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### Multimodality approach towards individualized non-small cell lung cancer treatment

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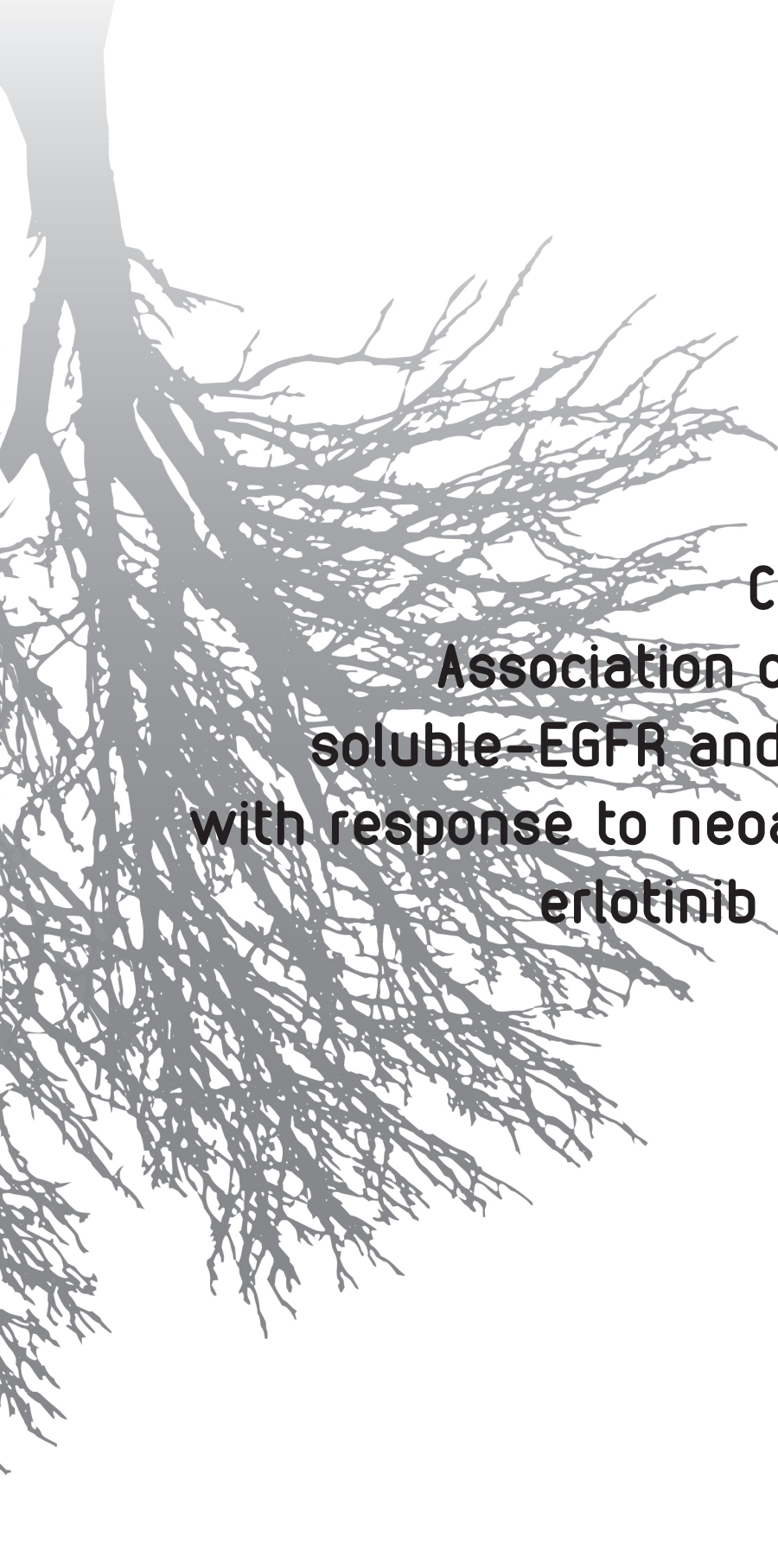
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**Chapter V**  
**Association of serum**  
**soluble-EGFR and ligands**  
**with response to neoadjuvant**  
**erlotinib in NSCLC**

**ABSTRACT**

*Background* | Epidermal Growth Factor Receptor (EGFR) ligands such as transforming growth factor- $\alpha$  (TGF $\alpha$ ), amphiregulin (ARG), insulin growth factor (IGF1) and soluble EGFR have been described as predictors of either response or resistance to EGFR-Tyrosine Kinase Inhibitors (TKIs). The aim of this study was to test these serum markers in a prospective neoadjuvant trial, in which NSCLC patients received 3-weeks of preoperative erlotinib.

*Methods* | Patients were treated with erlotinib for 21 days before radical resection. Serum marker levels of s-EGFR, TGF $\alpha$ , ARG, IGF1, IGFBP3 were determined in available pre-treatment (baseline) samples and samples during treatment. For evaluation of response, we used metabolic data during treatment, as measured by FDG-PET and compared to baseline scan according to EORTC criteria. Predefined cut-offs were used.

*Results* | Of 37 patients, serum samples and scans were available (n=27 baseline and during treatment). Metabolic partial response was seen in 6 patients. A baseline high s-EGFR (>54.95 $\mu$ g/l) was predictive for response (p=0.04). ARG (p=0.18), TGF $\alpha$  (p=0.65), IGF1 (p=0.71) and IGFBP3 (p=0.62) were not significantly related to metabolic response. All metabolic responders showed a decrease in s-EGFR during treatment with a mean decrease of 4.4  $\mu$ g/l.

*Conclusion* | Of potentially interesting serum markers, only high baseline levels of soluble EGFR are associated with response to erlotinib in early stage NSCLC. Furthermore, in patients with metabolic response, s-EGFR decreased during treatment.

## BACKGROUND

Inhibiting the epidermal growth factor receptor (EGFR) pathway by blocking the tyrosine kinase domain with small molecule inhibitors (EGFR-TKIs) has become a valid treatment option for some non-small cell lung (NSCLC) patients (1-4). It has become clear that this oncogenic pathway is often mutated and the presence of mutations in EGFR is predictive for EGFR-TKI response (5-8). This has resulted in trials studying EGFR-TKIs as first line therapy in NSCLC patients with EGFR-mutations (9,10). However, acquisition of (adequate) tumor tissue is not always possible, and some patients without mutations may also benefit from EGFR-TKI treatment (11-13). As systemic treatment options in NSCLC patients are limited and targeted therapy is expensive, identification of easily obtainable predictive markers remains essential.

Pursuing this goal, we previously tested serum concentrations of EGFR and insulin-like growth factor (IGF) ligands for their predictive potential in advanced NSCLC patients treated with erlotinib/gefitinib in comparison to matched, advanced NSCLC, patients without TKI treatment (14,15). We found that low concentrations of transforming-growth factor alpha (TGF $\alpha$ ) and high concentrations of amphiregulin (ARG) were associated with longer survival in patients treated with erlotinib or gefitinib compared to controls. Furthermore, high serum-EGFR (s-EGFR) concentrations were associated with a better overall survival in advanced NSCLC patients treated with erlotinib or gefitinib (14,16,17). The studies were exploratory and the study of Kappers et al and Vollebergh et al were both retrospective.

The aim of this study was therefore to validate our prior results using data from a prospective neoadjuvant trial, in which operable NSCLC patients received 3-weeks treatment with erlotinib before surgical resection. The objective was to validate the association of ligand concentrations and response to neoadjuvant erlotinib. Overall toxicity and response data of the neoadjuvant study have been presented before (18). In this analysis, concentrations of s-EGFR, ARG, TGF $\alpha$ , IGF1 and insulin-like growth factor binding protein-3 (IGFBP3) in serum collected prior to erlotinib treatment and during treatment were studied in relation to metabolic response as measured by FDG-PET imaging.

## PATIENTS AND METHODS

This current study is a sub-study of the neoadjuvant erlotinib (NEL) trial (18). Patients were treated in the NEL-study, an open-label, non-comparative phase II study performed in four hospitals in The Netherlands (EudraCT number 2006-003927-35). The protocol was approved by each local institutional ethical review board and written informed consent was obtained from each patient before the start of the study. First, 15 patients with resectable NSCLC from a selected (“enriched”) population (two or more of the following features: female, adenocarcinoma, non-smoker, Asian) were enrolled.

After evaluation of treatment in these patients by the safety committee, another 45 unselected patients were included. All 60 patients received, prior to resection, neoadjuvant erlotinib daily during an intended course of three weeks. Toxicity and response data of this study have been reported and showed tumor response in 14/60 patients (23%), of whom 3 (5%) had near-complete response (18,19). Of the NEL-study population (n=60), a sub-study was conducted with serum samples of 37 patients. Serum was available both before treatment (baseline) and between day 14 and 21 of erlotinib treatment in 27 patients; in another 10 patients only baseline samples were available. Besides, serum samples of 22 untreated control patients were available. These patients were treated for resectable NSCLC, in the same period, but did not receive erlotinib or other neoadjuvant treatment. The control patients were matched for patient and tumor characteristics.

### *Eligibility*

Patients with newly diagnosed resectable NSCLC, over 18 years of age, could enter the study. Pathological staging occurred according to the 7th TNM staging edition. Patients had to be fit for surgery with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, neither pregnant nor breastfeeding. The diagnosis had to be histologically proven or highly probable based on medical history, chest X-ray, spiral CT-scan, bronchoscopy and [18F]-FDG-Positron Emission Tomography (PET scan) (20). Clinical staging was performed using CT scan, FDG-PET and, (in case of suspected tumor positive nodes) additional endoscopic ultrasound guided fine needle aspiration cytology (EUS-FNA) or mediastinoscopy. Exclusion criteria were continuation of smoking (21), ophthalmologic abnormalities (especially those causing dry eyes), unwillingness or inability to wear glasses instead of contact lenses during the erlotinib treatment, or prior malignancy treated with HER1/EGFR inhibitors.

### *Treatment schedule*

Preoperative treatment consisted of one tablet of 150 mg erlotinib daily for a period of 3 weeks. FDG-PET scans were performed before the start of therapy and after 21 days, accompanied by a diagnostic CT scan. Surgical resection involved a radical resection of the tumor, preferably by lobectomy, and regional lymph node dissection.

### *Assessment of response*

Resected tumor specimens and imaging data were sent to the Netherlands Cancer Institute (NKI-AVL) for central review, quantification and analyses. CT and FDG-PET scans performed after treatment with erlotinib were compared to baseline scans, by one radiologist and one nuclear physician, respectively. PET imaging was performed using a hybrid system (GeminiTF, Philips, Eindhoven, the Netherlands). Uptake was quantified using the maximum standardised uptake value (SUVmax) (maximum activity concentration of FDG divided by the injected dose and corrected for the body weight of the patient). Metabolic tumor response was assessed following EORTC criteria (22).

The resection specimens were scored for residual vital tumor and the presence of morphological signs of therapy-induced regression such as foam cell reaction, giant cell reaction, cholesterol clefts and fibrotic alterations (Junker classification (23)). As described before, it remains difficult to ascertain whether the degree of necrosis and fibrosis reflects extensive tumor heterogeneity and spontaneous necrosis or treatment effect (18). Since more detailed quantification of response in the setting of targeted therapy for early stage NSCLC is lacking, change in metabolic activity, measured by FDG-PET, was used for response evaluation (24).

#### *Serum analyses*

Serum samples were stored at -30°C and all markers were tested simultaneously. s-EGFR, ARG, TGF $\alpha$ , IGF1 and IGFBP3 were measured as previously published (14,15). When cut-off levels were used, we only tested previously determined cut-offs. For s-EGFR this value was 54.95 $\mu$ g/L, for ARG 9.49  $\mu$ g/L, for TGF $\alpha$  21.69  $\mu$ g/L, for IGF1 11.95nmol/L and for IGFBP3 2.92mg/L (15,25). To calculate the IGF1:IGFBP3 ratio, IGFBP3 (mg/L) was converted to molar concentrations with a conversion factor of 34.78. The cut-off of the ratio was predefined at 0.1696. The prior studies on the markers mentioned above did not include serum samples during treatment; therefore we studied these as continuous values only. Differences between baseline and treatment serum were calculated and a decrease was indicated if the value of treatment serum was lower than at baseline.

#### *Mutation analyses*

Mutation testing was performed centrally at the certified lab of the NKI-AVL. EGFR and KRAS mutation status was determined (Roche Diagnostics, Pleasanton, California, USA) in the postoperative material by isolating DNA from fresh tumor tissue and formalin-fixed paraffin-embedded tumor samples as previously published (26).

#### *Statistical analyses*

Serum markers levels were mainly tested as continuous values. If predefined cut-offs were used, levels were reported as high or low. Associations among the individual markers and response were calculated using Fisher exact tests and Mann-Whitney U tests. Correlations between serum marker value and metabolic response were calculated and reported as the p-values according to the non-parametric Spearman rank correlation tests.

## RESULTS AND DISCUSSION

Sixty patients with operable early stage NSCLC were included in the NEL-study. Forty-two patients completed 21 days of erlotinib treatment using 150mg/day. Seven patients used erlotinib 150mg/day for 15-18 days. In four patients the dose was reduced to 100mg/day and seven patients stopped erlotinib prematurely due to toxicity (after 8 - 15 days).

In this current serum study, baseline serum and FDG-PET evaluations were available in 37 patients treated with erlotinib. Besides, serum samples of 22 untreated control patients were available. Clinical characteristics for all patients with available serum for marker analysis are listed in Table 1. All 37 patients were treated with erlotinib for a median of 21 days (range 9- 29 days). Following EORTC criteria, 8 patients were classified as metabolic partial responders (SUVmax decrease of -25 to -80%). Of 27 patients, serum samples were available during treatment; correlation of change of marker level and response could therefore be analyzed for 27 patients. In these 27 patients, serum samples as well as FDG-PET/CT were available both before treatment (baseline) and between day 14 and 21 of erlotinib treatment. Of the in total 8 metabolic responders, 6 were identified in the subgroup of 27 patients.

### *Serum markers*

The range and median of all marker levels was in accordance with our previous study's (Supplementary Table 1), except for IGFBP3 which had a higher median than previously reported. Only two patients had a value below the cut-off. Baseline serum marker distributions for all patients are summarized in Table 2. No differences in median and range were observed between the treated group and the untreated control group. Figure 1 shows baseline marker levels and change during treatment in relation to metabolic response in 27 patients for the markers s-EGFR, ARG, TGF $\alpha$  and IGF. IGFBP3 and the IGF1: IGFBP3 ratio was not included in the figures, due to the higher levels of IGFBP3 as reported above. High baseline levels of s-EGFR were significantly correlated with PET-response ( $p=0.04$  Spearman rank). All six responders had s-EGFR levels above 59  $\mu\text{g/l}$ . ARG, TGF $\alpha$ , IGF1 and IGFBP3 showed no significant associations with PET response with a p-value of respectively 0.98, 0.66, 0.74 and 0.77 (Table 3 and Figure 1). A repeated analysis was performed including the baseline serum marker levels of all erlotinib treated patients; with the serum of 10 extra erlotinib treated patients a significant association was continued with high s-EGFR in metabolic responders. Two of these 10 patients had a metabolic response. For the total 8 metabolic responder the significant correlation was stable ( $p=0.03$ ).

Differences between marker levels during treatment and baseline levels were studied in 27 patients (Figure 2). Decrease of s-EGFR during treatment showed a trend in correlation with response on PET-CT ( $p=0.09$ ). The relative change of SUVmax as measured by PET during treatment was minus 18% (as compared to baseline SUVmax) in patients with a decrease of s-EGFR, as compared to 3% in patients with an increase

( $p=0.04$ ). The other three markers did not show a significant relation of change in serum levels during treatment and metabolic response.

Table 1. Patient characteristics and serum marker outcome

		<b>PR</b> <b>N = 6</b>	<b>SD</b> <b>N = 17</b>	<b>PD</b> <b>N = 4</b>	<b>All PET</b> <b>N = 27</b>	<b>Control</b> <b>N = 22</b>	<b>Baseline only</b> <b>N=10</b>
<b>Histology</b>							
	Large cell	2 ( 33%)	2 ( 12%)	1 ( 25%)	<b>5 ( 19%)</b>	<b>4 ( 18%)</b>	1 (11%)
	Squamous cell	1 ( 17%)	3 ( 18%)	1 ( 25%)	<b>5 ( 19%)</b>	<b>2 ( 9%)</b>	3 (33%)
	Adenocarcinoma/BAC	3 ( 50%)	12 ( 71%)	2 ( 50%)	<b>17 ( 63%)</b>	<b>14 ( 64%)</b>	6 (60%)
	BAC	0 ( 0%)	0 ( 0%)	0 ( 0%)	<b>0 ( 0%)</b>	<b>2 ( 9%)</b>	0 (0%)
<b>Gender</b>							
	Male	2 ( 33%)	8 ( 47%)	2 ( 50%)	<b>12 ( 44%)</b>	<b>10 ( 45%)</b>	4 (40%)
	Female	4 ( 67%)	9 ( 53%)	2 ( 50%)	<b>15 ( 56%)</b>	<b>12 ( 55%)</b>	6 (60%)
<b>Age (yrs)</b>							
	Median/ range	62	61	60	<b>62</b>	<b>62</b>	63
	(Range)	(47 - 67)	(49 - 75)	(51 - 67)	<b>(47 - 75)</b>	<b>(33 - 82)</b>	(36-71)
<b>Smoking History</b>							
	Never	2 ( 33%)	1 ( 6%)	0 ( 0%)	<b>3 ( 11%)</b>	<b>1 ( 5%)</b>	3 (30%)
	Former	2 ( 33%)	12 ( 71%)	3 ( 75%)	<b>17 ( 63%)</b>	<b>14 ( 64%)</b>	4 (40%)
	Current	2 ( 33%)	4 ( 24%)	1 ( 25%)	<b>7 ( 26%)</b>	<b>7 ( 32%)</b>	3 (30%)
<b>PA stage</b>							
	IA	4 ( 67%)	4 ( 24%)	2 ( 50%)	<b>10 ( 37%)</b>	<b>7 ( 32%)</b>	3 (30%)
	IB	1 ( 17%)	2 ( 12%)	0 ( 0%)	<b>3 ( 11%)</b>	<b>4 ( 18%)</b>	2 (20%)
	IIA	0 ( 0%)	1 ( 6%)	0 ( 0%)	<b>1 ( 4%)</b>	<b>2 ( 9%)</b>	1 (10%)
	IIB	0 ( 0%)	3 ( 18%)	0 ( 0%)	<b>3 ( 11%)</b>	<b>3 ( 14%)</b>	1 (10%)
	IIIA	1 ( 17%)	5 ( 29%)	1 ( 25%)	<b>7 ( 26%)</b>	<b>1 ( 5%)</b>	3 (30%)
	IIIB	0 ( 0%)	0 ( 0%)	0 ( 0%)	<b>0 ( 0%)</b>	<b>2 ( 9%)</b>	0 (0%)
	IV	0 ( 0%)	2 ( 12%)	1 ( 25%)	<b>3 ( 11%)</b>	<b>3 ( 14%)</b>	0 (0%)
<b>EGFR mutation</b>	Positive	3 (50%)	1 (6%)	0 ( 0%)	<b>4 (15%)</b>	<b>2 (9%)</b>	1 (10%)
<b>K-Ras mutation</b>	Positive	0 ( 0%)	6 (35%)	0 ( 0%)	<b>6 (22%)</b>	<b>4 (18%)</b>	2 (22%)

Abbreviations: **PR**= partial response, **SD**= stable disease, **PD**= progressive disease. **BAC**= Broncho alveolar carcinoma. **PA stage**= pathological stage according to the 7th TNM edition

Supplement Table 1. Serum marker values in erlotinib patients.

	Baseline serum levels Total erlotinib population	Baseline serum levels	During treatment serum levels
	N = 37	N=27	N = 27
<b>s-EGFR</b>			
Range	37.7 - 71.7	37.7 - 71.7	42.1 - 72
Mean	58.0	57.3	56.7
Response	65.1	65.5	60.1
No response	56.3	55.4	55.8
<b>ARG</b>			
Range	< 3.0 - 5000	3.0 - 1465	< 3.0 - 1516
Median	7.0	7.0	6.2
Response	13.7	16.2	19.8
No response	6.0	6.0	4.6
<b>TGF<math>\alpha</math></b>			
Range	< 3.0 - 63.8	3.0 - 63.80	< 3.0 - 65.3
Mean	17.7	18.1	20.2
Response	19.4	23.0	21.3
No response	17.5	17.4	19.9
<b>IGF1</b>			
Range	9.0 - 51.4	9.30 - 51.38	9.5 - 48.22
Mean	19.0	19.4	20.4
Response	22.3	23.7	24.0
No response	18.2	18.4	19.4
<b>IGFBP33</b>			
Range	2.4 - 5.9	2.36 - 5.90	2.4 - 6.5
Mean	4.1	4.1	4.2
Response	4.5	4.3	4.6
No response	4.0	4.1	4.0
<b>IGF ratio</b>			
Range	0.06 - 0.28	0.06 - 0.28	0.05 - 0.26
Mean	0.14	0.14	0.14
Response	0.14	0.15	0.14
No response	0.13	0.13	0.14

Table 2: Baseline marker distributions according to metabolic response.

		PR	SD	PD	Total	P-value	Control	Baseline only
		N = 6	N = 17	N = 4	N = 27	metabolic response	N = 22	N=10
<b>sEGFR</b> ng/L	Median	65	55	57	57	<b>0.04</b>	55	61
	(Range)	(59 - 72)	(38 - 71)	(53 - 60)	(38 - 72)		(41 - 72)	(42-69)
	High/Low	6/0	8/9	3/1	17/10		7/3	
<b>ARG</b> ng/L	Median	12	5.9	9.9	7	0.98	2.9	12.7
	(Range)	(2.9 - 48)	(2.9 - 1500)	(2.9 - 170)	(2.9 - 1500)		(2.9 - 4100)	(<3-5000)
	High/Low	3/3	5/12	2/2	10/17		3/7	
<b>TGF</b> ng/L	Median	14	12	15	14	0.66	15	12
	(Range)	(2.9 - 64)	(3.5 - 37)	(9.9 - 16)	(2.9 - 64)		(3.3 - 70)	(<3-42)
	High/Low	1/5	5/12	0/4	6/21		4/6	
<b>IGF1</b> nmol/L	Median	19	16	20	16	0.74	17	17
	(Range)	(14 - 51)	(9.3 - 42)	(14 - 38)	(9.3 - 51)		(8.9 - 41)	(9-29)
	High/Low	6/0	14/3	4/0	24/3		9/1	
<b>IGFBP3</b> mg/L	Median	4.3	3.6	4.9	4.0	0.77		3,8
	(Range)	(3.7-5.5)	(2.4-5.9)	(3.7-5.6)	(2.4-5.9)			(2.9-5.3)
	High/Low	6/0	16/1	4/0	26/1		9/1	
<b>IGF ratio</b>	Median	0.12	0.13	0.10	0.12	0.96		0.15
	(Range)	(0.07-0.28)	(0.06-0.21)	(0.79-0.20)	(0.07-0.18)			(0.07-0.17)
	High/Low	1/5	4/13	2/2	7/20		2/7	

Abbreviations PR= partial response, SD= stable disease, PD= progressive disease, sEGFR: soluble endothelial growth factor receptor, TGFa, transforming growth factor alpha-, ARG, amphiregulin; IGF1, insulin-like growth factor-1, IGFBP3, insulin-like growth factor binding protein-3.

*Mutation status*

Mutations status was assessed in all 37 patients receiving erlotinib treatment with available baseline serum. EGFR was mutated in 5 patients (13.5%), KRAS in 8 (21.6%). Four out of five (80%) patients with EGFR-mutated tumors showed high sEGFR baseline levels (Table 4). ARG baseline levels did not seem to be associated with either EGFR or KRAS mutations, while low TGFa baseline levels was present in 4 out of 5 EGFR-mutated patients. IGF1 was high in 4 out of 5 EGFR mutated patients, IGFBP3 was high in all 5 and the IGF ratio was low in all 5. No further statistics were applied on mutation level.

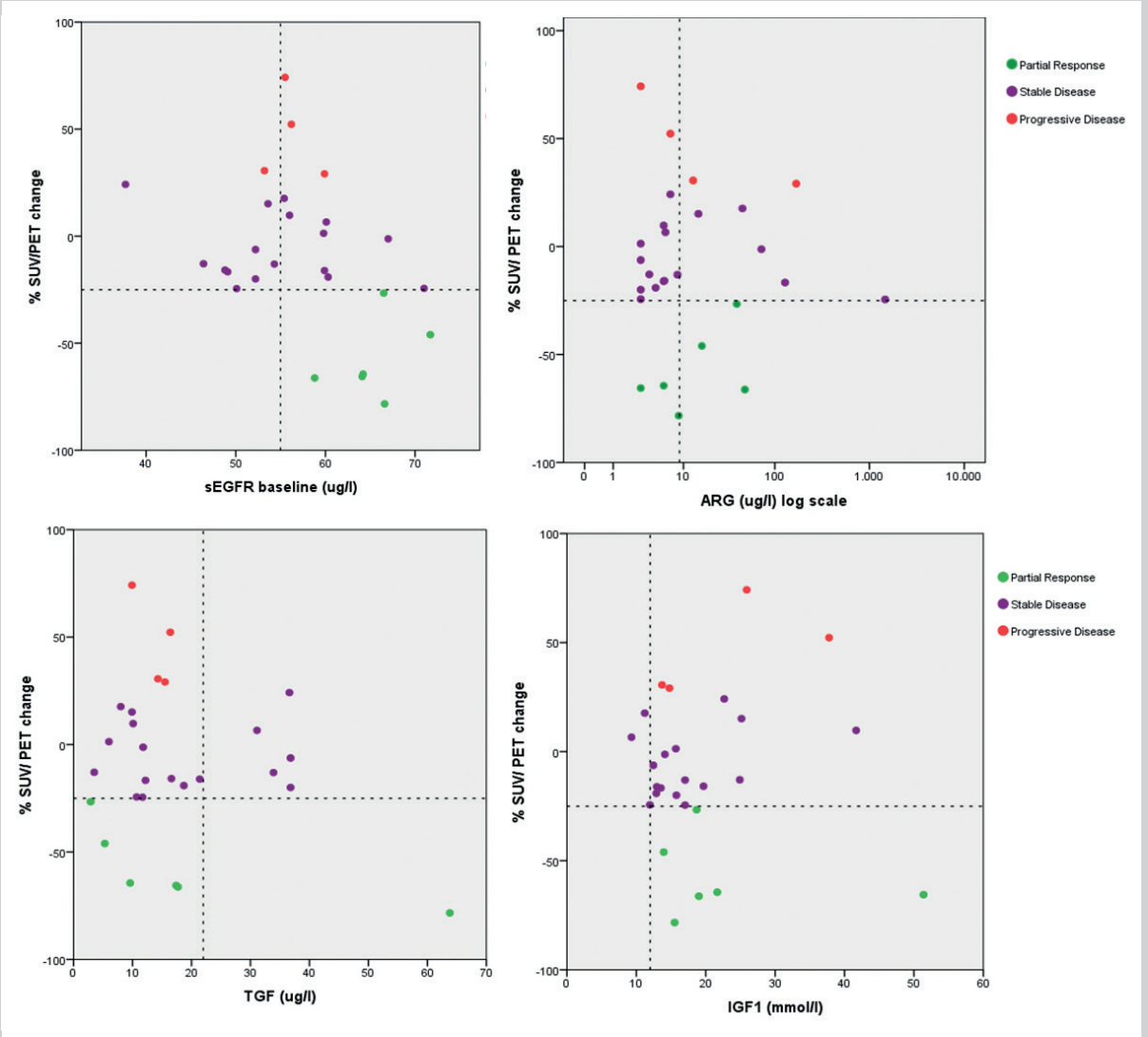


Figure 1: Relative PET-change vs. serum marker-baseline distributions in 27 patients, treated neoadjuvant with erlotinib.

Table 3: Absolute change in marker distributions between baseline and treatment serum levels according to metabolic response for 27 patients treated with erlotinib.

	<b>PR N = 6</b>	<b>SD N = 17</b>	<b>PD N = 4</b>	<b>Total N = 27</b>	<b>P value</b>
<b>sEGFR</b> ng/L					
Median difference	-7	-1.6	2	-1.8	0.09
(Range: decrease - increase)	(-10 - 9.3)	(-16 - 20)	(-5.5 - 13)	(-16 - 20)	
<b>ARG</b> ng/L					
Median difference	11	-11	68	-3.4	0.73
(Range: decrease - increase)	(-24 - 3200)	(-88 - 59)	(-47 - 650)	(-88 - 3200)	
<b>TGF</b> ng/L					
Median difference	20	-3.8	11	-2.8	1.00
(Range: decrease - increase)	(-39 - 54)	(-60 - 250)	(-21 - 190)	(-60 - 250)	
<b>IGF1</b> nmol/L					
Median difference	4.6	5.3	-2.6	5.1	0.26
(Range: decrease - increase)	(-17 - 31)	(-11 - 53)	(-31 - 53)	(-31 - 53)	
<b>IGFBP3</b> mg/L					
Median difference	0.5	0.2	-0.25	0.10	0.52
(Range: decrease - increase)	(-1.2 - 3.0)	(-1.3 - 0.8)	(-0.3 - 0.8)	(-1.3 - 0.8)	
<b>IGF ratio</b>					
Median difference	-0.01	-0.01	0.00	-0.01	0.40
(Range: decrease - increase)	(-0.03 - 0.06)	(-0.07 - 0.08)	(-0.04 - 0.03)	(-0.7 - 0.8)	

Table 4. Outcome baseline serum markers split out for mutation status, using predefined cut-off levels

		<b>Double WT</b> N=24	<b>EGFR +</b> N=5	<b>KRAS +</b> N=8
<b>sEGFR</b>	Low	8	1	4
	High	16	4	4
	Mean (range)	57.8 (42-72)	59.4 (52-60)	56 (38-71)
<b>ARG</b>	Low	13	2	5
	High	11	3	3
	Median	7 (<3-3427)	12.7 (6-48)	7.8 (<3-5000)
<b>TGF <math>\alpha</math></b>	Low	18	4	5
	High	6	1	3
	Mean (range)	16.8 (<3-37)	22.1 (8-64)	21.0 (3.5-42)
<b>IGF1</b>	Low	2	1	1
	High	22	4	7
	Mean (range)	20.3 (9-51)	16.5 (11-22)	18.7 (12-25)
<b>IGFBP</b>	Low	1	0	1
	High	23	5	7
	Mean (range)	4.2 (2-6)	4.1 (3-5)	3.8 (3-6)
<b>IGFratio</b>	Low	17	5	6
	High	7	0	2
	Mean (range)	0.13 (0.06-0.28)	0.11 (0.08-0.14)	0.15 (0.06-0.21)

## DISCUSSION

This study shows that high baseline levels of soluble EGFR are associated with response to erlotinib in early stage NSCLC. Furthermore, in patients with metabolic response, s-EGFR levels decreased during treatment. Concentrations and their changes during treatment of TGF $\alpha$ , ARG and IGF1 were not predictive for response. For IGFBP3, the value measured in this patient group was higher than the previous measured values in advanced NSCLC patients. In that study IGFBP3 was predictive for progression free survival, regardless of treatment, which might explain the level difference in different stages of NSCLC.

The need for predictive markers is greater than ever in this era of personalised medicine. Informative markers can reduce unnecessary toxicity and cost. Especially in lung cancer, selecting patients for targeted therapy using a serum test would be an advantage, as collecting (representative) tumor tissue may be difficult and require bronchoscopic or transthoracic biopsies.

In a previous study in patients treated with erlotinib or gefitinib for stage III/IV NSCLC, higher levels of baseline s-EGFR were associated with improved survival and with lower risk of progressive disease within three months (14). S-EGFR is thought to reflect the absolute number of activated EGF receptors that can be inhibited, explaining a relation to EGFR-TKI treatment response in both the present study and the study of Kappers et al. Decreasing levels of s-EGFR during response to EGFR-TKIs have been reported in patients with advanced NSCLC (27). In that study, responders showed a s-EGFR decrease at time of best response compared to baseline level by more than  $-3.6 \mu\text{g/l}$ .

Other serum markers, TGF $\alpha$ , ARG, IGF1 and IGFBP3, were not informative for response evaluation. Two studies have reported that patients with baseline high TGF $\alpha$  or low ARG level did not benefit from TKI treatment (28,29), assuming they could have a predictive value. In our study both responders and non responders were found having high TGF $\alpha$  levels or low ARG levels, indicating that these levels do not predict for treatment response.

Whether these markers (TGF $\alpha$ , ARG, IGF1 and IGFBP3) do not have any predictive value remains uncertain. Although the number of patients in our study is limited, the overlap of test results between responders and non-responders makes a substantial link with response to neoadjuvant EGFR-TKIs unlikely. Another explanation for the discrepancy is the different population of patients (in this study mainly early stage NSCLC as compared to other studies with more advanced stages). Furthermore, previous studies have used other outcome measures (survival, stable disease). In this study, we used a metabolic measure for response. Defining response in the setting of targeted therapy for early stage NSCLC is challenging, since tumor volume reduction (RECIST) is not expected to occur within short term and pathologic standards to qualify for regression to TKI therapy are lacking (18,30). There is an ongoing discussion on the relation between response evaluation and prediction of survival in patients with NSCLC receiving neoadjuvant therapy, however early metabolic evaluation during treatment is more appropriate than other modalities (24).

## CONCLUSION

Currently in NSCLC, mutation status is the main factor used to select patients for TKI treatment. However, some patients do not benefit from EGFR-TKI treatment despite the presence of EGFR mutations, while some other patients do benefit without (known) mutation. Whether this is due to intra- and intertumor heterogeneity and sampling or whether it is due to false-negative or false-positive tests caused by methodological errors, or whether there are patients with other molecular aberrations benefitting from EGFR-TKIs, is unclear. Furthermore, systemic antineoplastic treatment may affect mutation status and EGFR overexpression.

If s-EGFR does reflect the amount of activated receptors that can be inhibited, this biomarker test may be a more functional indicator to select patients for EGFR-TKI treatment, since it potentially selects patients with an activated EGFR-pathway regardless of mechanism of activation. In addition, it is a relatively simple test, which can be repeated during treatment to evaluate decrease. The cut-off level of 54.95 µg/l was derived from former studies, but may be suboptimal. In another study a cut-off level of serum EGFR of 55,42 µg/L was reported (31). Using this slightly higher cut-off level did not make a substantial difference in outcome for the current dataset. More data are needed to refine this cut-off level and further test s-EGFR for treatment selection and monitoring.

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