Utility of Insulin-like Growth Factor Receptor-1 Expression in Gefitinib-Treated Patients with Non-small Cell Lung Cancer

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Abstract. *Background: Insulin-like growth factor receptor 1 (IGF1R) is a proposed mechanism of resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors. Newer agents targeting this pathway make it of clinical interest. This study evaluates the IGF1R expression in regard to outcomes and molecular markers of EGFR activity in lung cancer patients treated with gefitinib. Materials and Methods: Gefitinib-treated patients with sufficient archived tissue were included. The IGF1R activity was measured by immunohistochemistry and the EGFR by immunohistochemistry, fluorescent in situ hybridization, and gene mutation testing. Logistic regression and cox proportional hazards models were used. Results: A total of 83 patients were included in the study: 71% were positive for IGF1R expression which was not associated with EGFR parameters or clinical outcomes. Exploratory analyses showed counter-intuitive improved outcomes with co-expression of IGF1R and EGFR. Conclusion: IGF1R expression measured by immunohistochemistry does not appear to be related to gefitinib resistance.*

Initial phase II trials testing epidermal growth factor receptor tyrosine kinases (EGFR-TKIs) gefitinib and erlotinib, showed objective responses in approximately 10% of patients with non-small cell lung cancer (NSCLC) (1, 2). Subsequently, superior survival was observed with erlotinib in unselected, previously treated patients with NSCLC, and gefitinib was associated with better progression-free survival as well as a trend for longer overall survival (3, 4). *EGFR*-activating mutations have emerged as important predictors for progression-free survival and response in previously untreated patients with advanced NSCLC (5). Although present in about half of non-smoking-related NSCLCs, the prevalence of activating *EGFR* mutations in North America and Europe, overall, is only approximately 10% (6). In the phase III trials of gefitinib and erlotinib, however, disease stabilization was observed in approximately 35% of patients with NSCLC treated with an EGFR-TKI, benefiting many *EGFR* gene mutation-negative patients (3, 4).

Molecular profiles that could identify patients most likely to have disease stabilization or rapid progression on EGFR-TKIs would be beneficial by bringing more effective therapies earlier in the treatment course of such patients. The insulin-like growth factor receptor 1 (IGF1R) is a proposed mechanism for resistance to EGFR-TKIs (7-10). IGF1R is a transmembrane tyrosine kinase which, through its downstream signaling, controls the cell size, the growth stimulation and inhibits apoptosis (11). Activation of IGF1R and loss of IGF-binding proteins have been associated with cellular proliferation, survival, transformation, and metastasis (11-15). Recently monoclonal antibodies targeting the IGF1R and the small molecule IGF1R-TKIs have become available.

The objective of our retrospective study was to evaluate the potential relationship of the IGF1R expression with the response, progression-free and overall survival in patients with advanced NSCLC treated with gefitinib. Additional objectives included the determination of the relationship of IGF1R expression with EGFR by immunohistochemistry (IHC) and fluorescent *in situ* hybridization (FISH) with *EGFR*-activating mutations.

Materials and Methods

Patients and clinical assessment. A total of 150 patients with NSCLC treated for more than one week with gefitinib on the Gefitinib Expanded Access Program were included in this study, although only 83 out of the 150 patients had sufficient tissue available for analysis. Institutional Review Board approval was obtained for the retrospective tissue analyses and the clinical correlations. All patients had histologically confirmed stage IV NSCLC that relapsed or progressed after at least one platinum containing chemotherapy regimen, or were not considered to be

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candidates for conventional cytotoxic therapy. Non-smoking status was defined by lifetime consumption of <100 cigarettes. Overall response rate (RR) was assessed according to the RECIST criteria. The progression-free interval (PFI) and the overall survival (OS) were measured in months from the start of gefitinib treatment to the time of disease progression or untill death. Progressive disease was defined as progression within 70 days of treatment.

Immunohistochemistry. IHC and FISH were performed on 5.0 μm sections of formalin-fixed paraffin-embedded tumor tissue or sections from cytology cell blocks. A Ventana ES Histo-stainer (Ventana Medical Systems, Tuscon, AZ, USA) was used with supplied diaminobenzidine and avidin-biotin conjugated immunoperoxidase chemistry to stain the single best tissue block as determined by the largest amount of well-fixed histologically viable tumor. EGFR was stained with the M3563 mouse monoclonal antibody at 1:200 dilution (Lot 087; Kako Corp, Carpinteria, CA, USA) and IGF1R was stained using the Clone 24-31, specific for the extracellular alpha subunit of IGF1R (Thermo Fisher Scientific, Barrington, IL, USA). Both EGFR frequency and intensity of staining of all tumor cells on each slide were estimated on a scale of 0 to 4 by a clinical pathologist, blinded to clinical patient data. Frequency of positive staining in fewer than 1% of tumor cells was scored as 0, 1%-10% as 1, 11%-35% as 2, 36%-70% as 3, and over 70% as 4. Only cell membrane-associated staining was considered for EGFR and IGF1R. An EGFR IHC score was calculated by multiplying the intensity by the frequency. Prior to statistical analysis, IGF1R staining was classified as zero or positive, with positivity defined as any IGF1R staining present in the examined sample. Analyses were run again using the Ludovini definition of IGF1R positivity (16).

EGFR in situ hybridization and mutational analysis. *EGFR* and centromere 7 (CEN7) probes were utilized to examine *EGFR*/cell, CEN7/cell and *EGFR*/CEN7. Specimen slides were hybridized with two-color FISH probe solutions (Vysis Spectrum Orange LSI and EGFR/Spectrum Green CEP7; Abbott Molecular Incl, Des Plains, IL, USA). The methods for *EGFR* gene mutation analysis (exon 19 and exon 21) and FISH score calculations have been described elsewhere (17).

Statistical analysis. The associations between response to gefitinib (yes/no) and categorical covariates were tabulated and the Fisher's exact test was used to measure their significance. The Kaplan-Meier method was used to estimate the probability of survival as a function of time. Survival differences among comparator groups were analyzed by the log-rank test. Logistic regression and Cox proportional hazards models were used to select and model the effect of molecular markers and other predictors on OS and PFI, respectively. All analyses were performed using Version 9.1.3 of the SAS software (SAS Institute, Cary, NC, USA) and all reported *p*-values are two-sided with a level of <0.05 as accepted significance.

Results

Patients' characteristics. A total of 83 out of 150 Expanded Access Program patients had sufficient tissue available for IGF1R analysis. Each patient was treated with gefitinib, taken orally at a dose of 250 mg daily. The median age of this group was 68 years and 48% were women. There were 12 non-smokers in the group, and 57% had Eastern Cooperative Oncology Group performance status (PS) of 0-1. The most frequent histological subtype was adenocarcinoma, and it was present in 56 (67.5%) of the analyzed patients. RR, median PFI, and median overall survival (OS) on gefitinib therapy for the 83 patients with sufficient tissue for this analysis were: 14.46%, 3.1 months (2.1-4.0), and 7.3 months (5.7-9.7), respectively. Smoking was associated with shortened PFI and OS (p=0.0112 and p=0.0443, respectively) although there was a larger percentage of *EGFR* mutation carriers in the non smokers compared with the smokers (66.7% and 20%, respectively). The one year progression-free survival rate was 17%.

IGF1R expression: clinical and molecular characteristics. A positive IGF1R score (at least 1% of tumor cells positive) was seen in 71.08% of the tissue samples. There was no apparent relationship between IGF1R positivity and age, gender, histology, or PS, although an analysis of adenocarcinoma histology subtyping was not performed (Table I). There was a marginally higher rate of IGF1R positivity in smokers (p=0.096), and smoking was associated with both IGF1R positivity and EGFR IHC positivity using the Ludovini definitions of IGF1R positivity and EGFR IHC positivity (16).

Eightyone patients were evaluated for EGFR protein expression by IHC. *EGFR* gene copy number, and chromosome 7 copy number (c7) were evaluated by FISH. Exon 19 and 21 *EGFR* mutation analyses were carried out in 58 cases. Both IGF1R protein expression by IHC, EGFR expression by IHC and FISH analyses were performed in 78 cases.

There were no significant associations between IGF1R positivity and EGFR IHC, *EGFR* gene copy number, *EGFR* gene mutation, or other markers associated with the EGFR pathway. There was an association between IGF1R positivity and high chromosome 7 copy number (chromosome 7>3, p=0.011). An exploratory analysis of high EGFR IHC, *EGFR* gene copy number, and chromosome 7 gene amplification with the product of IGFR1 intensity (0-4) and frequency (0-4) did show a statistically significant correlation of increasing EGFR IHC and FISH-related parameters with increasing IGFR-1 score (p=0.002, p=0.01, and p=0.002, respectively).

IGF1R expression and clinical outcomes. There was no statistically significant association between IGF1R positivity alone and PFI or OS by the log-rank analysis (p=0.783 and p=0.20, respectively). The combination of IGF1R positivity and EGFR IHC positivity, as defined by Ludovini *et al*. (16), was associated with improved one-year PFI (27% *vs*. 10% for negative IGF1R and EGFR, log-rank p=0.0273) (see Figure 1). *EGFR* gene mutation in exon 19 or exon 21 was significantly associated with prolonged progression-free survival (p=0.0025).

Table I. *IGF1R protein expression according to clinical characteristics.*

Characteristic	IGF1R negative	IGF1R positive	*p*-Value (Fisher exact test)
N	24	59	
Male (%)	58%	52.60%	0.81
Median age, years	68	68	
Adenocarcinoma (%)	62.50%	69.50%	0.61
Smokers (%)	96%	80%	0.096

A total of 67 out of the 150 patients enrolled in the Expanded Access Trial did not have sufficient tissue available for IGF1R staining. Patients with sufficient tissue for analysis had prolonged PFI and OS on gefitinib therapy ($p<0.0001$ and $p<0.0002$ by log-rank analysis, respectively).

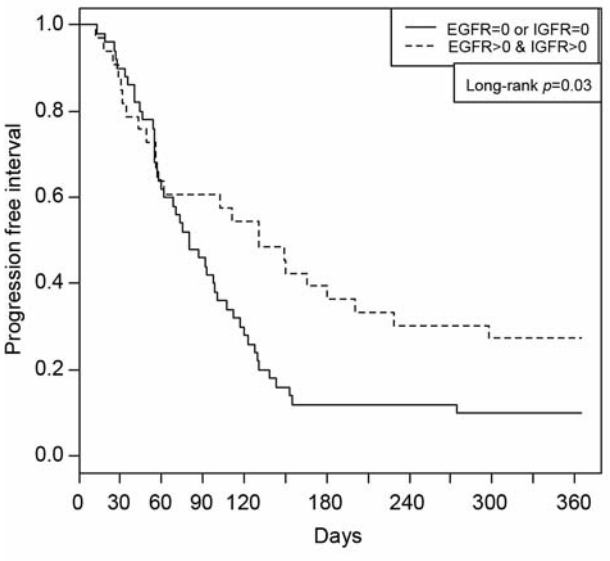

Figure 1. *Progression-free interval by IGF1R and EGFR expression.*

Discussion

The IGF pathway is complex, involving interactions between receptors, ligands, binding proteins, downstream enzymes, and other membrane-bound tyrosine kinases including the EGFR (12, 18-20). Bellil *et al*. correlated gene expression profiles in cell lines with highly activated insulin receptor IGF1R pathways with worse outcome in surgically resected NSCLC patients (13). Models also suggest that signaling through the IGF1R pathway might be a mechanism of resistance to EGFR TKI (8-10, 13, 14, 21, 22). Despite the potential importance of this pathway, there is relatively little information in regard to other molecular markers deemed important in treating NSCLC patients with EGFR-targeted therapy. These considerations provided the rationale to study the frequency of IGF1R expression and its potential relationship to outcomes in gefitinib-treated patients and correlate expression with other molecular markers that are downstream or in cross-talk with the IGF1R pathway.

Our study classified tumors as being positive for IGF1R if at least one cell was stained with the Thermo Fisher Scientific antibody and 71% of the specimens tested positively for IGF1R. Novus (Novus Biologicals, Littleton, CO, USA) and Ventana G11 (CONFIRM; Ventana Medical Systems, Tuscon, AZ, USA) antibodies have also been used to study IGF1R expression in NSCLC. IGF1R positivity, defined by the product of the percentage of stained cells and the intensity of staining (1-4) >99, was present in 39-76.4% of patients studied using the Novus antibody and in 84% of patients using the Ventana antibody. Lee *et al*. used the Vector antibody (Vector Laboratories, Burlingame, CA, USA) on stage I tumors and found 12.7% of positive cases (intensity 1+ or greater on a scale of 0-3+) (23). Dziadziuszko *et al*. tested surgically resected tumors with the Lab Vision antibody (Lab Vision Neomarkers, Fremont, CA, USA) and found that 36% of patients had tumors with at least 10% of cells staining for IGF1R (24). Finally, Hurbin *et al*. stained samples from 80 patients with adenocarcinoma with bronchoealveolar features and found an IHC positivity rate of 43%, using the G11 Ventana antibody (10) (see Table II).

The prognostic role of IGF1R expression in NSCLC is not yet clear. Wynes *et al*. found prolonged progression-free and OS in surgically resected NSCLC patients with increased copy numbers of *IGF1R* detected by silver *in situ* hybridization (SISH) (25). Lee *et al*. and the University of Colorado group, did not see this correlation with IHC staining of IGF1R using the LabVision Neomarkers and the Ventana antibodies, respectively (23, 24). Earlier work with the Novus IGF1R antibody suggested a negative correlation of IGF1R staining with OS, particularly for patients with stage I tumors, but later work suggested that the Novus antibody did not correlate with mRNA expression of *IGF1R,* as well as did the Ventana antibody (r=0.37; $p<0.001$) (24, 26). Using probes developed at the University of Colorodo, this same group did find that high *IGF1R* gene copy numbers (gene amplification and high polysomy) correlated with improved OS in resected patients (3-year survival 58% *vs*. 47%, $p=0.024$) (24). In a large retrospective study, IGF1R IHC expression using the Novus antibody on 369 surgically resected NSCLC tumor specimens did not correlate with survival (27). Another large study totaling 811 surgically resected NSCLC patients from two patient datasets showed a correlation of higher expression of cytoplasmic IGF1R and worse survival only in one dataset (28) (see Table II). Gene expression of IGF1R has not correlated with response or survival in erlotinib treated NSCLC patients harboring an EGFR-activating gene mutation (29).

Table II. *Summary of IGF1R clinical and molecular correlates.*

Investigator	No. of patients	IGF-1R method	Percent positive	Squamous histology correlation	EGFR correlation	Stage correlation	Progression free survival	Overall survival
Present study *	83	IHC (Thermo Fisher Scientific)	71%	–	p=0.011 (Cen7>3)	–	p=0.763	p=0.647
Cappuzzo *et al.** (30)	77	IHC (Novus)	39%	–	p=0.65(FISH) p=0.23 (mutation)	p=0.1	p=0.8	p=0.013
Dziadziuszko *et al.* (24)	177	IHC (Ventana)	84%	p<0.001	p<0.001 (IHC)	p=0.03	–	p=0.40
	181	FISH (Ventana)	48%	p=0.581	NS	p>0.05	p<0.05	p=0.024
	114	mRNA	–	p=0.006	NS	NS	–	p=0.25
Wynes *et al.* (25)	189	SISH (Ventana)	–	p=0.008	–	–	p=0.0014	p=0.0211
Cappuzzo *et al.* (27)	369	IHC (Novus)	76.4%	p=0.04	–	p=0.41	–	p=0.98
Ludovini *et al.* (16)	125	IHC (Lab Vision Neomarkers)	36%	p=0.59	p=0.033 (IHC)	p=0.04 (tumor size)	p=0.4	p=0.11
Lee *et al.* (20)	71	IHC (Biosource)	12.7%	p>0.05	–	–	–	p>0.05
Kim *et al.* (28)	811	IHC (Cell Signal)	–	p<0.0001	–	–	NS	p=0.312, p=0.042 for two data sets
Hurbin *et al.* (10)	80	IHC (Ventana)	43%	Mucinous adenocarcinoma (p<0.00001)	Inverse r=–0.512 (p<0.0001)	–		

NS, Not stated; IHC, immunohistochemistry; SISH, silver in-situ hybridization; FISH, fluorescent *in situ* hybridization; Cen7, centromere 7. *Patients received gefitinib.

The results of our retrospective trial suggested that patients with IGF1R and EGFR expression may have prolonged PFI when treated with gefitinib therapy. These results are similar to those of Cappuzzo *et al.* who showed prolonged OS for the highest quartile of IGF1R expression in advanced NSCLC patients treated with gefitinib therapy (30). These results remain counterintuitive, as one would expect the IGF1R pathway to impart resistance to EGFR tyrosine kinase therapy. In a study of 34 patients with adenocarcinoma with broncheoalveolar features, the opposite result was seen; in this retrospective analysis, IGF1R expression correlated with the mucinous subtype (p<0.0001) and with disease progression (p=0.0209) (10). Taken together, IHC measurement of IGF1R activity is likely not the best method for selecting patients for EGFR-TKI therapy. Recent results suggest that the circulating levels of IGF-1 may be predictive of benefit from targeting the IGF1R pathway in patients treated with both cytotoxic chemotherapy and EGFR-TKI. In randomized phase II and III trials comparing carboplatin and paclitaxel with or without figitumumab, superior survival was observed with figitumumab in patients with high levels of circulating IGF-1 (31, 32). In a study comparing two doses of R1507 monocloncal antibody against IGF1R *versus* erlotinib plus placebo, the addition of R1507 failed to show an improvement in 12-week progression-free survival in unselected patients. Circulating levels of IGF-1 were analyzed in 165 out of the 171 enrolled patients. Those with levels higher than the median had a 46% progression-free rate at 12 weeks, compared to an 18% progression-free rate in patients with IGF-1 levels below the median (33). Although we await results of other clinical trials targeting the EGFR and IGF1R pathway, it appears that serum markers of the IGF pathway will likely be more useful than IHC evidence of IGF1R expression on tissue specimens.

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