical analyses were performed using JMP® 13 software (SAS Institute Inc., Cary, NC, USA). Differences were considered significant at $p \le 0.05$.

Results

Patient characteristics. Table I shows the characteristics of the 38 patients enrolled in this study. Ages of the patients ranged from 55 to 75 years (average=65 years). Twenty patients (52.6%) took afatinib as their 1st EGFR-TKI, whereas 11 (28.9%) and seven (18.4%) took afatinib as their 2nd or 3rd TKI, respectively. The average length of afatinib treatment period was 241 (±197.8) days. All patients started afatinib treatment at a dose of 40 mg/day, but only four (10.5%) could continue at the initial dosage.

Association of ABCB1 and ABCG2 genotypes with afatinib-induced AEs. We divided patients into T/T and non-T/T groups for *ABCB1* SNPs 1236, 2677, and 3435, and into A/A and non-A/A groups for *ABCG2* SNP 421. Table II presents the demographic and clinical data of patients in the two groups of each SNP. The prevalence of all AEs was 89.5%; the prevalence of diarrhoea was 60.5% and that of rash was 42.1%. Of patients with diarrhoea, 9.0% had grade 1, 27.3% had grade 2, 59.1% had grade 3, and 4.5% had grade 4 (the total does not equal to 100% because of rounding). Of patients with rash, 12.5% had grade 1 and 87.5% had grade 2. The other 12 AEs included fever, loss of appetite, hepatic dysfunction, and renal dysfunction. Patients with T(A)/T(A) at *ABCB1* 2677 were significantly more prone to diarrhoea (14/16, 87.5%) than those with the non-T(A)/T(A) genotype (8/22, 36.4%) (*p*=0.003). No significant difference was observed with the other SNPs. For patients with the *ABCB1* 2677 T(A)/T(A) genotype, the high prevalence of diarrhoea led to a shorter period before the first dose reduction: 26.1±44.0 days with (T(A)/T(A)), 62.8±89.4 days with non-T(A)/T(A); *p*=0.12). However, the *ABCB1* 2677 T(A)/T(A) genotype group had a higher proportion of males and more patients with lower BSA than the non-T(A)/T(A) group. Thus, we performed multiple regression analyses with gender, BSA, T/T of *ABCB1* 2677 and 3435 as factors to predict diarrhoea (Table III). The *ABCB1* 2677 T(A)/T(A) genotype showed a significantly higher odds ratio (*p*=0.002). Even with the Kaplan-Meier curve, the incidence of first diarrhoea was significantly higher in the *ABCB1* 2677 T(A)/T(A) group (*p*=0.006)

Table II. *Comparisons among genotypes.*

	ABCB1									ABCG2		
	C1236T			G2677T(A)			C3435T			C421A		
	T/T (n=15)	Non T/T (n=23)	*p*-Value	T(A)/T(A) (n=16)	Non T(A)/T(A) (n=22)	*p*-Value	T/T (n=7)	Non T/T (n=31)	*p*-Value	A/A (n=4)	Non A/A (n=34)	*p*-Value
Male/Female	5/10	11/12	0.51	11/5	5/17	0.008	3/4	13/18	1	2/2	14/20	1
Age	64.4±7.4	66.1±11.8	0.37	62.6±11.4	67.5±8.9	0.12	63.3±9.7	65.9±10.4	0.56	59.3±4.5	66.1±10.4	0.1
BSA	1.60±0.22	1.59±0.18	0.87	1.72±0.15	1.50±0.17	<0.001	1.69±0.13	1.57±0.2	0.09	1.61±0.13	1.59±0.2	0.79
ECOG PS (≥2/<2)	1/14	2/21	1	2/14	1/21	0.56	1/6	2/29	0.48	0/4	3/31	1
AST (≥30/<30)	3/12	4/19	1	5/11	2/20	0.11	1/6	6/25	1	1/3	6/28	1
ALT (≥40/<40)	1/14	2/21	1	3/13	0/22	0.07	1/6	2/29	0.48	1/3	2/32	0.29
T-Bil (≥1/<1)	4/11	4/19	0.69	3/13	5/17	1	1/6	7/24	1	0/4	8/26	0.56
SCr (≥0.8/<0.8)	4/11	11/12	0.31	8/8	7/15	0.32	2/5	13/18	0.68	2/2	13/21	1
ALB (≥4/<4)	5/10	3/20	0.22	6/10	2/20	0.05	3/4	5/26	0.15	0/4	8/26	0.56
Adverse effect	14 (93.3%)	20 (87.0%)	1	16 (100.0%)	18 (81.8%)	0.12	7 (100.0%)	27 (87.1%)	1	4 (100.0%)	30 (88.2%)	1
Diarrhoea	9 (60.0%)	13 (56.5%)	1	14 (87.5%)	8 (36.4%)	0.003	6 (85.7%)	16 (51.6%)	0.2	2 (50.0%)	20 (58.8%)	1
(≥grade 3)	4 (44.4%)	10 (76.9%)	0.19	8 (57.1%)	6 (75.0%)	0.65	3 (50.0%)	11 (68.8%)	0.62	1 (50.0%)	13 (65.0%)	1
Rush (Paronychia)	6 (40.0%)	10 (43.5%)	1	6 (37.5%)	10 (45.5%)	0.74	2 (28.6%)	14 (45.2%)	0.68	1 (25.0%)	15 (44.1%)	0.62
Others	7 (46.7%)	5 (21.7%)	0.16	8 (50.0%)	4 (18.2%)	0.08	5 (71.4%)	7 (22.6%)	0.02	2 (50.0%)	10 (29.4%)	0.58
Period until 1st dose reduction (Day)	42.6±68.8 (n=14)	47.6±42.6 (n=20)	0.31	26.1±44.0	62.8±89.4 (n=18)	0.12	11.4±3.0	54.4±79.9 (n=27)	0.06	15.6±8.3	49.5±77.1 (n=30)	0.53
Dosing period (Day)	199.8±164.7	269.2±217.4	0.54	224.4±186.9	253.9±210.4	0.69	219.7±203.9	245.9±200.2	0.92	237.8±248.1	241.4±195.2	0.82

(Figure 1). However, median values of time to first diarrhoea with *ABCB1* 2677 T(A)/T(A) and non-T(A)/T(A) were 13 days (IQR=9.75-17.5) and 10 days (IQR=6.5-17.5), respectively, which did not differ significantly.

Discussion

In this study, patients with *ABCB1* 2677 T(A)/T(A) had a significantly higher incidence of diarrhoea than non-T(A)/T(A) patients. Other SNPs reported to be capable of altering the function of drug transporters–*ABCB1* C1236T, C3435T, and *ABCG2* C421A–had less effect on the incidence of AEs. This result differs from existing findings of the effect of gene polymorphisms on afatinib. Hayashi *et al*. (9) reported that the afatinib plasma concentration was higher and diarrhoea was more severe in patients carrying the A allele of *ABCG2* C421A. However, in this study, *ABCB1* G2677T(A) was not investigated.

The effects of *ABCB1* polymorphisms on pharmaco-kinetics have been reported for many drugs, including

Table III. *Multivariable logistic regression analysis to predict afatinib-induced diarrhoea.*

Risk factor of diarrhoea	Odds ratio (95%Cl)	*p*-Value
Male	0.26 (0.02-4.46)	0.33
BSA↑	0.59 (0.003-126)	0.85
ABCB1 2677 T(A)/T(A)	46.6 (2.2-989)	0.002
ABCB1 3435 T/T	0.44 (0.02-9.9)	0.61

molecular-targeted drugs (6). One of the main functions of *ABCB1* is the first-pass exclusion of orally administered drugs by releasing drugs from the epithelium of the bile duct, small intestine, and colon. *ABCB1*-mediated drug disposition can be influenced by modulation of its gene expression and activity. Presence of 2677 T and 3435 T alleles decreases the expression level of *ABCB1* in liver tissue (10). In this study, only the *ABCB1* 2677 polymorphism was associated with diarrhoea. Lamba *et al*.
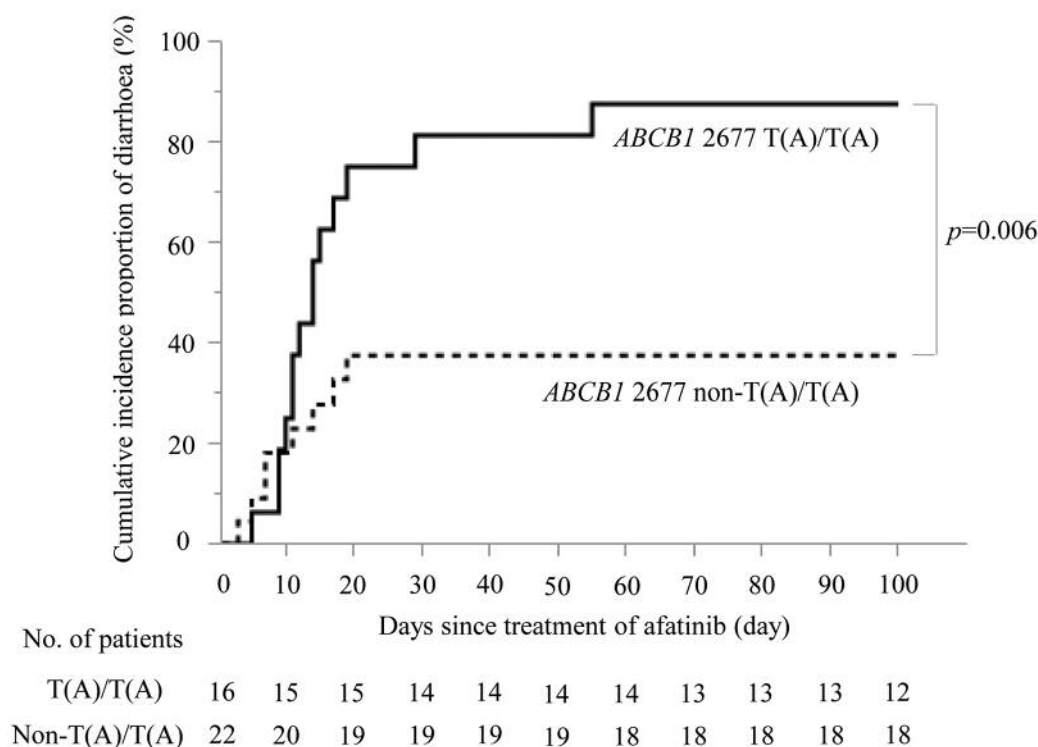
Figure 1. *Cumulative incidence proportion of first diarrhoea after afatinib treatment.*

(11) reported that patients with the *ABCB1* 2677 G/G genotype had higher P-gp expression in their intestines than those with the *ABCB1* 2677 T/T genotype. When the level of P-gp protein decreases because of the presence of functional SNPs, the first-pass exclusion effect decreases and the plasma concentration of the anticancer agent increases, which might cause strong AEs.

The exact mechanisms by which EGFR-TKIs cause diarrhoea remain unclear, but they are considered to involve multiple processes, such as changes in digestive tract movements or intestinal flora, and excess chloride secretion (12). In this study, more than half of the patients treated with afatinib (55.2%) suffered from diarrhoea. Appropriate management of diarrhoea therefore plays a key role in whether the physician is able to continue treatment with this effective drug. Yang *et al*. (13) reported that an initial afatinib dose of 30 mg daily provided a similar response rate and progression-free survival as an initial dose of 40 mg daily, and resulted in a significantly lower incidence of diarrhoea. Furthermore, a low starting dose of afatinib therapy showed promising clinical efficacy and good tolerability, but patients were not assessed for gene polymorphisms (14). Our study suggests the need to initiate treatment with low doses of afatinib for *ABCB1* 2677 T(A)/T(A) patients.

The primary limitation of this study was that it was a small sample size, single-centre, retrospective study. Additionally, only a limited number of SNPs were analysed, and the small sample size prevented us from performing statistical analysis of afatinib concentrations in the blood with respect to genotype. A prospective study is needed that has a sufficiently large sample size to assess how drug concentrations in the blood are associated with genotype.

Our results suggest that afatinib-induced diarrhoea leading to dose reduction, may be associated with the genetic polymorphism *ABCB1* 2677 T(A)/T(A). Afatinib has a high response rate with uncommon mutations except T790M and those in exon 20 (15). Therefore, afatinib is thought to have an important role among other EGFR-TKI therapies. However, compared with other EGFR-TKIs, afatinib is associated with a higher incidence of diarrhoea. By taking genetic polymorphisms into account and properly managing the dose of afatinib, it will be possible to reduce the number of patients suffering from diarrhoea.

Conflicts of Interest

Authors' Contributions

C.N., K.K., Y.N., S.K. and N.S.-A. conceptualized the study, drafted the initial manuscript, and reviewed and revised the manuscript. R.S., T.N., K.T. and S.K. collected data, carried out the initial analyses, and reviewed and revised the manuscript. All Authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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References

1 Yang JC, Shih JY, Su WC, Hsia TC, Tsai CM, Ou SH, Yu CJ, Chang GC, Ho CL, Sequist LV, Dudek AZ, Shahidi M, Cong XJ, Lorence RM, Yang PC and Miller VA: Afatinib for patients with lung adenocarcinoma and epidermal growth factor receptor mutations (LUX-Lung 2): a phase 2 trial. Lancet Oncol *13*: 539-548, 2012. PMID: 22452895. DOI: 10.1016/S1470-2045(12)70086-4

2 Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, Geater SL, Orlov S, Tsai CM, Boyer M, Su WC, Bennouna J, Kato T, Gorbunova V, Lee KH, Shah R, Massey D, Zazulina V, Shahidi M and Schuler M: Phase III study of afatinib orcisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol *31*: 3327-3334, 2013. PMID: 23816960. DOI: 10.1200/JCO.2012.44.2806

3 Takeda M, Okamoto I and Nakagawa K: Pooled safety analysis of EGFR-TKI treatment for EGFR mutation-positive non-small cell lung cancer. Lung Cancer *88*: 74-79, 2015. PMID: 25704957. DOI: 10.1016/j.lungcan.2015.01.026

4 Terada T and Hira D: Intestinal and hepatic drug transporters: pharmacokinetic, pathophysiological, and pharmacogenetic roles. J Gastroenterol *50*: 508-519, 2015. PMID: 25773773. DOI: 10.1007/s00535-015-1061-4

5 Wind S, Schnell D, Ebner T, Freiwald M and Stopfer P: Clinical pharmacokinetics and pharmacodynamics of afatinib. Clin Pharmacokinet *56*: 235-250, 2017. PMID: 27470518. DOI: 10.1007/s40262-016-0440-1

6 Wolking S, Schaeffeler E, Lerche H, Schwab M and Nies AT: Impact of genetic polymorphisms of ABCB1 (MDR1, P-Glycoprotein) on drug disposition and potential clinical implications: Update of the literature. Clin Pharmacokinet *54*: 709-735, 2015. PMID: 25860377. DOI: 10.1007/s40262-015-0267-1

7 Imai Y, Nakane M, Kage K, Tsukahara S, Ishikawa E, Tsuruo T, Miki Y and Sugimoto Y: C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. Mol Cancer Ther *1*: 611-616, 2002. PMID: 12479221.

8 Hamada A, Sasaki J, Saeki S, Iwamoto N, Inaba M, Ushijima S, Urata M, Kishi H, Fujii S, Semba H, Kashiwabara K, Tsubata Y, Kai Y, Isobe T, Kohrogi H and Saito H: Association of ABCB1 polymorphisms with erlotinib pharmacokinetics and toxicity in Japanese patients with non-small-cell lung cancer. Pharmacogenomics *13*: 615-624, 2012. PMID: 22462752. DOI: 10.2217/pgs.11.176

9 Hayashi H, Iihara H, Hirose C, Fukuda Y, Kitahora M, Kaito D, Yanase K, Endo J, Ohno Y, Suzuki A and Sugiyama T: Effects of pharmacokinetics-related genetic polymorphisms on the side effect profile of afatinib in patients with non-small cell lung cancer. Lung Cancer *134*: 1-6, 2019. PMID: 31319966. DOI: 10.1016/j.lungcan.2019.05.013

10 Shou W, Wang D, Zhang K, Wang B, Wang Z, Shi J and Huang W: Gene-wide characterization of common quantitative trait loci for ABCB1 mRNA expression in normal liver tissues in the Chinese population. PLoS One *7*: e46295, 2012. PMID: 23050008. DOI: 10.1371/journal.pone.0046295

11 Lamba J, Strom S, Venkataramanan R, Thummel KE, Lin YS, Liu W, Cheng C, Lamba V, Watkins PB and Schuetz E: MDR1 genotype is associated with hepatic cytochrome P450 3A4 basal and induction phenotype. Clin Pharmacol Ther *79*: 325-338, 2006. PMID: 16580901. DOI: 10.1016/j.clpt.2005.11.013

12 Yang JC, Reguart N, Barinoff J, Köhler J, Uttenreuther-Fischer M, Stammberger U, O'Brien D, Wolf J and Cohen EE: Diarrhea associated with afatinib: An oral ErbB family blocker. Expert Rev Anticancer Ther *13*: 729-736, 2013. PMID: 23506556. DOI: 10.1586/era.13.31

13 Yang CJ, Tsai MJ, Hung JY, Lee MH, Tsai YM, Tsai YC, Hsu JF, Liu TC, Huang MS and Chong IW: The clinical efficacy of Afatinib 30 mg daily as starting dose may not be inferior to Afatinib 40 mg daily in patients with stage IV lung adenocarcinoma harboring exon 19 or exon 21 mutations. BMC Pharmacol Toxicol *18*: 82, 2017. PMID: 29237484. DOI: 10.1186/s40360-017-0190-1

14 Yokoyama T, Yoshioka H, Fujimoto D, Demura Y, Hirano K, Kawai T, Kagami R, Washio Y, Ishida T, Kogo M, Tomii K, Okuno T, Akai M, Hirabayashi M, Nishimura T, Nakahara Y, Kim YH, Miyakoshi C, Yoshimura K, Hirai T and Kyoto Thoracic Oncology Research Group (KTORG): A phase II study of low starting dose of afatinib as first-line treatment in patients with EGFR mutation-positive non-small-cell lung cancer (KTORG1402). Lung Cancer *135*: 175-180, 2019. PMID: 31446992. DOI: 10.1016/j.lungcan.2019.03.030

15 Yang JC, Sequist LV, Geater SL, Tsai CM, Mok TS, Schuler M, Yamamoto N, Yu CJ, Ou SH, Zhou C, Massey D, Zazulina V and Wu YL: Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. Lancet Oncol *16*: 830-838, 2015. PMID: 26051236. DOI: 10.1016/S1470-2045(15)00026-1