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Original Research

Dosing to rash? – The role of erlotinib metabolic ratio from patient serum in the search of predictive biomarkers for EGFR inhibitor-mediated skin rash

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Abstract ***Aim:*** The aim of this study was to investigate if biomarkers of individual drug metabolism, respectively, the erlotinib/O-desmethyl-erlotinib metabolic ratio, may be a predictive factor for the severity of erlotinib-mediated skin rash in epidermal growth factor receptor (EGFR) inhibitor-treated patients suffering from epithelial cancers. This is especially important since it is known that the severity of skin rash has a prognostic value on outcome and survival in cancer patients experiencing skin rash under treatment with EGFR inhibitors.
Methods: From 2008 to 2014, 96 patients, n = 63 suffering from histologically confirmed non-small-cell lung cancer and n = 33 from pancreatic adenocarcinoma were observed for the occurrence and severity of skin rash after the onset of treatment with erlotinib. The primary

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Predictive biomarker

end-points (occurrence and severity of skin rash, progression-free survival [PFS] and overall survival [OS]) were analysed with regard to erlotinib and its metabolite O-desmethyl-erlotinib trough serum concentrations measured at 4 weeks after onset of therapy by the use of correlation, multiple regression and survival analysis.

Results: Occurrence of skin rash was associated with PFS ($p = 0.0042$) and OS ($p = 0.017$) in the overall cohort of erlotinib-treated cancer patients. Drug-metabolising activity assessed by the erlotinib/O-desmethyl-erlotinib metabolic ratio was correlated with severity of skin rash ($p = 0.023$) and as well highly associated with both PFS ($p = 2.1 \times 10^{-4}$) and OS ($p = 5.8 \times 10^{-5}$).

Conclusion: The erlotinib/O-desmethyl-erlotinib metabolic ratio reflecting the individual metabolic activity of erlotinib correlated with the severity of skin rash and outcome in patients treated with EGFR tyrosine kinase inhibitors. The metabolic ratio determined in serum may be used for therapeutic monitoring in erlotinib treatment and decisions on individual dosing to rash in rash-negative patients.

1. Introduction

Erlotinib is a small-molecule tyrosine kinase inhibitor. It is orally active, highly selective and a potent inhibitor of the epidermal growth factor receptor (HER1/EGFR) tyrosine kinase. Overexpression of EGFR plays a pivotal role in tumour growth and progression, mainly by promoting cell proliferation, angiogenesis, invasion and metastasis [1]. Erlotinib can inhibit EGFR-dependent tumour cell proliferation in vitro and also cell cycle progression in the G1 phase at nanomolar concentration [2,3].

Erlotinib is currently approved for the treatment of EGFR-mutated non-small-cell lung cancer (NSCLC), second-line treatment of EGFR-wild-type NSCLC and advanced pancreatic adenocarcinoma (PACA) [4,5]. The pathway how erlotinib works to combat cancer is not clearly known yet. Erlotinib binds selectively to the ATP binding site of the EGFR tyrosine kinase expressed on the cell surface of both normal and cancer cells and inhibits the intracellular molecules of the EGFR pathway. After oral administration, erlotinib is extensively metabolised by CYP3A4, CYP3A5, CYP1A1 and CYP1A2 isoforms of P450 enzyme [6]. The main active metabolite of erlotinib is O-desmethyl-erlotinib that is produced by O-demethylation of the side chains and represents approximately 5–10% of the circulating erlotinib [7,8].

Skin rash and diarrhoea are the most frequent adverse events of EGFR inhibitor treatment. Skin toxicities associated with erlotinib include papulopustular rash, xerosis, hair and nail abnormalities, and pruritus [9–11]. However, the presence and severity of skin rash shows a positive association with patients' outcomes, rendering skin rash a potential marker of drug efficacy [9,12,13].

Numerous studies investigated the underlying mechanism of EGFR inhibitor-induced skin rash in cancer patients. In a previous analysis, we showed that genetic variation within the *EGFR* gene (-216G/T and -191C/A) and its downstream signalling partner *PIK3CA* might predict the EGFR inhibitor-associated skin toxicity in patients treated with either small-molecule tyrosine kinases (erlotinib and gefitinib) or monoclonal antibodies (cetuximab and panitumumab) [14]. We also reported that patients carrying *HLA-A*02:01* or *HLA-A*03:01* alleles had a lower chance to develop skin toxicity [15]. Furthermore, there is some evidence that inflammation caused by cytokines like CXCL8 may also be crucial for individual EGFR inhibitor-induced skin toxicity and overall survival (OS) [16].

Population-based pharmacokinetic and exposure safety analyses showed that EGFR inhibitor drug exposure is related to rapid development of skin toxicity [17]. Similar results were reported from Lu and colleagues [18] who described a statistically significant correlation between drug exposure and skin toxicity and by White-Koning et al. [19] who found a positive relationship between an increase in drug exposure with increasing grade of skin toxicity.

Within the current study, we intend to examine the relationship between clinical and pharmacokinetic parameters relevant for the severity and onset of skin toxicity in cancer patients treated with erlotinib. Specifically, we aim to elucidate the role of erlotinib and its active metabolite O-desmethyl-erlotinib, measured at trough concentrations in serum and evaluate their potential to act as predictive biomarkers for skin rash and outcome of EGFR-targeted therapy.

2. Methods

2.1. Patients

The study was designed as a prospective, multicentre pharmacovigilance study as described elsewhere [13],

including 96 erlotinib-treated patients suffering from NSCLC or advanced PACA. All patients received first-time treatment with erlotinib 150 mg as a single agent or at 100 mg in combination with gemcitabine.

The study protocol was approved by the local ethics committees of the University Ulm and the Ludwig-Maximilians-University Munich. Patients were enrolled at both university hospitals and one medical practice specialised for the treatment of cancer patients. All patients scheduled for inclusion in the clinical study gave written informed consent.

Primary inclusion criteria were histologically confirmed cancer disease and first-time treatment with EGFR inhibitors. The grade of skin rash was rated according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0, using a four-staged scale [20] and were recorded weekly within the first 4 weeks after treatment initiation. Severity of adverse drug reactions (ADRs) were judged by the highest grade observed over the study period. Any pharmacological interventions against ADRs were recorded. OS was described as the time in days from start of treatment to death from any cause. Follow-up was performed for 12 months after initial EGFR inhibitor therapy and patients were censored at the last date when they were known to be alive hereafter. The baseline demographics of the study population are summarised in Table 1.

Table 1
Summary of patient characteristics

Patient characteristics	Category/class	Count (n = 96)	%
Age (y), median (range)	Men	69 (43–86)	
	Women	68 (48–84)	
Sex	Female	31	32.3
	Male	65	67.7
BMI (y), median (range)		25.1 (14.2–42.4)	
Smoking status	Never	29	31.2
	Former	52	55.9
	Present	12	12.9
	Unknown	3	
Tumour	Lung cancer (NSCLC)	63	65.6
	Pancreatic cancer (PACA)	33	34.4
Clinical stage (UICC)	IIa	2	2.2
	III	0	0.0
	IIIa	1	1.1
	IIIb	11	12.0
	IV	78	84.8
	Unknown	4	
Skin rash grade (NCI – CTCAE)	0	20	20.8
	1	32	33.3
	2	37	38.6
	3	7	7.3
	4	0	0.0
Diarrhoea grade (NCI – CTCAE)	0	61	63.5
	1	21	21.9
	2	12	12.5
	3	1	1.0
	4	1	1.0

Abbreviation: BMI, body mass index; NSCLC, non-small-cell lung cancer; PACA, pancreatic adenocarcinoma; UICC, Union for International Cancer Control; NCI – CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events

2.2. Blood sampling and measurement of drug concentration

Blood samples were drawn at 4 weeks from the beginning of the treatment before application of the last erlotinib dose.

Serum samples were obtained following standard procedures as previously described [16] and trough concentrations of erlotinib (OSI-774) and its active metabolite O-desmethyl-erlotinib (OSI-420) in serum were determined by using a high-performance liquid chromatography tandem mass spectrometry method. A detailed description of the sample preparation and drug concentration measurement is given in the Supplementary data.

2.3. Statistics

In a first step, the clinical variables were tested for association with the end-points maximal skin rash, maximal diarrhoea and OS. Normally distributed variables were compared with Student t-test, analysis of variance and Pearson correlation, whereas Mann–Whitney U test, Kruskal–Wallis H test and Kendall rank-correlation coefficient were used for non-normally distributed quantities and ordinal metrics. Time to progression (TTP) was calculated with censoring of patients who died before progression and progression-free survival (PFS) as the time from start of treatment to the earliest of TTP and death from any cause: PFS = min(TTP, OS). Survival distributions between different patient groups were compared in case of Kaplan–Meier analysis with the non-parametric log-rank test due to the nature of our censored data.

In the second step, we used multiple regression analyses to analyse the effect and interactions among the clinical variables and the end-point maximal skin rash. To assess the instantaneous risk of death of the covariates, the corresponding hazards were calculated using Cox proportional-hazard regression model. Statistical analyses were performed with SPSS Statistics 21 and R v3.01 including the libraries coin v1.0-23 and multcomp v1.3-6.

3. Results

3.1. Descriptive statistics

After 4 weeks of erlotinib therapy, skin rash and diarrhoea (all grades) occurred with a frequency of 79.2% and 36.5%, respectively. The incidence of grade 3–4

adverse reactions was 7.3% for skin rash and 2.1% for diarrhoea. Mean time of onset to maximal rash was 17.5 (±8.4) d and to maximal diarrhoea 15.2 (±9.9) d. Sixty-eight (89%) out of the 76 patients who experienced skin rash were subsequently treated with antihistamines, antibiotics, or glucocorticoids for relief of rash symptoms; 11 (31%) out of 35 study participants received supportive treatment with loperamide or laudanum against diarrhoea. Erlotinib treatment had to be stopped due to skin rash in two cases and due to diarrhoea in one case. The median duration of treatment with erlotinib until blood sampling was 27 d (according to the study protocol with trough levels taken at 4 weeks after start of treatment).

3.2. Inferential statistics – clinical results

Skin rash and diarrhoea were weakly correlated only within the subgroup with NSCLC (n = 63, p = 0.05, tau = 0.22) but not within the subgroup with PACA (n = 33, p = 0.85, tau = 0.03).

Gender differences in the severity of skin rash were seen with men experiencing more often higher grades of skin rash than women (p = 0.0078, tau = −0.25) and patients without any smoking history showed higher grades of skin rash than former smokers and these, in turn, than present smokers (p = 0.011, tau = −0.24) (Fig. 1a,b).

All other clinical variables (tumour type, tumour stage, age, and body mass index [BMI]) were not significantly correlated with severity of skin rash or diarrhoea. Especially, there were no significant differences in the severity of skin rash and diarrhoea between the NSCLC and PACA patient groups.

The erlotinib trough concentrations were 1.27 (±1.30) μg/ml in the 150-mg dose group (NSCLC patients) and 0.73 (±0.40) μg/ml in the 100-mg dose group (PACA patients). The concentrations of the metabolite O-desmethyl-erlotinib were 183 (±338) ng/ml in the 150-mg dose group and 69 (±72) ng/ml in the 100-mg dose group. As expected, the erlotinib and the O-desmethyl-erlotinib serum level were highly correlated among themselves ($p = 2.2 \times 10^{-16}$, tau = 0.76). There were no differences in the erlotinib or O-desmethyl-erlotinib trough levels between men and women or with regard to the other clinical parameters using bivariate analyses.

For depicting the individual metabolic activity, the metabolic ratio (erlotinib/O-desmethyl-erlotinib) was calculated. The metabolic ratio was slightly higher in the 100-mg dose group versus in the 150-mg dose group (p = 0.020), while in the 100-mg dose group gemcitabine was used in combination with erlotinib in the treatment of PACA patients and in the 150-mg dose group erlotinib monotherapy was given to NSCLC patients. Severity of skin rash was significantly associated

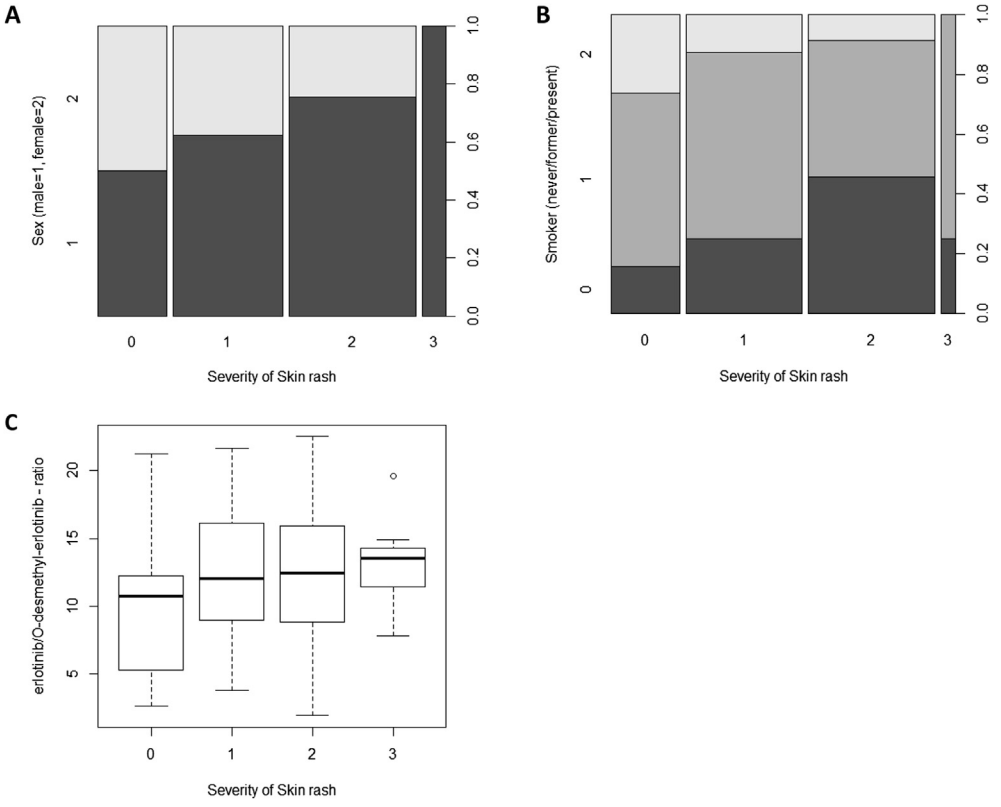

Fig. 1. (a–c) Significant correlations between primary outcome variable severity of skin rash and the clinical parameters sex, smoking status and the erlotinib/O-desmethyl-erlotinib ratio.

Table 2
Fitted regression model for skin rash: best subset selection of predictors with respect to AIC (null deviance: 97.784 on 94 degrees of freedom and residual deviance: 78.580 on 89 degrees of freedom).

Variable	Estimate	SE	p-Value
Intercept	−4.469	2.1920	0.042
Diarrhoea	1.096	0.6574	0.095
Sex, female (reference male)	1.868	1.484	0.208
BMI	0.123	0.077	0.112
Erlotinib/O-desmethyl-erlotinib ratio	0.262	0.104	0.012
Sex:erlotinib/O-desmethyl-erlotinib ratio	−0.250	0.131	0.057

Abbreviation: AIC, Akaike information criterion; BMI, body mass index; SE, standard error.

with the erlotinib/O-desmethyl-erlotinib ratio using a linear trend test ($p = 0.023$) (Fig. 1c), but not with the erlotinib trough levels or with O-desmethyl-erlotinib serum levels. No association between drug concentrations, erlotinib/O-desmethyl-erlotinib ratio and diarrhoea (as an EGFR inhibition independent adverse effect) was observed.

Multiple regression analysis to predict skin rash was based on the most striking clinical variables (diarrhoea, gender, age, BMI, tumour type, and erlotinib/O-desmethyl-erlotinib ratio) up to and including all pairwise interactions among the explanatory variables. In case of controlling for all these model parameters, the erlotinib/O-desmethyl-erlotinib ratio was the only significant predictor for skin rash ($p = 0.030$) that also remained a significant main effect ($p = 0.012$) in the fitted model (Table 2).

3.3. Survival analyses

Median PFS and OS were similar between tumour types with a median PFS of 92 (90–107) d and a median OS of 233 (180–313) d across tumours (Table 3a). As already known, severity of skin rash differed with respect to PFS ($p < 0.0042$) and OS ($p < 0.017$) (Table 3b). O-desmethyl-erlotinib serum levels ($p = 0.012$) and the erlotinib/O-desmethyl-erlotinib ratio ($p = 5.8 \times 10^{-5}$) showed a statistically significant correlation with survival outcome in Kaplan–Meier analysis (Fig. 2). Lower O-desmethyl-erlotinib serum level and accordingly a higher erlotinib/O-desmethyl-erlotinib ratio came along with an improved OS. O-desmethyl-erlotinib ($p = 0.032$) and the erlotinib/O-desmethyl-erlotinib ratio ($p = 2.1 \times 10^{-4}$) were as well significantly correlated to PFS.

The Cox proportional-hazard regression model comprised the unspecific baseline function over time and the most striking clinical variables in turn. The adjusted hazard ratio (HR) associated with erlotinib/O-desmethyl-erlotinib ratio for OS was 0.89 with a 95% confidence interval (CI) of 0.83–0.95 ($p = 0.0013$) (Table 4a). After stepwise variable elimination, three parameters were left as significant predictors in the optimal model: age (HR 0.97, 95% CI [0.95–0.99]), BMI (HR 0.94, 95% CI [0.89–0.99]) and the erlotinib/O-desmethyl-erlotinib ratio (HR 0.91, 95% CI [0.87–0.96]) (Table 4b).

4. Discussion

To date, evaluation of therapy-induced dermatologic toxicities is the method of choice to predict response, efficacy and survival in patients treated with EGFR inhibitors in EGFR-wild-type NSCLC [21–23]. Accordingly, increasing EGFR inhibitor dosage until the occurrence of rash has been discussed as a rational management strategy under the term ‘treatment to rash’ in several studies [24,25] illustrating the need for reliable

Table 3a
PFS and OS separated by tumour type

Tumour	PFS (d), mean ± SD	PFS (d), median (95% CI)	OS (d), mean ± SD	OS (d), median (95% CI)
Lung cancer (NSCLC)	135 ± 12	92 (90–107)	224 ± 16	236 (127–337)
Pancreatic cancer (PACA)	113 ± 13	91 (75–128)	233 ± 20	222 (151–314)
Across tumours	128 ± 9	92 (90–107)	227 ± 13	233 (180–313)

Abbreviation: CI, confidence interval; NSCLC, non-small-cell lung cancer; OS, overall survival; PACA, pancreatic adenocarcinoma; PFS, progression-free survival; SD, standard deviation. PFS and OS times are restricted with an upper limit = 360 d.

Table 3b
PFS and OS separated by grades of skin rash

Skin rash	PFS (d), mean ± SD	PFS (d), median (95% CI)	OS (d), mean ± SD	OS (d), median (95% CI)
Grade 0	85 ± 9	87 (61–92)	172 ± 25	135 (83–247)
Grade I	128 ± 17	91 (82–111)	198 ± 23	162 (96–327)
Grade II	148 ± 16	128 (92–168)	262 ± 18	312 (215–NA)
Grade III	164 ± 40	137 (91–183)	325 ± 21	360 (256–NA)
Grade I–III	132 ± 9	105 (91–133)	241 ± 14	278 (180–360)

Abbreviation: CI, confidence interval; OS, overall survival; NA, not applicable; NSCLC, non-small-cell lung cancer; PFS, progression-free survival; SD, standard deviation. PFS and OS times are restricted with an upper limit = 360 d.

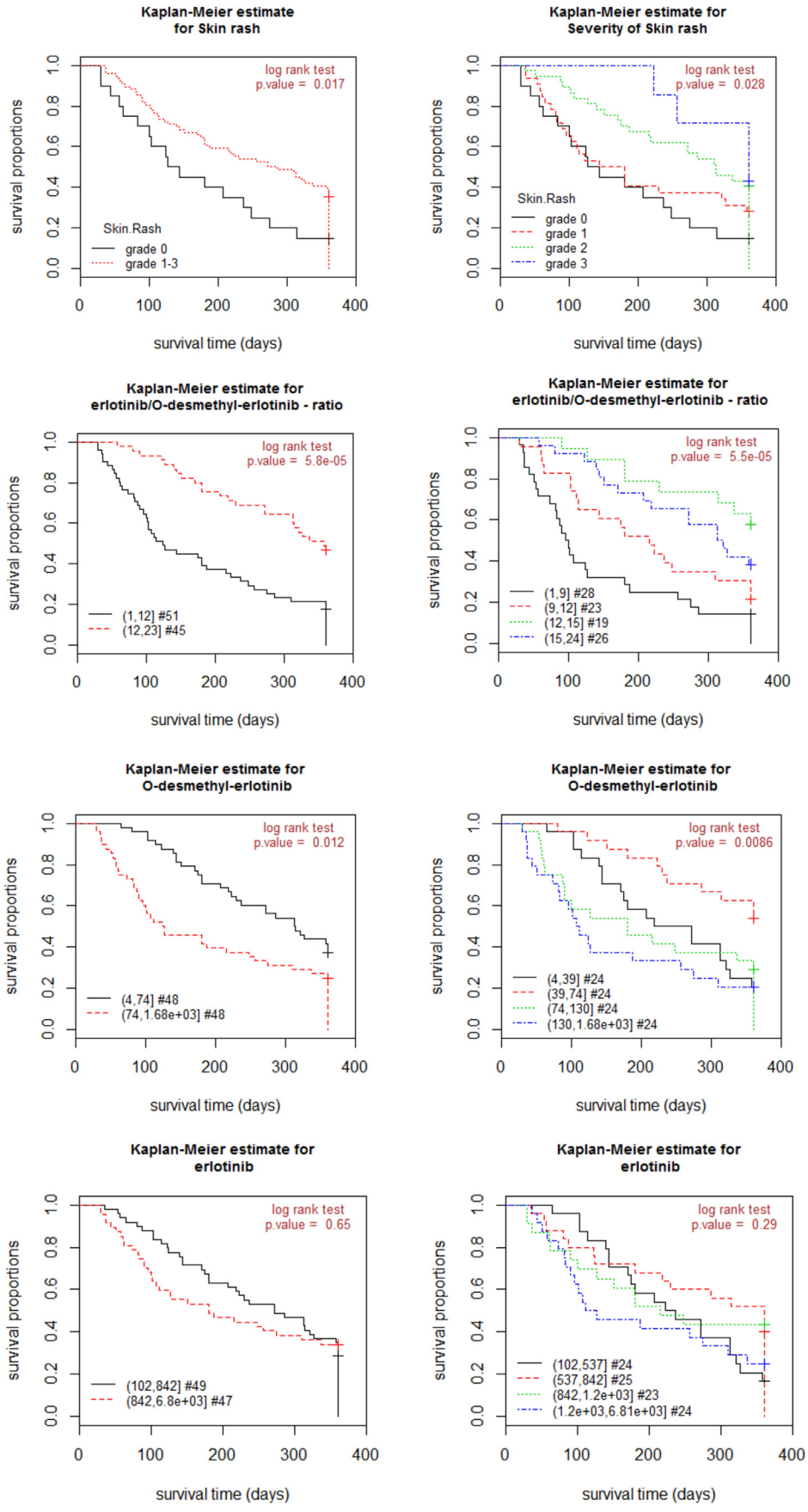

Fig. 2. Survival curves across all patients for skin rash, erlotinib/O-desmethyl-erlotinib ratio, O-desmethyl-erlotinib and erlotinib. The left hand side shows the survival curves for dichotomised and the right hand side for quartered range of predictor values.

Table 4a
Cox regression model examining the relationship between survival and the most important clinical covariates (n = 95, number of events = 66, score log-rank test = 25.7 on 9 degrees of freedom, p = 0.0023).

Variable	HR	95% CI	p-Value
Tumour PACA (reference NSCLC)	1.28	0.74–2.23	0.37
Skin rash	0.70	0.36–1.35	0.29
Diarrhoea	1.08	0.63–1.87	0.78
Sex, female (reference male)	0.84	0.47–1.49	0.55
Age	0.97	0.95–1.00	0.065
BMI	0.93	0.86–0.99	0.032
Erlotinib	1.00	1.00–1.00	0.66
O-Desmethyl-erlotinib	1.00	1.00–1.00	0.29
Erlotinib/O-desmethyl-erlotinib ratio	0.89	0.83–0.95	0.0013

BMI, body mass index; CI, confidence interval; HR, hazard ratio; NSCLC, non-small-cell lung cancer; PACA, pancreatic adenocarcinoma.

Table 4b
Optimal Cox regression model: best subset selection of predictors with respect to AIC (n = 95, number of events = 66, score log-rank test = 22.29 on 3 degrees of freedom, p = 5.7×10^{-5})

Variable	HR	95% CI	p-Value
Age	0.97	0.95–0.99	0.044
BMI	0.94	0.89–0.99	0.046
Erlotinib/O-desmethyl-erlotinib ratio	0.91	0.87–0.96	4.5×10^{-4}

AIC, Akaike information criterion; BMI, body mass index; CI, confidence interval; HR, hazard ratio.

predictors of which patient will profit from higher dosing to obtain rash symptoms. In the present study, we observed that the individual metabolic activity measured as metabolic ratio, rather than trough serum levels or daily dose, was associated with the severity of skin rash and that the metabolic ratio as well as the concentration of the erlotinib metabolite but not the trough concentration of erlotinib itself were correlated with improved treatment outcomes in lung and pancreatic cancers and may act as markers for effective target inhibition. In particular, lower metabolic activity (lower O-desmethyl-erlotinib concentrations) was associated with better PFS and OS in our patients. This means that higher metabolism of erlotinib in some patient may be responsible for lower exposure to the drug over the time, and therefore may be counteracted by increasing the erlotinib dose. However, as a therapeutic drug monitoring parameter that is practicable in clinical practice, the metabolic ratio may be used that can be obtained with a single blood sample at the time point before the next dose of erlotinib as trough level. The erlotinib/O-desmethyl-erlotinib ratio was correlated with the severity of skin rash, and patients with a low metabolic ratio indicating fast erlotinib metabolism may be suited for dosing-to-rash strategies because they risk to have low erlotinib exposure.

Skin rash and other class-related side-effects occur 1–2 weeks after initiation of therapy with a maximum intensity at 2–3 weeks [25,26]. According to our study protocol, measurement of erlotinib and O-desmethyl-erlotinib serum levels was performed at 4 weeks after start of treatment. Thus, in order to state if the metabolic ratio is really predictive for the severity of skin rash, serum concentrations should be studied in a prospective manner with sampling times earlier such as 1 week after start of treatment.

By using F-statistics (in order to compare models containing either the erlotinib/O-desmethyl-erlotinib ratio as an additional predictor to skin rash or not), we saw an additional predictive effect of the erlotinib/O-desmethyl-erlotinib ratio to skin rash in terms of PFS and OS in our study sample. A further advantage of erlotinib/O-desmethyl-erlotinib ratio as biomarker in comparison to skin rash might be that it is an earlier predictor because serum concentrations of erlotinib and its metabolites are already in steady state 1 week after start of treatment (with a median half-life of erlotinib of 1.5 d). This hypothesis has to be indeed confirmed as well as the predictive value of the erlotinib/O-desmethyl-erlotinib ratio has to be validated in further prospective studies.

Another interesting finding is the association between higher severities of skin rash in men than in women but without differences in OS in the erlotinib-treated cohort of patients. Erlotinib is metabolised mainly by CYP3A4 which shows differences in metabolic activity between men and women, with higher metabolic activity and consequently higher systemic clearance – when weight adjusted – of CYP3A4 substrates in women [27]. In our study, the median age of men and women was 69 and 68 years, respectively, and no sex differences were obvious in the drug exposure and erlotinib/O-desmethyl-erlotinib ratio, thus signifying that there was no major difference in erlotinib metabolism activity between men and women. The differences in severities of skin rash might be due to hormonal reasons that are made accountable for gender differences in skin with respect to skin physiology, skin immunology and wound healing [28] and are in concordance with the observation of more inflammatory lesions in male acne patients [29]. Since the sex differences in the severity of skin rash did not translate into differences in OS, it seems to be a stronger skin phenotype of EGFR inhibitors in men but not a stronger EGFR inhibition.

In accordance with the literature [30,31], we also observed lower skin rash in active smokers as well as in former smokers than in non-smokers. Hamilton et al. showed, e.g., that the bioavailability of erlotinib in smokers is 2.8-fold lower than in non-smokers regardless of the dose, and C_{max} in smokers was only two-third of that of non-smokers [32].

Median OS did not differ significantly between NSCLC and PACA in our study. The median OS was 236 d for NSCLC and 222 d for PACA and were in the range reported in the literature [4,21].

So far, while being active, the EGFR inhibitory activity and receptor affinity of the primary erlotinib

metabolite O-desmethyl-erlotinib is not well characterised. To date, no K_i values for O-desmethyl-erlotinib are known, thus we do not know to what extent O-desmethyl-erlotinib contributes to the receptor inhibition or interacts in competition with erlotinib.

In summary, serum erlotinib/O-desmethyl-erlotinib metabolic ratio was shown to correlate with PFS, OS and the severity of skin rash, in that way that high metabolic activity lowers the occurrence of skin rash and therefore might be a marker for individual dosing strategies in individuals being identified as fast metabolisers. More severe skin rash is correlated to treatment outcome in EGFR-targeted therapy with erlotinib. Its validation in larger cohorts is a prerequisite in order to establish a new surrogate marker useful in therapeutic drug monitoring for everyday clinical routines of EGFR-targeted therapy.

Conflict of interest statement

Stefan Boeck received research funding and honoraria for scientific presentations from Roche. No conflicts of interest have to be declared by the other authors.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejca.2015.11.022.

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