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Multimodality approach towards individualized non-small cell lung cancer treatment

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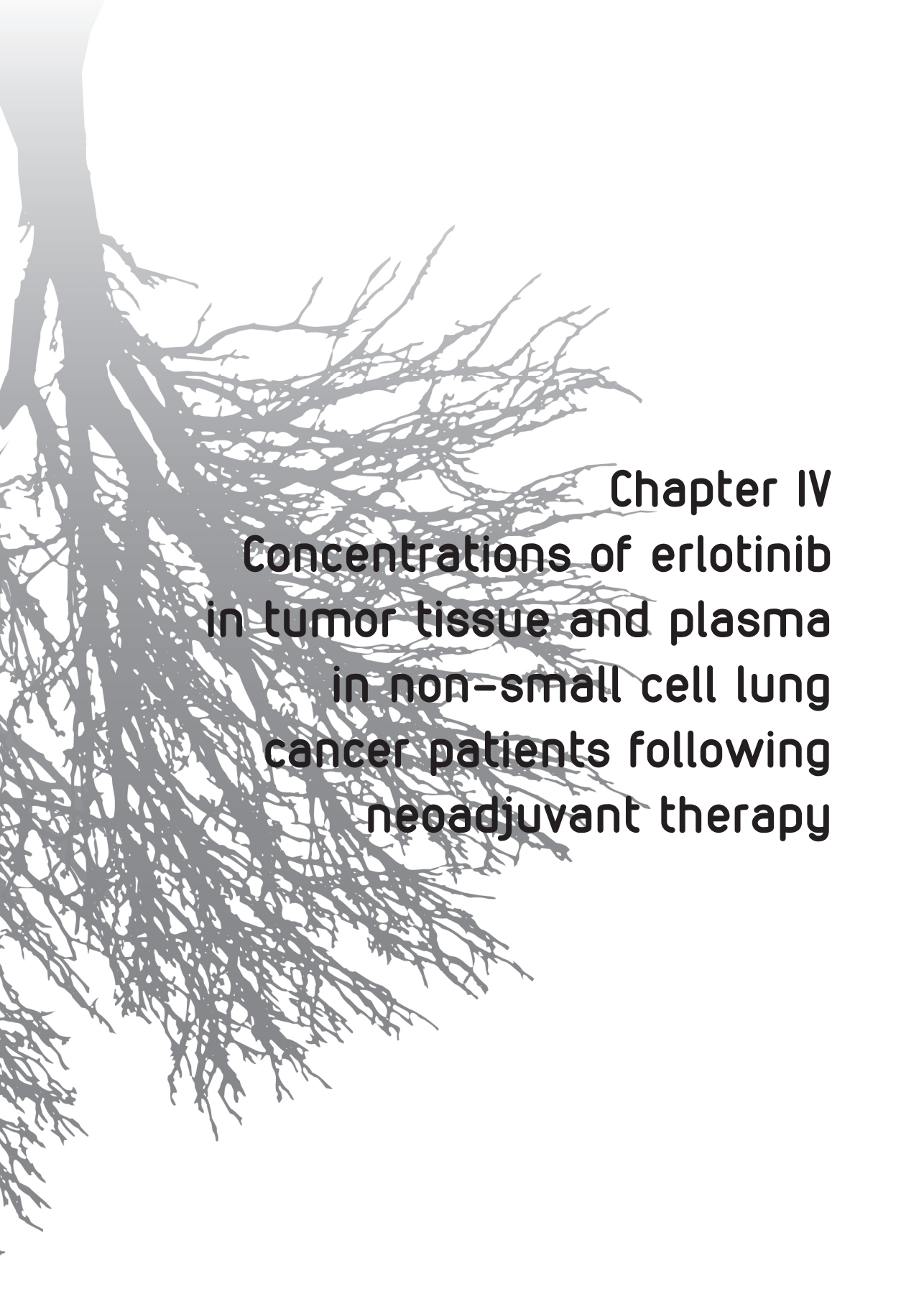
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Chapter IV
Concentrations of erlotinib
in tumor tissue and plasma
in non-small cell lung
cancer patients following
neoadjuvant therapy

ABSTRACT

Objectives | Tumors may not optimally respond to systemic therapy if minimal effective therapeutic levels are not reached within the tumor. Erlotinib has mainly been studied in the adjuvant or palliative setting and, therefore, little is known about erlotinib tumor penetration. The purpose of this exploratory study is to investigate lung tumor tissue concentrations after neoadjuvant erlotinib therapy for non-small cell lung cancer (NSCLC).

Patients and Methods | Patients were treated preoperatively with erlotinib (150 mg QD for 3 weeks) up to 48 hours prior to surgery. Plasma samples were collected during treatment. Surgical resection involved radical resection of the lung tumor and tumor biopsies were frozen directly after surgery. Erlotinib and O-desmethyl erlotinib concentrations in lung tumor tissue and plasma were determined using high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS).

Results | Thirteen evaluable patients were included. The mean plasma and lung tumor tissue erlotinib levels were 1222 ng/mL (standard deviation (SD) 678) and 149 ng/g (SD 153), respectively. In two individual patients, erlotinib and O-desmethyl erlotinib concentrations in lung tumor tissue were detectable up to 13 days and 7 days after erlotinib intake, respectively. Mean erlotinib tissue concentrations extrapolated to a time point directly after intake of erlotinib were approximated at >200 ng/g tissue, which is above the reported IC_{50} of wild-type EGFR (183 ng/mL).

Conclusion | No strong accumulation of erlotinib in lung tumor tissue was observed. Nevertheless, extrapolated intratumoral concentrations during erlotinib therapy were above the IC_{50} of wild-type EGFR.

INTRODUCTION

The epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) erlotinib is a targeted agent which has been approved for second-line treatment of patients with non-small cell lung cancer (NSCLC) regardless of the EGFR genotype and in first-line treatment of patients with activating mutations in EGFR (1). It has been established that the magnitude of the pharmacological effect of erlotinib (EGFR tyrosine kinase inhibition) in vitro is concentration dependent (2). Moreover, in clinical studies trough plasma concentrations of erlotinib and its metabolite (O-desmethyl erlotinib) have been correlated with treatment outcome (3). The minimal effective therapeutic level of erlotinib for wild-type EGFR, as deduced from half maximal inhibitory concentration (IC_{50}) in vitro after correction for plasma protein binding is 183 ng/mL (2,4). Penetration of drugs into tumor tissue is affected by different factors including tumor vascularisation, plasma protein binding, drug efflux pumps in tumor cells and intratumoral drug metabolism (5-9). Tumors may not optimally respond to systemic therapy if minimal effective therapeutic levels are not reached within the tumor (2). Since erlotinib has been studied mainly in the adjuvant or palliative setting, little is known about tumor penetration in NSCLC (10-12). Due to tumor location and risk of complications it is not common to obtain (repeated) tumor samples of NSCLC patients following systemic treatment. However, the introduction of neoadjuvant (preoperative) treatment with targeted agents enables the collection of tumor material during resection to get insight into tissue penetration of erlotinib. Therefore, an exploratory, observational study was performed within a multicenter phase II study of neoadjuvant erlotinib monotherapy in early stage NSCLC patients (13). The purpose of this study was to investigate erlotinib plasma and lung tumor tissue concentrations after neoadjuvant erlotinib therapy.

PATIENTS AND METHODS

This exploratory study was part of a larger multicenter phase II trial performed in the Netherlands (13). The study protocol was approved by the institutional review board and was conducted in accordance with guidelines established by the World Medical Association Declaration of Helsinki.

Eligibility

Patients with newly diagnosed resectable NSCLC over 18 years of age could enter the study. Patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and were neither pregnant nor breastfeeding. The diagnosis had to be histologically proven or highly probable (> 95%) based on medical history, chest X-ray, spiral CT-scan, bronchoscopy and [18F]-FDG-Positron Emission Tomography (PET scan). Exclusion criteria were continuation of smoking, prior malignancy treated with HER1/EGFR inhibitors, ophthalmologic abnormalities

(especially those causing dry eyes) or the unwillingness or inability to wear glasses instead of contact lenses during treatment.

For a patient to be evaluable in the present pharmacological study, a tissue sample collected after erlotinib therapy and data on the duration of the interval between last intake of erlotinib and surgery had to be available.

Treatment schedule

Preoperative treatment consisted of 150 mg erlotinib once daily for a period of at least 3 weeks. Surgical resection involved a radical resection of the tumor, preferably by lobectomy, and regional lymph nodes (at least three hilar and three mediastinal lymph node stations). Erlotinib was stopped at least 48 hours prior to surgery to prevent wound complications at resection. The treatment duration of three weeks was chosen to fit within the “preoperative window”, thereby not delaying surgery.

Pharmacokinetics of tumor tissue and plasma

Plasma samples were collected in the afternoon between day 14 and 21 of the erlotinib treatment. Patients were instructed to take erlotinib at dinnertime, therefore, time between intake and blood collection was between 18 and 24 hours. All plasma samples were snap frozen and stored at -80°C until analysis. The resection specimens were snap frozen at -80°C directly after surgery until further processing. A 50 to 150 mg specimen from each resected tumor was weighted accurately. Subsequently, an accurate volume of 500 to 1500 µL of drug-free human plasma was added to obtain samples containing 100 mg of lung tumor tissue per 1.0 mL of plasma. Lung tumor tissue homogenate was prepared by using a rotor/stator-type mechanical homogenizer for minimal three minutes per sample. Tissue homogenate samples were stored at nominally -20°C until analysis.

Bio-analytical quantification of erlotinib and its metabolite, O-desmethyl erlotinib, in plasma and lung tumor tissue homogenates was performed by using high-performance liquid chromatography and detection with tandem mass spectrometry (HPLC-MS/MS) as described previously (14). The lower limit of detection (LLOD) of the assay for both compounds was established at 2.0 ng/mL and 20 ng/g in plasma and tumor tissue samples, respectively.

Mutational analysis

EGFR and KRAS mutational status were determined in the postoperative material by isolating DNA from fresh tumor tissue and formalin-fixed paraffin-embedded tumor samples (Roche Diagnostics, Pleasanton, California, USA).

Statistical analyses

Descriptive statistics were used to summarize the patient characteristics and erlotinib and O-desmethyl erlotinib plasma and tissue concentrations.

RESULTS

Patients' characteristics

Between December 2006 and November 2010 tumor tissue and plasma samples were collected from 14 NSCLC patients receiving erlotinib preoperatively. One patient was not evaluable, since no data on time interval between last erlotinib dose and surgery were available. Clinical and histological data for all evaluable patients are listed in Table 1.

Plasma and tumor tissue concentrations

An overview of measured erlotinib and N-desmethyl erlotinib concentrations in plasma and lung tumor tissue samples of all patients is presented in Table 2. Mean plasma trough erlotinib and O-desmethyl erlotinib levels were 1222 ng/mL (SD 678) and 179 ng/mL (SD 140), respectively. Mean lung tumor tissue levels of erlotinib were 149 ng/g

Table 1. Patient characteristics

Characteristics	Total (n=13)
Gender (number of pts (%))	
Male	5 (39)
Female	8 (61)
Age (y) (mean (range))	63 (47-74)
Tumor histology (number of pts (%))	
Adenocarcinoma	10 (77)
Squamous cell carcinoma	1 (8)
Large cell carcinoma	2 (15)
Smoking status (number of pts (%))	
Never smoker	3 (23)
Former smoker	7 (54)
Current smoker	3 (23)
KRAS mutation status (number of pts (%))	
Negative	11 (85)
Positive	2 (15)
EGFR mutation status (number of pts (%))	
Negative	10 (77)
Positive	3 (23)
Median erlotinib treatment duration (median number of days)	20
Median period between erlotinib treatment and surgery (median number of days)	6

Table 2. Erlotinib and O-desmethyl erlotinib concentrations in plasma and lung tumor tissue.

Patient	Plasma concentration (ng/mL)		Tumor tissue concentration (ng/g)		Time interval between last erlotinib intake and surgery (days)
	Erlotinib	O-desmethyl erlotinib	Erlotinib	O-desmethyl erlotinib	
1*	1407	161	105	<LLOD	6
2*	NA	NA	441	57.7	3
3	701	85.7	<LLOD	<LLOD	7
4	993	83.0	450	187	1
5	2521	520	52.0	<LLOD	3
6	1189	162	77.6	<LLOD	4
7	633	52.6	33.2	<LLOD	10
8	862	126	48.3	<LLOD	3
9	782	76.0	197	46.9	7
10	NA	NA	<LLOD	<LLOD	19
11	2440	348	84.8	<LLOD	4
12	596	212	110	<LLOD	13
13*	1321	141	39.3	<LLOD	7
Mean	1222	179	149	79.2	7
SD	678	140	153	78.0	4.9

* represents a patient with EGFR mutation. LLOD, lower limit of detection (2.0 ng/mL in plasma samples and 20 ng/g in tissue samples); NA, not available.

(SD 153). The median number of days between last erlotinib administration and surgery was 6 days with a range from 1 to 19 days. In one patient erlotinib tissue concentrations were quantifiable up to at least 13 days after intake of erlotinib. In two patients erlotinib tissue concentrations (collected 7 and 19 days after intake of erlotinib) were below the LLOD (20 ng/g). O-desmethyl erlotinib concentrations were quantifiable in three patients up to 7 days after intake of erlotinib with mean lung tumor tissue concentrations of 79.2 ng/g (SD 78.0). The relation between trough plasma levels and tumor tissue concentrations in the available samples was indeterminate, taking into account the time point of tissue collection.

Figure 1 shows the erlotinib and O-desmethyl erlotinib lung tumor tissue concentrations versus the time interval between the last erlotinib dose and tumor tissue collection during surgery. Using extrapolation, lung tumor tissue concentrations during erlotinib therapy (time after erlotinib intake = 0 days) were approximated at 200 ng/g tissue, which is above the IC_{50} level of wild-type EGFR inhibition by erlotinib (183 ng/mL). No correlation between EGFR mutational status and intratumoral erlotinib concentrations was found.

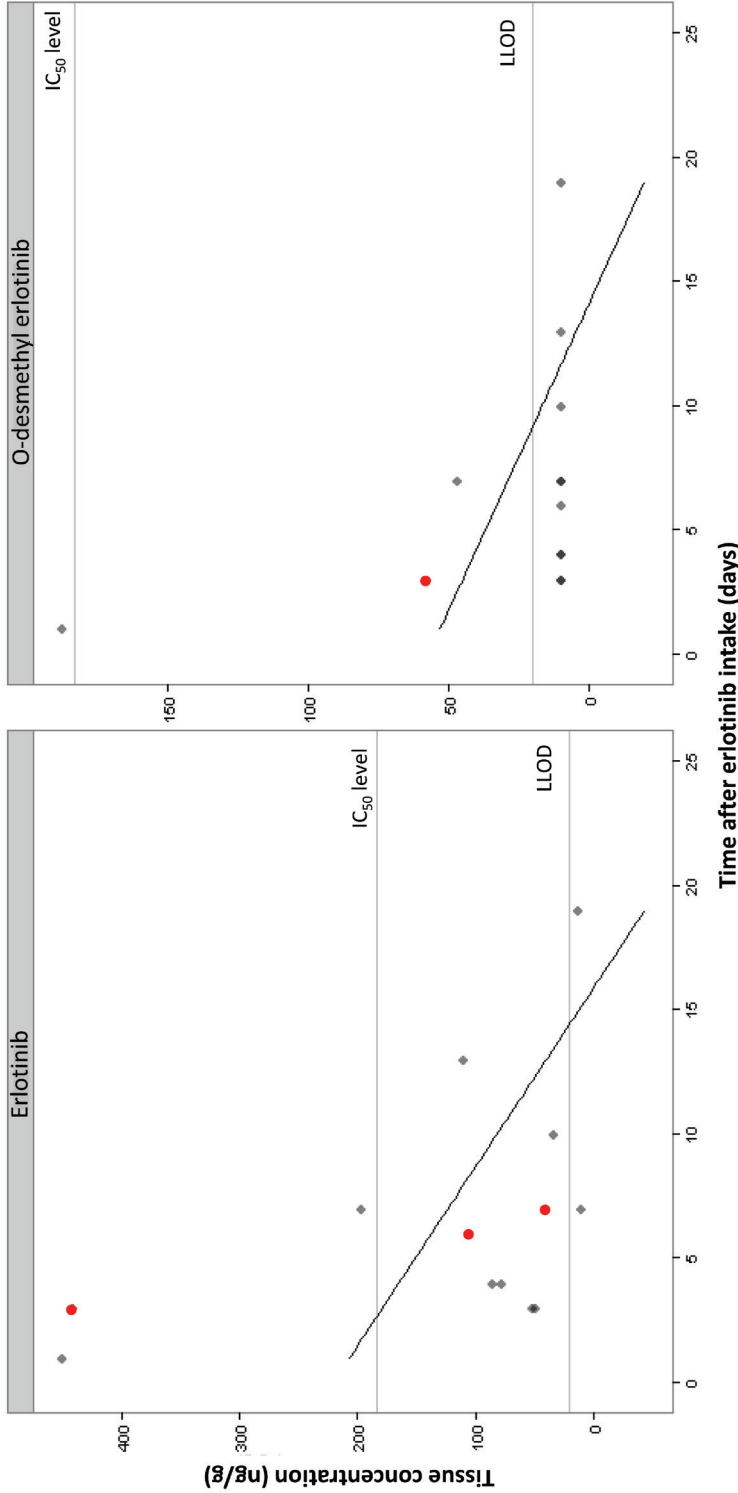


Figure 1. Tumor tissue concentrations of erlotinib and O-desmethyl erlotinib of patients (n=13) plotted against the interval between erlotinib administration and tumor tissue collection during surgery. The red dots represent intratumoral concentrations in patients with EGFR mutation (n=3). The horizontal grey lines represent the lower limit of detection (20 ng/g) and the minimal effective therapeutic concentration (IC₅₀ level:183 ng/mL). The black line represents the linear regression line with correlation coefficients (R) of -0.481 (p=0.096) and -0.451 (p=0.122) for erlotinib and O-desmethyl erlotinib, respectively.

DISCUSSION

This exploratory study was conducted to investigate erlotinib concentrations in lung tumor tissue after neoadjuvant erlotinib therapy. Only limited data of erlotinib and O-desmethyl erlotinib concentrations in lung tumor tissue were available thus far. Concentrations in lung tumor tissue were detectable up to 13 days (for erlotinib) and 7 days (for O-desmethyl erlotinib) after drug intake. The extrapolated tumor tissue concentration of approximately 200 ng/g was lower than the mean plasma concentrations of 1222 ng/mL, thus, no strong accumulation of erlotinib in tumor tissue was observed. Nevertheless, these extrapolated tumor concentrations were above the the IC_{50} level of wild-type EGFR inhibition by erlotinib (183 ng/mL).

A drawback of assessment of intratumoral drug concentrations is the availability of only one tissue sample per patient per time point (15,16). Therefore, it is not possible to investigate changes in tumor levels over time. In previously reported studies of erlotinib and gefitinib, lung tumor tissue was obtained during tyrosine kinase inhibitor (TKI) treatment (10-12). In our observational study, however, treatment with erlotinib was interrupted at least 48 hours before surgery to decrease any risk of surgical complications. In addition, some patients discontinued neoadjuvant treatment earlier due to toxicity. Therefore, we could only measure erlotinib and O-desmethyl erlotinib concentrations in lung tumor tissue samples that were collected at different time points up to 19 days after administration of erlotinib.

Available data concerning TKI levels in lung tumor tissue, including data of the present study, showed wide variability. Variability in tumor tissue concentrations can be partly the consequence of variable plasma levels. The observed plasma concentrations and corresponding inter-patient variability within our study were comparable to previously reported trough plasma concentrations by Hidalgo et al. (1200 ng/mL (\pm SD 620)) (3). No correlation between trough plasma levels and tumor tissue concentrations could be established, due to differing time points of sample collection.

Heterogeneity within one tumor may exist causing variable drug penetration within different tumor areas (9). For instance, vital tissue is supposed to have a better blood supply than non-vital tissue (fibrotic and necrotic tissue). Furthermore, a treatment response may result in a different tumor penetration in the remaining tumor (9). Additionally, contamination of tissue samples with blood clots can affect measured intratumoral TKI levels as was experienced in the study of Lassman et al (15). In our study no blood clots were observed in tumor samples, however, tumor heterogeneity of samples could not be ruled out as this is inherent to tumor tissue analysis (17).

A part of the lung tumor tissue samples showed undetectable amounts of erlotinib (15.4%) and O-desmethyl erlotinib (76.9%). The lower limit of detection of our assay for erlotinib and O-desemethyl erlotinib in tissue samples (20 ng/g) was well below the the IC_{50} level of wild-type EGFR inhibition by erlotinib (183 ng/mL) (2,4). Therefore, exact quantification of concentrations below 20 ng/g was not supposed to be clinically relevant.

Due to the observational setting, tissue collection occurred at various time points after last erlotinib intake. Therefore, direct comparison of erlotinib and O-desmethyl erlotinib tissue concentrations between patients within our study and with previously reported data was not possible. Tumor tissue concentrations were extrapolated to a time point directly after intake of erlotinib and estimated at >200 ng/g tissue. In a previous study including 3 patients with NSCLC and 1 patient with laryngeal cancer treated with erlotinib (150 mg QD for 9 days), samples of tumors resected within 90 minutes after erlotinib intake showed mean erlotinib and O-desmethyl erlotinib tumor concentrations of 1185 ng/g (range 94.0 - 3028) and 160 ng/g (range 125 - 184), respectively (12,18).

In our study no indication for accumulation of erlotinib in lung tumor tissue was observed independent of EGFR mutational status, which was consistent with reported data (tissue to plasma ratio's (n=4) of 0.05 - 1.61 and 0.88 - 1.30 for erlotinib and O-desmethyl erlotinib, respectively) (12,18). In contrast, for gefitinib, an other EGFR-TKI, lung tumor concentrations during gefitinib therapy in 23 patients with NSCLC were 40-fold elevated compared to plasma concentrations which suggested that gefitinib strongly accumulated in lung tumor tissue (10). Since physicochemical properties of gefitinib and erlotinib are quite similar, this difference remains unexplained. Possibly, interaction with drug efflux pumps P-glycoprotein (P-gp) or Breast Cancer Resistance Protein (BCRP) in tumor cells for which erlotinib is a substrate (6,19,20) or metabolism of erlotinib by CYP1A1/1A2 may be involved (7,21). Nevertheless, even without tissue accumulation 150 mg QD erlotinib leads to lung tumor tissue concentrations in NSCLC exceeding the IC_{50} level of wild-type EGFR inhibition.

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