Cancer Therapy: Clinical

Association of Epidermal Growth Factor Receptor Polymorphism, Skin Toxicity, and Outcome in Patients with Squamous Cell Carcinoma of the Head and Neck Receiving Cetuximab-Docetaxel Treatment

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Abstract

Purpose: Cetuximab, a monoclonal antibody targeting epidermal growth factor receptor (EGFR), has shown clinical efficacy in squamous cell carcinoma of the head and neck with prolonged progression-free (PFS) and overall survival (OS). In this study, we analyzed whether cetuximab-induced skin rash was correlated with distinct polymorphisms within the *EGFR* gene known to modulate EGFR expression, ligand binding, or signaling activity.

Experimental Design: Fifty-one patients enrolled in a single-arm phase II multicenter study for second-line treatment of recurrent or metastatic squamous cell carcinoma of the head and neck with cetuximab/docetaxel were genotyped for two genetic variations in the *EGFR* gene, a point substitution G→A in exon 13 resulting in an amino acid substitution in position 521 (EGFR-R521K) and a CA repeat (CA-SSR) polymorphism in intron 1. Association between genotypes and incidence/grade of skin rash was determined by Fisher's exact test. The predictive value of genotypes for PFS and OS was determined using the log-rank test.

Results: Overall, 21 patients (41%) developed skin rash with grade >1 within 6 weeks of treatment. The common EGFR-R521K genotype (G/G) was significantly associated with increased skin toxicity ($P = 0.024$) and showed a trend toward reduced risk of tumor progression (hazard ratio, 0.55; 95% confidence interval, 0.27-1.08; $P = 0.08$), whereas no correlation of the EGFR-R521K genotype with OS could be observed ($P = 0.20$). No significant interaction between CA-SSR polymorphism and skin toxicity, PFS, or OS could be detected.

Conclusions: Our study revealed an influence of the EGFR-R521K genotype on skin toxicity and suggested its relation to clinical activity of cetuximab/docetaxel treatment. *Clin Cancer Res; 16(1); 304–10. ©2010 AACR.*

Epidermal growth factor receptor (EGFR) and its ligands play a fundamental role in signaling transduction pathways involved in DNA repair, tumor cell survival, proliferation, and metastasis. In addition, EGFR overexpression in tumor tissue has frequently been associated with poor clinical outcome. In squamous cell carcinoma of the head and neck (SCCHN), overexpression of EGFR has been shown (1, 2) and associated with decreased response to therapy (3, 4) and reduced disease-free and overall

survival (OS; ref. 4, 5). Because of its prevalence and its crucial role in the pathogenesis, targeting EGFR has become a rational approach for treatment of SCCHN. Indeed, the combination of cetuximab with radiotherapy (6) or platinum-containing chemotherapy regimens (7) has already shown significant improvement of treatment outcome. In search for biomarkers allowing prospective identification of patients with significant benefit of EGFR targeting therapy, the role of gene amplification, protein expression levels, and activating mutations in EGFR itself or in key molecules downstream its specific signaling pathway have been evaluated by numerous studies. They revealed significant association between these biomarkers and the efficacy of treatment in lung (8–11) and colorectal cancer (12, 13). However, activating mutations in EGFR and downstream signaling molecules are rather rare in SCCHN and, together with EGFR expression levels, seem not to influence treatment efficacy in SCCHN (14, 15). However, the latter study was the first to reveal a significant association between the development of skin rash of at least

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Translational Relevance

Anti–epidermal growth factor receptor (EGFR) therapy with cetuximab for treatment of squamous cell carcinoma of the head and neck has shown promising results even in patients with recurrent/metastatic disease who experienced disease progression on platinum therapy. As potential mechanisms that may reduce sensitivity to cetuximab treatment, EGFR amplification as well as activating K-ras mutations have been identified in colorectal cancer, but such genetic aberrations are rarely detected in squamous cell carcinoma of the head and neck. Here, we show for the first time that a germline polymorphism of *EGFR* (EGFR-R521K), which has been associated with EGFR ligand binding and its mitogenic activity, could predict the occurrence of cetuximab-related skin toxicity, the most frequent adverse side effect of cetuximab treatment that has also been associated with its clinical efficacy. Indeed, patients with the G/G genotype of EGFR-R521K who significantly developed skin rash more often showed a trend to prolonged progression-free survival on cetuximab/docetaxel treatment. Larger clinical trials are needed to confirm and validate our preliminary findings.

grade 2 and OS of SCCHN patients treated with erlotinib (15), an observation subsequently made also in other tumor entities (16). The molecular basis for this association is still unresolved; however, two genetic variations in the *EGFR* gene affecting either EGFR expression levels (17) or ligand binding affinity (18) have been proposed as possible cause. The first one, a highly polymorphic microsatellite sequence in intron 1 (9-23 CA simple sequence repeat here called CA-SSR) has been shown to influence the expression levels of EGFR in a way that the longer the CA repeat, the lower the EGFR expression is (19) and the less effective EGFR targeting is in SCCHN cell line models (17). The second one is a single nucleotide polymorphism (G→A) in exon 13 (called EGFR-R521K in this study) that leads to an amino acid exchange from arginine to lysine with the EGFR-R521K variant (A genotype) showing less affinity to EGF and transforming growth factor-α and less mitogenic activity than the common G genotype (18). In the present study, we evaluated the potential of these two genetic variations to predict for the development of skin rash and treatment outcome in patients with advanced SCCHN receiving cetuximab/docetaxel treatment.

Materials and Methods

Patients and treatment. Eighty-four patients with histologically confirmed recurrent or initially metastatic SCCHN were enrolled in a phase II multicentric clinical trial for treatment with cetuximab/docetaxel. Further eligibility criteria were tumor relapse after cisplatin-containing

chemoradiotherapy or after platinum-containing first-line chemotherapy; no intermittent anticancer treatment since platinum failure; Eastern Cooperative Oncology Group performance status 0 to 1; adequate bone marrow, liver, and renal function; and signed written informed consent. Eligible patients received a maximum of six cycles of 35 mg/m^2 of docetaxel administered on day 1, 8 and 15, repeated on day 29 in the absence of disease progression or severe toxicity. Cetuximab was administered at an initial dose of 400 mg/m^2 followed by subsequent weekly doses of 250 mg/m^2 until disease progression or severe toxicity. Tumor assessment was done after every 8 wk. Evaluation of treatment response was done according to Response Evaluation Criteria in Solid Tumors. Skin toxicity was recorded according to National Cancer Institute common toxicity criteria, version 3. Highest recorded grade of skin rash during the first cycle of treatment was used for evaluation. All patients for whom skin reactions during treatment had been documented and from whom tumor biopsy material was available (*n* = 51 from 5 of 10 participating clinical study centers) were included in the accompanying translational research study presented here.

DNA extraction and EGFR polymorphism analysis. Genomic DNA was extracted from paraffin-embedded tissue samples using QIAmp DNA FFPE Tissue kit (Qiagen) according to the manufacturer's instruction. DNA content and quality was determined using the Nanodrop 1000 spectrophotometer. The EGFR-R521K polymorphism (rs11543848) was analyzed by RFLP-PCR as described previously (20). Briefly, for amplification of the designated region in exon 13 of the EGFR gene, the forward primer (5′-TGC TGT GAC CCA CTC TGT CT-3′) and reverse primer (5′-CCA GAA GGT TGC ACT TGT CC-3′) were used. Each PCR was done in a total volume of 50 μL containing 150 ng genomic DNA, 5 μL 10× PCR buffer including 1.5 mmol/L MgCl$_2$ (Roche), 200 nmol/L of each primer, 1 μL Nucleotide Mix (Roche) equivalent to 200 nmol/L of each nucleotide, and 2.5 units of Taq DNA Polymerase (Roche). After initial denaturation at 94°C for 3 min, the reaction was carried out at 94°C denaturation for 30 s, 58.6°C annealing for 30 s, and 72°C elongation for 30 s for a total of 30 cycles. Twenty microliters of PCR product was digested overnight with BstNI restriction enzyme (New England Biolabs) at 60°C. Fragments were separated on a 4% agarose gel (NuSieve, Lonza). Results were visualized by staining gels for 45 min with Sybr Green (Sigma). For validation of RFLP-PCR results, EGFR-R521K genotypes from four samples were confirmed by sequence analysis of the PCR product.

Genotyping of EGFR CA-SSR (rs11568315) was done by PCR and consecutive double-strand sequencing. Primers to amplify the CA repeat region were designed using the Primer3 program. The forward primer 5′-GGG CTC ACA GCA AAC TTC TC-3′ and reverse primer 5′-AAG CCA GAC TCG CTC ATG TT-3′ were used.

Statistical analysis. The influence of EGFR polymorphisms on the incidence/severity of skin toxicity, as the

primary hypothesis of this study, and on the disease control rate [DCR; partial remission (PR) and stable disease (SD)] was assessed using Fisher's exact test. The level of significance was set at $P < 0.05$. The P values from the additional statistical analyses of associations between EGFR polymorphisms and progression-free survival (PFS) or OS were regarded as exploratory because the cohort size had not the statistical power for such type of analysis. PFS was calculated from the date of initiation of cetuximab/docetaxel therapy to the date of disease progression, the date of death if it occurred before documented progression, or the date of last contact. OS was calculated from the date of treatment initiation to the date of death or the date of last contact. Comparison of PFS and OS from different genotype groups was done using the Kaplan-Meier method and significance was determined using the log-rank test. Statistical analyses were carried out using the StatView software (version 5.0.1, SAS Institute, Inc.).

Results

Relationship between EGFR genotypes and patient characteristics. The relative distribution of EGFR genotypes and their association with patient characteristics are given in Table 1. For EGFR-R521K, the relative frequency of A and G genotypes was comparable with that reported by the HapMap consortium for a Caucasian reference cohort: G/G ($n = 24$, 47%) was the most common genotype and 27 patients showed the variant form, of which 4 (7%) were homozygous (A/A) and 23 (45%) were heterozygous (G/A). No significant association of the G or A genotype

with sex, age, tumor localization, or initial tumor stage was observed (Table 1).

The number of CA repeats within the microsatellite region of EGFR intron 1 comprised between 14 and 21. As reported for healthy controls as well as SCCHN patients, we observed a predominance of 16 CA repeats (21). In detail, the allelic distribution was as follows: 33%, 16-CA; 17.6%, 15-CA; 16.7%, 17-CA; 12.7%, 20-CA; 9.8%, 18-CA; 4.9%, 19-CA; 3.9%, 21-CA; and 0.9%, 14-CA. We used a length of ≤16 CA repeats in the shorter allele as cutoff for the definition of two genotype groups because this cutoff has been shown to distinguish between EGFR expression levels (19) and between the response of non–small cell lung carcinoma (NSCLC) patients to gefitinib treatment (22, 23). No significant association of CA-SSR with sex, age, tumor site, or stage was observed (Table 1).

EGFR genotypes and therapy-induced skin toxicity. Evaluation of EGFR polymorphisms that have been shown to influence the expression of EGFR (19) or its affinity to EGF/transforming growth factor-α (18) was made and associated with the development of skin toxicity during the first cycle of treatment. Patients were stratified in two groups according to skin toxicity of grade 0 to 1 or grade >1 because by using this cutoff, a significant association between CA-SSR and skin rash has recently been identified in lung cancer patients treated with gefitinib (24). In our cohort, a correlation between the allelic length of the CA-SSR and the occurrence of skin rash was not observed ($P = 0.99$; Table 2). In contrast, the A genotype (A/A or G/A) of EGFR-R521K was significantly associated with a lower incidence of skin rash grade >1 compared with the G/G genotype ($P = 0.024$; Table 2).

Table 1. Baseline patient characteristics according to EGFR polymorphisms

Factor	Total	R521K A/A or G/A genotype	R521K G/G genotype	P	CA-SSR >16	CA-SS ≤ R16	P
Sex (*n*)							
Female	11	7	4		7	4	
Male	40	20	20	0.50	18	22	0.32
Age							
Median (SD)	60 (8)	59 (7)	64 (9)	0.40	63 (8)	59 (8)	0.23
Tumor site (*n*)							
Oral cavity	11	4	7		5	8	
Oropharynx	16	8	8		4	12	
Hypopharynx	14	7	7		5	9	
Larynx	1	1	0		0	1	
Other	3	2	1	0.71	1	2	0.93
Tumor stage at initial diagnosis (*n*)							
I	8	5	3		6	2	
II	2	1	1		2	0	
III	2	1	1		1	1	
IV a	27	13	14		9	18	
IV b	3	0	3		2	1	
IV c	4	3	1		3	1	
Not available	5	4	1	0.40	2	3	0.20

Table 2. Association of EGFR polymorphisms with skin toxicity

Factor	Total	R521K A/A or G/A genotype	R521K G/G genotype	P	CA-SSR > 16	CA-SSR ≤ 16	P
Skin rash (*n*)							
Grade 0/1	30 (14/16)	20	10		15	15	
Grade 2/3	21 (20/1)	7	14	0.024	10	11	0.99

Skin toxicity and EGFR-R521K as predictors for response, PFS, and OS. Because the efficacy of cetuximab, either as monotherapy in metastatic colorectal cancer (25) or combined with radiotherapy in SCCHN (6) or chemotherapy in NSCLC (FLEX study ref. 26),[3] has been positively associated with incidence/grade of skin toxicity, we evaluated whether the occurrence/severity of skin toxicity was associated with response, PFS, or OS in our study cohort. Response rate for all 51 patients was 12% and the DCR (DCR = PR + SD) was 55%. Median PFS and OS were 4.1 and 7.5 months, respectively. There was a trend for an association between skin toxicity and treatment efficacy: in patients without skin rash (grade 0), PR and DCR were 7% and 28%, 13% and 63% for those with a skin toxicity of grade 1, and 14% and 67% for those with a skin toxicity of grade >1, respectively (with *P* = 0.07 for DCR). Although we observed a trend toward longer PFS and OS for those patients experiencing any kind of rash, the grade of skin toxicity itself had no influence on PFS or OS (Fig. 1).

The major advantage of a molecular biomarker other than skin rash for the prediction of the efficacy of EGFR targeting would be that such molecular marker could be determined before initiation of cetuximab treatment and, thus, could be included in the algorithm of treatment decision. Since observing a significant association of the EGFR-R521K genotype with skin rash (Table 2), we finally asked whether EGFR-R521K was also associated with outcome of SCCHN patients treated with cetuximab/docetaxel. In the group of patients with the A genotype of the EGFR-R521K (A/A or G/A), PR and DCR was 7% and 44% compared with 17% and 67% in patients with the G genotype of EGFR-R521K (*P* = 0.16 for DCR). Furthermore, patients with the A genotype of the EGFR-R521K showed a trend to shorter PFS (hazard ratio, 0.55; 95% confidence interval, 0.28-1.08; log-rank: *P* = 0.08; Fig. 2A) but the EGFR-R521K genotype had no influence on OS (hazard ratio, 0.68; 95% confidence interval, 0.36-1.29; log-rank: *P* = 0.20; Fig. 2B), probably due to the small cohort size. As for skin rash, we did not observe an association of the CA-SSR genotype (≤16 CA repeats) with DCR (*P* = 0.49), PFS (log-rank: *P* = 0.36), or OS (log-rank: *P* = 0.69).

Discussion

In the present study, we found that the occurrence of skin rash under EGFR antibody treatment of patients with advanced head and neck cancer was associated with the EGFR-R521K genotype. Although the underlying molecular mechanisms remain unclear, an attenuated antibody binding to the variant form of EGFR might be the cause for less skin rash and shorter PFS and OS in patients with A genotype. Indeed, structural analysis of the molecular interaction between the Fab fragment of cetuximab and the extracellular domain of EGFR revealed that amino acid exchanges at critical interaction sites dramatically influenced binding affinity not only of EGF itself but also of cetuximab (27). However, because the effect of an arginine-to-lysine exchange at codon 521 had not been tested in this previous study, the interaction of the EGFR-R521K genotype with cetuximab binding affinity remains unresolved.

Thus far, EGFR-R521K has not been associated with the occurrence of skin rash in EGFR antibody regimens. Three recent studies failed to observe a correlation between EGFR-R521K and skin toxicity in NSCLC patients treated with gefitinib (24, 28) or erlotinib (29). These differences in results could be explained by the different EGFR-targeting agents used in their and our study: tyrosine kinase inhibitors such as gefitinib or erlotinib bind to the intracellular tyrosine kinase domain of the EGFR and should therefore not interfere with the extracellular ligand binding domain affected by the EGFR-R521K; thus, an influence of a polymorphism would only be related to the binding of the ligand or cetuximab to the receptor and not to the inhibitory function of the tyrosine kinase inhibitor. A second explanation might be a difference based on the ethnic background of patients (Asian versus Caucasian), which has been shown to significantly affect the activity of EGFR-targeting therapy for genetically ill-defined reasons (30). Of course, differing results about the association of EGFR-R521K with skin toxicity may also arise from differences in treatment regimens due to potential confounding effects by docetaxel. However, an influence of docetaxel in our cohort seems very unlikely because skin toxicity is a very rare event in treatment with docetaxel and, if observed at all, manifests itself rather as erythema at hands and feet and not as the clinically typical EGFR inhibition–related rash (7).

Increased affinity between EGF or transforming growth factor-α and the EGFR of R521K common genotype leading to an enhanced transcription of *fos*, *myc*, and *jun* has initially

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[3] U. Gatzemeier, J. von Pawel, I. Vynnchenko, et al., presented at the 2008 Chicago Multidisciplinary Symposium in Thoracic Oncology.

been shown by Moriai and coworkers (18). Subsequently, several studies identified the EGFR-R521K genotype as independent prognostic factor. Zhang et al. (31) found that colorectal cancer patients with G/G genotype of EGFR-R521K had a higher risk of local tumor recurrence than patients with G/A or A/A genotype. In accordance with this observation, Wang et al. (32) defined EGFR-R521K as a key determinant factor for tumor recurrence of stage II/III colorectal cancer after curative surgery. Given that the prognostic value of EGFR-R521K results from its interference with the affinity of EGFR ligand binding and signaling activity, patients with the unfavorable G/G genotype and thus increased EGFR pathway activity should mostly benefit from EGFR-targeting therapy, at least if further genetic alterations such as activating *raf* or *ras* mutations rendering ligand-mediated EGFR activation irrelevant did not occur. Indeed, even in the small patient cohort of our study, we observed a trend to prolonged PFS in patients carrying the G genotype (Fig. 2), which would support our hypothesis of preferential activity of EGFR targeting in this patient cohort. The small sample size of our study clearly limits our conclusions on the association of EGFR-R521K with clinical efficacy of cetuximab. However, support for our hypothesis of increased activity of cetuximab in patients carrying the G genotype of EGFR-R521K comes from a recent report on patients with metastatic colorectal cancer receiving cetuximab monotherapy who had a better survival if they had the G/G or G/A compared with A/A genotype of EGFR-R521K (33).

Based on our results, it is tempting to speculate that a predictive molecular biomarker with the characteristics of EGFR-R521K would have several advantages compared with the observation of skin rash after treatment initiation. First, given an association of EGFR-R521K with decreased cetuximab binding, it might identify patients for whom the dose of cetuximab potentially would have to be intensified. Second, the results from our study and those from previous reports testing the predictive value of EGFR-R521K for the efficacy of cetuximab or tyrosine kinase inhibitors (24, 28, 29), respectively, suggest that EGFR-R521K

might be a drug-specific, but not class-specific, marker. It can thus be hypothesized that EGFR-R521K has the potential to identify patients who are poor responders to cetuximab and for whom treatment with tyrosine kinase inhibitors would be potentially more successful. Of course, both hypotheses, EGFR-R521K as a biomarker for the adaptation of dose and/or selection of the class of drug, will have to be tested in further preclinical and clinical studies.

In contrast to the observed association of EGFR-R521K with skin toxicity, we did not find such an association for CA-SSR despite *in vitro* evidence that this specific polymorphism interferes with EGFR expression levels of cell lines (19, 34) and their sensitivity to the growth-inhibitory effect of erlotinib (17). The results from the subsequent clinical evaluation of CA-SSR as predictive marker for skin toxicity and response to EGFR targeting therapy were less conclusive: in 84 patients with NSCLC, the analysis of the CA-SSR together with 14 further single nucleotide polymorphisms within the EGFR gene detected a significant association between the number of CA repeats and the response to gefitinib treatment if a cutoff of ≤16 CA repeats was used (22). A second study in a cohort of 58 NSCLC patients could detect such an association only if patients were stratified into two groups defined by having 16 CA repeats or any other number (23). Using a cutoff of ≤18 CA repeats for grouping 52 NSCLC patients, Huang et al. (24) found a significant association of the number of CA-SSR only with skin rash but not with tumor response. Finally, the evaluation of the relationship between CA-SSR and EGFR expression levels in SCCHN could detect an inverse correlation of CA repeat numbers and EGFR expression only in a subgroup of 76 patients who exhibited at least one 16 CA repeat allele (21). An association of CA-SSR genotype with patient survival was not found in either in this patient subgroup or in the total cohort. Although testing each of the proposed cutoffs for definition of two risk groups, a significant association between CA-SSR, skin toxicity, and outcome was not found, which speaks against the clinical potential of this genetic markers.
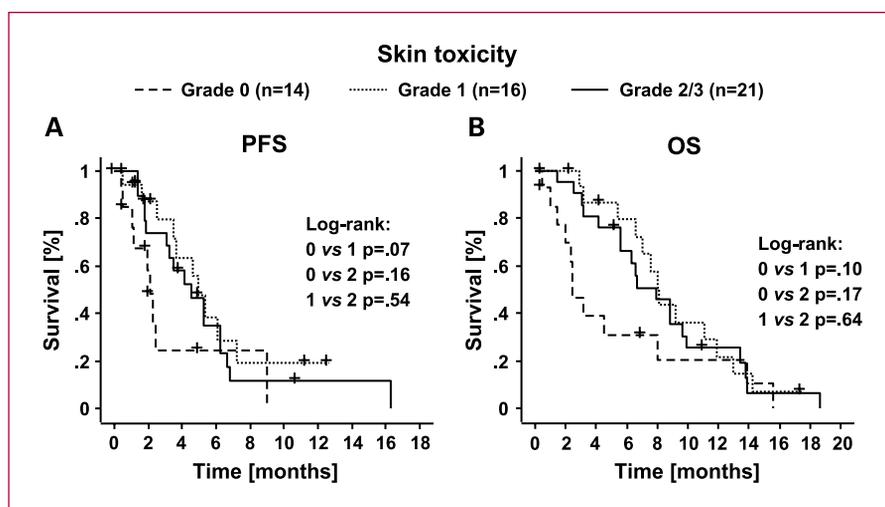
Fig. 1. SCCHN patients with any skin toxicity on docetaxel/cetuximab treatment show a trend for prolonged PFS and OS. Kaplan-Meier curves for PFS (*A*) and OS (*B*) of patients with skin toxicity grade 0, grade 1, or grade >1 are presented. *P* values for comparison of groups using the log-rank test are given.

Fig. 2. The EGFR-R521K genotype influenced PFS but not OS of SCCHN patients treated with docetaxel/cetuximab. Kaplan-Meier curves for PFS (*A*) and OS (*B*) of SCCHN patients with the A/A or G/A genotype (*solid line*) or the G/G genotype of the EGFR-R521K (*dotted line*) are presented, and *P* values for comparison of groups using the log-rank test are given.
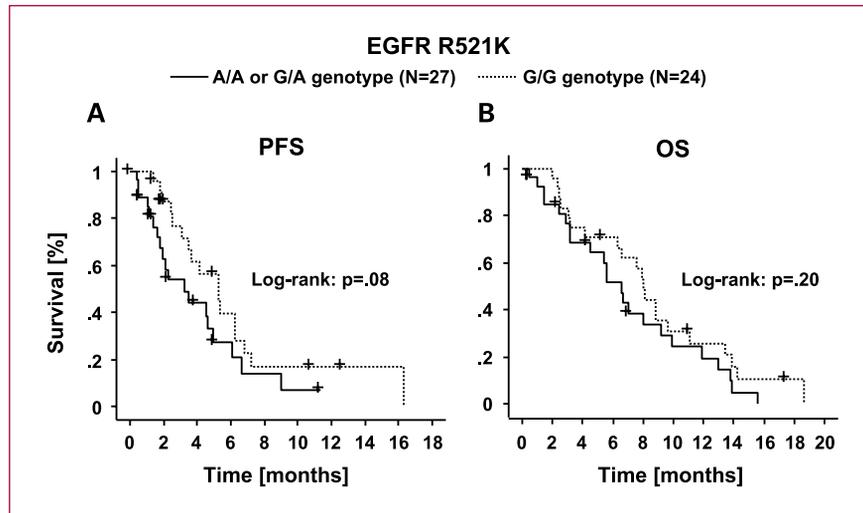
In conclusion, EGFR-R521K but not CA-SSR polymorphism might be an attractive predictor for the occurrence of cutaneous side effects. Its potential value in predicting efficacy under EGFR-targeting antibody treatment has to be validated in clinical trials including larger patient cohorts.

Disclosure of Potential Conflicts of Interest

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