VOLUME 28 · NUMBER 13 · MAY 1 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Insulin-like Growth Factor Receptor 1 (IGF1R) Gene Copy Number Is Associated With Survival in Operable Non–Small-Cell Lung Cancer: A Comparison Between IGF1R Fluorescent In Situ Hybridization, Protein Expression, and mRNA Expression

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Submitted June 17, 2009; accepted December 14, 2009; published online ahead of print at www.jco.org on March 29, 2010.

Supported by SPORE Grant No. P50 CA058187 (University of Colorado Cancer Center), Grant No. ST-23 (Medical University of Gdansk), by a grant from the International Association for the Study of Lung Cancer Fellowship (R.D.), and by a grant from Colorado Cancer League (F.R.H.).

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/10/2813-2174/$20.00

DOI: 10.1200/JCO.2009.24.6611

ABSTRACT

Purpose
The purpose of this study was to characterize insulin-like growth factor-1 receptor (IGF1R) protein expression, mRNA expression, and gene copy number in surgically resected non–small-cell lung cancers (NSCLC) in relation to epidermal growth factor receptor (EGFR) protein expression, patient characteristics, and prognosis.

Patients and Methods
One hundred eighty-nine patients with NSCLC who underwent curative pulmonary resection were studied (median follow-up, 5.3 years). IGF1R protein expression was evaluated by immunohistochemistry (IHC) with two anti-IGF1R antibodies (n = 179). EGFR protein expression was assessed with PharmDx kit. *IGF1R* gene expression was evaluated using quantitative reverse transcription polymerase chain reaction (qRT-PCR) from 114 corresponding fresh-frozen samples. *IGF1R* gene copy number was assessed by fluorescent in situ hybridization using customized probes (n = 181).

Results
IGF1R IHC score was higher in squamous cell carcinomas versus other histologies ($P < .001$) and associated with stage ($P = .03$) but not survival ($P = .46$). IGF1R and EGFR protein expression showed significant correlation ($r = 0.30$; $P < .001$). *IGF1R* gene expression by qRT-PCR was higher in squamous cell versus other histologies ($P = .006$) and did not associate with other clinical features nor survival ($P = .73$). Employing criteria previously established for *EGFR* copy number, patients with *IGF1R* amplification/high polysomy (n = 48; 27%) had 3-year survival of 58%, patients with low polysomy (n = 87; 48%) had 3-year survival of 47% and patients with trisomy/disomy (n = 46; 25%) had 3-year survival of 35%, respectively ($P = .024$). Prognostic value of high *IGF1R* gene copy number was confirmed in multivariate analysis.

Conclusion
IGF1R protein expression is higher in squamous cell versus other histologies and correlates with EGFR expression. IGF1R protein and gene expression does not associate with survival, whereas high *IGF1R* gene copy number harbors positive prognostic value.

J Clin Oncol 28:2174-2180. © 2010 by American Society of Clinical Oncology

INTRODUCTION

Lung cancer remains the leading cause of cancer-related deaths worldwide.[1] Novel therapeutic developments in non–small-cell lung cancer (NSCLC) have resulted in only minor improvement of patient outcomes. There is substantial evidence for involvement of insulin-like growth factor (IGF) pathway in the development and progression of multiple cancer types, and good preclinical evidence of antitumor efficacy of pathway blockade in NSCLC and several other tumors.[2,3] The IGF pathway is composed of two ligands (IGF1 and IGF2, both produced in the liver under the control of growth hormone), their binding proteins (IGFBP1-6), and two receptors (IGF1R and IGF2R).[3] IGF1R has the capability of signal transduction through intracellular tyrosine kinase linked

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to RAS/RAF/mitogen activated protein kinase (MAPK) pathway and phosphatidylinositol-3-kinase (PI3K)-Akt pathway.

A number of IGF1R inhibitors are currently being investigated in several phase I to III clinical trials. Monoclonal antibodies against IGF1R are the most advanced in clinical development. Phase I to II studies of these compounds indicated favorable toxicity profiles and promising activity.[4-7] Several lines of evidence suggest an association between epidermal growth factor receptor (EGFR) and IGF1R pathways. Results of preclinical studies indicate that both receptors may heterodimerize,[8] are capable of trans-phosphorylation,[9] and share the same adaptor proteins and downstream signaling pathways.[10] Activation of IGF1R is a well-documented mechanism of EGFR inhibitor resistance in vitro.[11] Several ongoing clinical trials explore the efficacy of combination of EGFR and IGF1R inhibitors in patients with lung cancer.

This study was aimed to evaluate the relationship between IGF1R and EGFR protein expression, *IGF1R* gene copy number, and IGF1R mRNA expression in a cohort of patients with NSCLC who underwent pulmonary resection, and to assess the prognostic value of these markers. Similar molecular features were previously evaluated for clinical efficacy of EGFR inhibitors by our group[12-15] and some are currently prospectively tested. These data will serve as background information for studies assessing predictive value of markers for sensitivity to IGF1R inhibitors.

PATIENTS AND METHODS

Patient Population

This study included 189 consecutive patients who underwent pulmonary resection at the Medical University of Gdansk (Table 1). The patients were staged with chest x-ray, chest and upper abdominal computed tomography, and mediastinoscopy if mediastinal lymphadenopathy was suspected on chest computed tomography (short lymph node axis > 1 cm). Other examinations were done if clinically indicated. Positron emission tomography was not available in this period, thus relatively high proportion of pathologic stage III patients (32%) and eight stage IV patients (4%) were included. Surgery consisted of lobectomy (56%), bilobectomy (7%), pneumonectomy (34%), or limited lung resection (3%). Systematic lymph node dissection was performed in all patients. Adjuvant chemotherapy was not routinely administered in the analyzed period per institutional guidelines. Pathologic stage III patients had unsuspected mediastinal involvement and were not managed with chemotherapy, except for seven patients who received platinum-based induction chemotherapy and three patients who received adjuvant chemotherapy. Pathologic stage IV patients were treated with palliative chemotherapy. Postoperative radiotherapy was administered in eight patients with positive postsurgical margins. Median follow-up was 5.3 years (range, 1.1 to 6.9 years). Five-year survival probability in this patient cohort was 38% (95% CI, 31% to 45%) making it comparable with other reported surgical series.[16] Tissue banking and research conduct was approved by institutional review boards of both institutions involved in the study. All patients provided informed consent and their identifying information was removed from the database.

Table 1. Patient Characteristics According to IGF1R IHC Score (Ventana G11 antibody) and *IGF1R* Gene Copy Number by Fluorescent In Situ Hybridization

	All Patients		IGF1R IHC ≤ Median		IGF1R IHC > Median			*IGF1R* Low Copy Number		*IGF1R* High Polysomy or Gene Amplification		
Variable	No.	%	No.	%	No.	%	*P*	No.	%	No.	%	*P*
Sex							.431					.304
Female	45	24	22	56	17	44		29	67	14	33	
Male	144	76	69	49	71	51		104	75	34	25	
Pathologic stage							.030					.275
I	77	41	45	63	27	37		51	68	24	32	
II	42	22	16	38	26	62		29	73	11	28	
III/IV	69	37	30	47	34	53		52	80	13	20	
Histology							< .001					.581
Squamous	103	54	33	33	67	67		75	74	26	26	
Adenocarcinoma	55	29	38	76	12	24		34	67	17	33	
Large cell	5	3	3	60	2	40		4	80	1	20	
NSCLC NOS	24	13	16	73	6	27		18	82	4	18	
Other	2	1	1	50	1	50		2	100	0	0	
Grade							.534					.224
1	20	12	7	35	13	65		16	80	4	20	
2	81	49	36	47	40	53		61	78	17	22	
3	63	38	29	49	30	51		39	66	20	34	
Smoking							.169					.701
No	9	5	7	78	2	22		6	67	3	33	
Yes	180	95	84	49	86	51		127	74	45	26	
Age							.685					.156
Median	71	38	65		63			66		62		
Range	118	62	37-81		47-85			37-85		44-77		

NOTE to Table 1. Missing data were excluded from the analysis.
Abbreviations: IGF1R, insulin-like growth factor-1 receptor; IHC, immunohistochemistry; NSCLC, non–small-cell lung cancer; NOS, not otherwise specified.

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Tissue Microarray, Immunohistochemistry, and the Scoring System

Material for analysis was derived from primary tumor tissue. Tissue microarrays were created using MaxArray customized tissue microarray service (Invitrogen, South San Francisco, CA). Three tissue cores of 1.5 mm diameter were obtained from different areas of primary tumor. Immunohistochemistry (IHC) evaluation was done using the Ventana G11 (CONFIRM, Ventana Medical Systems, Tucson, AZ) and Novus (#NB600-559, Novus Biologicals, Littleton, CO) anti-IGF1R antibodies. The Ventana G11 staining took place on the BenchMark XT from Ventana utilizing the ultraView detection kit and the primary antibody was incubated for 16 minutes. The Novus antibody was stained for 2 hours at room temperature at a 1:25 dilution after antigen retrieval. Envision Plus (DAKO Corp, Carpinteria, CA) was utilized as the detection kit and diaminobenzadine plus (DAKO) as the chromogen. For EGFR IHC, DAKO PharmDx kit was used as described previously.[15] For all assays, scoring was done according to the University of Colorado IHC scoring criteria (semiquantitative system with total score range 0 to 400).[15] Two observers (R.D., D.T.M.) independently scored each sample. Discrepant cases (IHC score $>$ 50) were reassessed by consensus meeting. For each patient, results from three cores (or fewer if depleted) were averaged. Results for each observer were averaged to obtain the final IGF1R IHC score. A tumor sample was considered positive if the score was above median and negative otherwise.

RNA, Primers, and Quantitative Real-Time Reverse-Transcriptase Polymerase Chain Reaction

Samples of primary tumors were fresh-frozen at the time of surgery and stored at −80°C. Total RNA was prepared from primary NSCLC tumors using the AllPrep DNA/RNA kit (Qiagen, Valencia, CA). From the initial cohort of 189 paraffin-embedded tumors, there were 114 corresponding fresh-frozen tumors with sufficient tumor cell content, quality, and quantity of extracted RNA. cDNA was transcribed from 1 μg RNA of each sample using Affinity-Script QPCR cDNA Synthesis Kit (Stratagene, La Jolla, CA). Quantitative real-time polymerase chain reaction was performed in 1/20th of the cDNA reaction using the Brilliant SYBRGreen QPCR Core Reagent Kit (Stratagene) in triplicates using StepOne real-time PCR System (Applied Biosystems, Foster City, CA). Primer sequences were as follows: IGF1R-1514F:TGAAAGTGACGTCCTGCATTTC, IGF1R-1587R:GGTACCGGTGCCAGGTTATG; β-actin-667F:GAGCGCGGCTACAGCTT, β-actin-1081R:TCCTTAATGTCACGCACGATTT. Amplification data were analyzed by StepOne software (Applied Biosystems, Foster City, CA), quantified according to a relative standard curve, normalized to β-actin expression, and multiplied by 1,000 for easier readout.

Gene Copy Number

IGF1R gene copy number was analyzed by fluorescent in situ hybridization (FISH) using probes developed in the University of Colorado Cytogenetics Core Facility. The scoring was tentatively carried out in 50 nonoverlapping nuclei per core. The following data were recorded: mean *IGF1R* gene and centromere 15 (CEP15) copy number per cell, number of cells with two or fewer, three, and four or more copies of *IGF1R* and CEP15 signals, and *IGF1R*/CEP15 ratio. The reading was done for each core in the tissue microarray and the core with highest average *IGF1R* gene copy number per cell was selected for each patient. Data were also analyzed using criteria established previously for *EGFR* copy number, with six categories according to ascending number of gene copies per cell.[17] Valid results were obtained for 181 patients (96%).

Statistical Analysis

Categoric variables were compared using χ^2 or Fisher's exact test, where appropriate. Continuous variables were compared using Mann-Whitney *U* test. Correlations between two continuous variables were analyzed using Spearman's rank correlation. Median follow-up time was calculated according to Schemper et al.[18] Survival curves were plotted using Kaplan-Meier method starting from date of surgery to date of death or last follow-up. Disease-free survival was defined from date of pulmonary surgery to date of local or distant progression, death of any cause, or date of last follow-up. Univariate survival analysis was performed with log-rank test and Cox proportional hazard regression. In multivariate Cox model, variables with $P \leq .20$ from Wald's test of

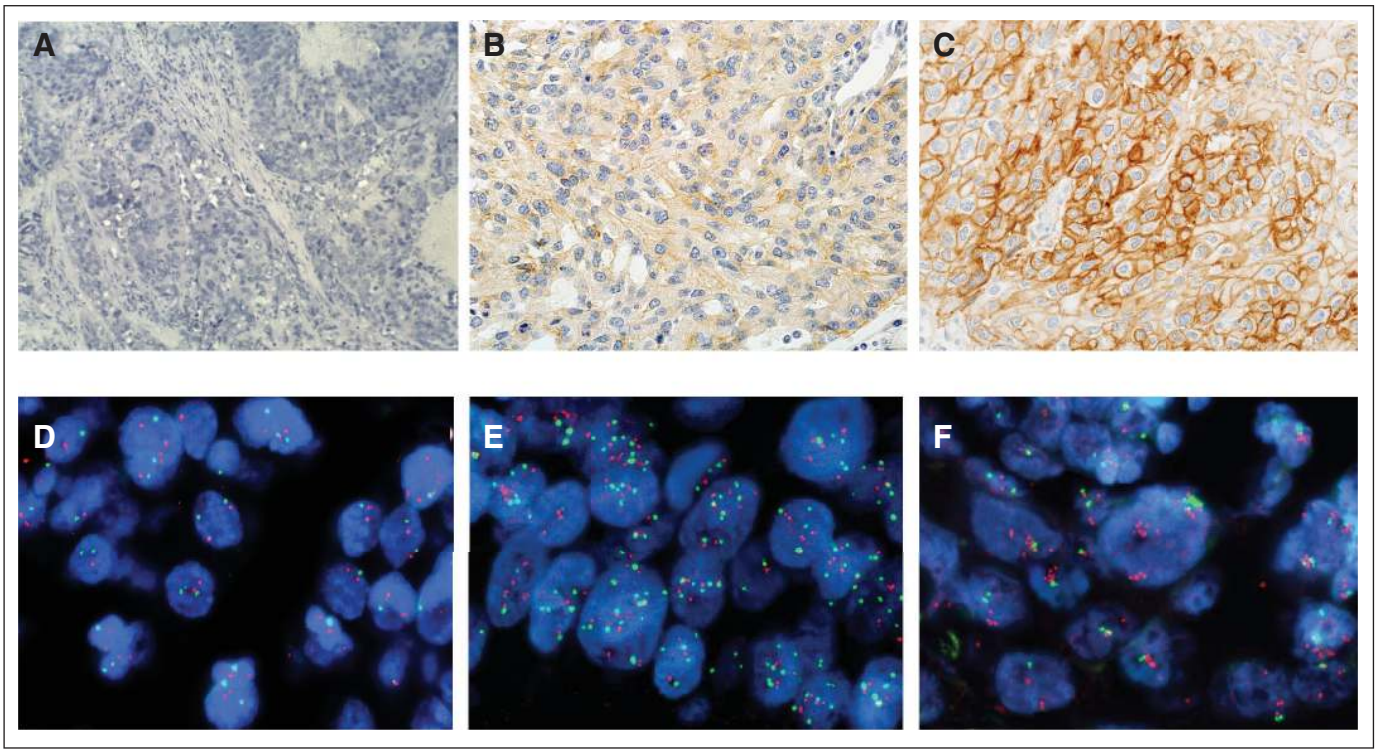

Fig 1. Examples of insulin-like growth factor-1 receptor (IGF1R) immunohistochemical staining with Ventana G11 antibody: (A) immunohistochemistry (IHC) score 0; (B) IHC score 105; (C) IHC score 350. *IGF1R* gene copy number assessment by fluorescent in situ hybridization: (D) low *IGF1R* gene copy number; (E) *IGF1R* high gene polysomy; (F) *IGF1R* gene amplification.

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univariate model were included. In an exploratory analysis, maximum log-likelihood function was used to select the most discriminative survival cutoff point of average *IGF1R* copy number per cell.[19,20] Significance level of $\alpha \leq .05$ was used without adjustment for multiple testing.

RESULTS

Expression of IGF1R and EGFR Protein by IHC

Results of IHC staining were obtained for 179 patients (95%). The pattern of immunostaining with Ventana G11 anti-IGF1R antibody showed predominantly membranous but also weak cytoplasmic staining (Fig 1C). The pattern of staining with Novus anti-IGF1R antibody was predominantly cytoplasmic. The IHC scores with the two antibodies showed weak inverse correlation ($r = -0.16$; $P = .04$; n = 177). A significant correlation was observed between levels of staining with Ventana antibody and of IGF1R mRNA ($r = 0.37$; $P < .001$; n = 109), but not between staining with Novus antibody and levels of IGF1R mRNA ($r = .002$; $P = .99$; n = 109). Given the above data, we concluded that the Ventana G11 antibody staining measures IGF1R protein expression more accurately and further analyses were performed only for this antibody.

Median IGF1R IHC score was 86 (range, 0 to 354). One hundred fifty samples (84%) showed IGF1R IHC score higher than 10. Associations of IGF1R protein expression and clinical and pathologic characteristics are presented in Table 1. IGF1R IHC score above median was found in 67% of squamous cell carcinomas as compared with 24% of adenocarcinomas ($P < .001$). Higher levels of expression were found in patients with more advanced stage ($P = .03$). A significant correlation between IGF1R and EGFR IHC score was found ($r = 0.30$; $P < .001$; n = 177). In subset analyses by histology, the correlation was significant for squamous cell carcinomas ($P = .028$) and for nonsquamous NSCLC types ($P = .040$).

Overall survival of patients with tumors showing high versus low IGF1R protein expression was similar (log-rank $P = .40$, hazard ratio [HR] adjusted for stage, age, sex, and histology, 0.92; 95% CI, 0.60 to 1.42; $P = .72$; Fig 2A). In exploratory analysis with other cutoff points to define IGF1R positivity, there was no difference in overall survival according to IGF1R protein expression (all *P* values not significant, data not shown). When tested as a continuous variable, IGF1R IHC score was not associated with survival difference (adjusted HR, 1.000; 95% CI, 0.997 to 1.002; $P = .74$). Patients with tumors showing high EGFR and IGF1R expression (both above median, n = 54) had outcome similar to all other patients (n = 125, log-rank $P = .71$). IGF1R protein expression was not associated with disease-free survival (data not shown).

IGF1R *gene copy number.* The median value of average *IGF1R* gene copy number per cell was 2.68. The pattern of *IGF1R* gene copy number was relatively homogenous throughout the cores obtained from the same tumor (average upper and lower 95% CIs for mean *IGF1R* gene copy number per cell represent 14% of this value), justifying the use of tissue microarray for gene copy number assessment. The median value of IGF1R/CEP15 ratio was 1.17. Average *IGF1R* gene copy number per cell analyzed as a continuous variable in the univariate Cox model showed positive significant association with survival (HR, 0.76; 95% CI, 0.61 to 0.95; $P = .014$). In the exploratory analysis, we used maximum log-likelihood function to select the most discriminative cutoff of average *IGF1R* gene copy number for survival difference. We found that the best discrimination was observed with cutoff of 4.0 gene copies per cell (HR, 0.38 for subset of patients with tumors with average of 4 or more copies per cell [21 patients; 12%] as compared with all other patients, 95% CI, 0.17 to 0.82, $P = .014$). The percentage of cells with 4 copies or more was originally proposed for *EGFR* gene copy number assessment for prediction of therapeutic

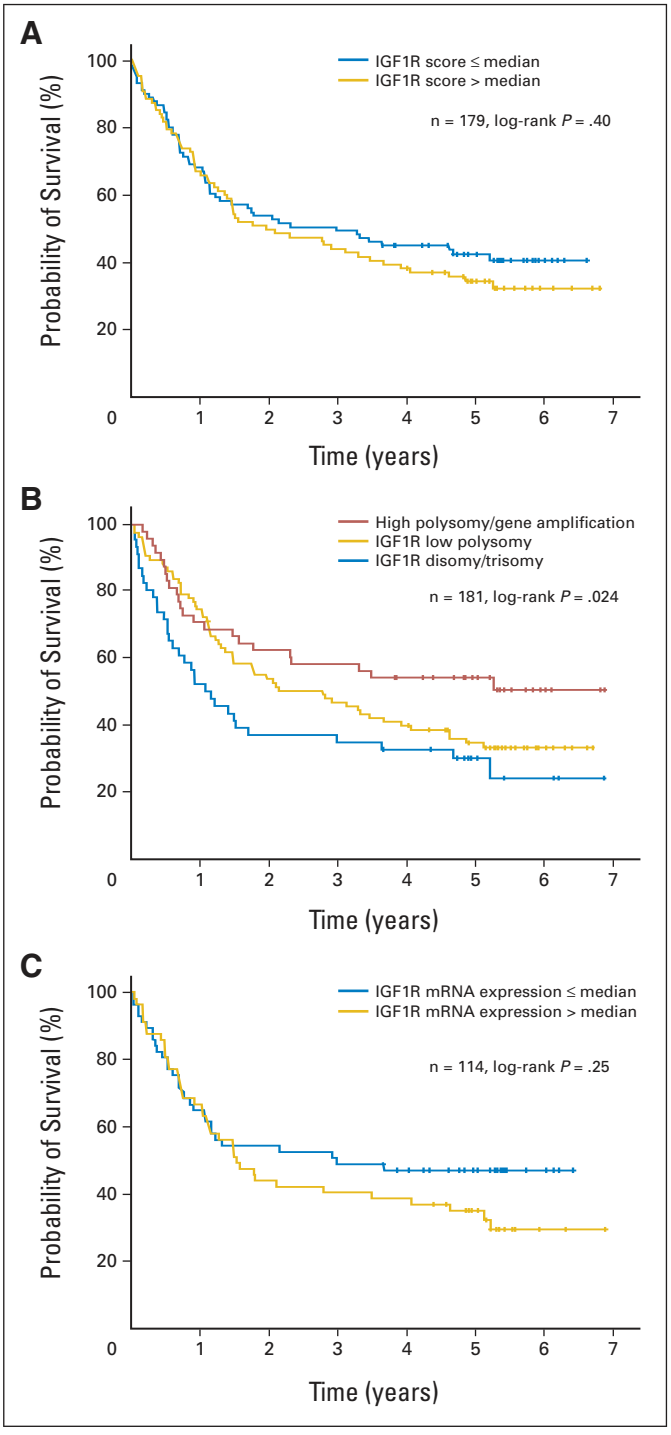

Fig 2. (A) Overall survival (OS) according to insulin-like growth factor-1 receptor (IGF1R) immunohistochemistry score with Ventana G11 antibody (cutoff by median). (B) OS according to *IGF1R* gene copy number by fluorescent in situ hybridization (cutoffs according to the criteria previously established for *EGFR* gene copy number).[17] (C) OS according to IGF1R mRNA expression (cutoff by median).

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Table 2. Univariate and Multivariate Analysis of Survival

Variable	Univariate Analysis			Final Multivariate Analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Pathologic stage						
I *v* II *v* III/IV	1.95	1.56 to 2.42	< .0001	1.93	1.54 to 2.43	< .0001
Histology						
Squamous *v* other	1.22	0.85 to 1.76	.28	—		
Age, years						
Continuous	1.020	1.000 to 1.040	.055	1.029	1.007 to 1.052	.010
Sex						
Female *v* male	1.38	0.88 to 2.15	.165	—		
IGF1R gene copy number						
Low copy number *v* low polysomy *v* high polysomy + gene amplification	0.70	0.53 to 0.90	.007	0.75	0.57 to 0.99	.039

Abbreviations: HR, hazard ratio; IGF1R, insulin-like growth factor-1 receptor.

benefit from EGFR inhibitors.[17] Applying the same criteria developed for *EGFR* gene copy number, five tumors (3%) were classified as carrying *IGF1R* gene amplification, 43 tumors (24%) showed high polysomy, 87 tumors (48%) had low polysomy, and 46 tumors (25%) had low gene copy number. Comparing tumors with high copy numbers (gene amplification and high polysomy) and other tumors, there was no difference in patient characteristics (Table 1). There was also no difference in clinical or pathologic characteristics when tumors with low polysomy, high polysomy, and gene amplification were grouped for comparison with tumors with low gene copy number (data not shown). Patients with tumors harboring high *IGF1R* gene copy numbers (amplification and high polysomy) had 3-year survival of 58% (95% CI, 44% to 72%) versus 47% (36% to 57%) for tumors with low polysomy and 35% (21% to 49%) for tumors with trisomy/disomy (log-rank $P = .024$; Fig 2B). Prognostic value of *IGF1R* gene copy number was confirmed in the multivariate analysis (Table 2) and was also observed for disease-free survival (data not shown). Examples of images with *IGF1R* gene amplification, high polysomy, and disomy are shown in Figures 1D to 1F.

IGF1R mRNA expression. Median relative IGF1R mRNA expression was 23.4 (range, 0.1 to 1,280). There was no association of IGF1R mRNA expression and clinical or pathologic characteristics when the expression was divided by median, except for a tendency toward higher proportion of patients with squamous cell histology in the subset with high IGF1R expression ($P = .089$; data not shown). When analyzed as continuous variable, IGF1R expression was significantly higher in squamous cell carcinomas versus other histologies (median relative expression 32.3 *v* 11.6; $P = .006$).

In the survival analysis, there was no difference according to median IGF1R mRNA expression (log-rank $P = .25$; Fig 2C). Survival HR for IGF1R mRNA expression when tested as a continuous variable and adjusted for sex, age, stage, and histology was 1.000 (95% CI, 0.999 to 1.001; $P = .35$).

Association between IGF1R *gene copy number and IGF1R protein expression.* Median IGF1R IHC score in tumors showing *IGF1R* gene disomy or trisomy was 60, for tumors with low polysomy was 93, and for tumors with high polysomy or gene amplification was 98 ($P = .007$; Fig 3). The proportion of tumors with IGF1R IHC score above median (> 86) for these three FISH categories with ascending *IGF1R* gene copy number was 36%, 52%, and 62%, respectively ($P = .038$).

DISCUSSION

Identification of biomarkers for selecting patients most likely to derive clinical benefit from IGF1R inhibitors is needed. In vitro studies showed that expression of IGF1R correlates with sensitivity to IGF1R inhibitors, such as BMS-536924.[21] An important initial step in predictive marker discovery for IGF pathway inhibitors is a descriptive characterization of *IGF1R* gene and protein aberrations in tumors from patients with NSCLC.

In this study, we used large-core tissue microarray with sufficient amount of tumor tissue for adequate protein and gene copy number assessment. However, we cannot exclude the possibility of missing some focal gene amplifications as compared with the whole tissue sections. The expression of IGF1R was evaluated by IHC using two antibodies, the Ventana antibody and the previously reported Novus anti-IGF1R antobody.[22,23] The pattern of IHC staining differed between the two antibodies. Importantly, the results obtained with the Ventana antibody correlated significantly with the mRNA expression

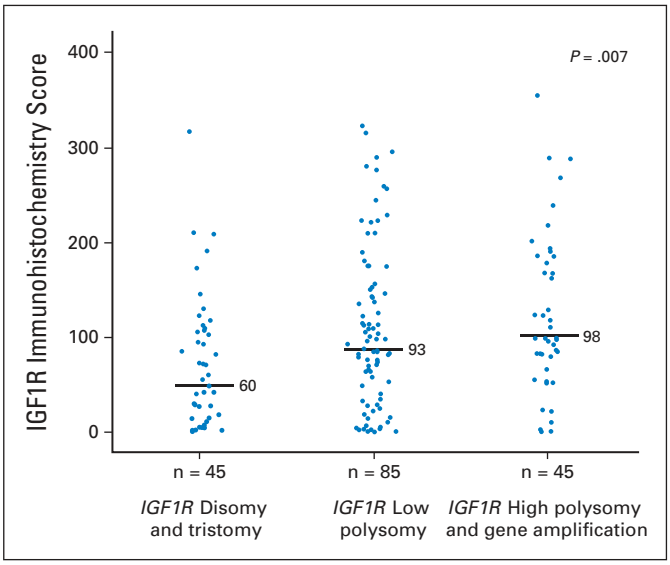

Fig 3. Insulin-like growth factor-1 receptor (IGF1R) immunohistochemistry score according to *IGF1R* gene copy number by fluorescent in situ hybridization.

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($P < .001$) whereas this correlation was not observed for Novus antibody ($P = .99$), suggesting that the former reagent provides a more accurate method for detection of the IGF1R protein. The reason for weak inverse correlation between the results obtained with two antibodies is unknown. Lack of association between anti-IGF1R Novus antibody staining and IGF1R mRNA expression is an indirect indication of poor specificity of this antibody. Different and unexpected staining pattern obtained with this antibody may indicate a possible cross-reactivity with other proteins.

Some ongoing clinical trials with IGF1R-targeting therapies suggest that IGF1R inhibitor activity associates with the histology of squamous cell carcinoma.[7] In this study, we have found that higher IGF1R protein expression is indeed observed in squamous cell carcinomas as compared with other NSCLC histologies. The same is observed for IGF1R mRNA expression, but not for *IGF1R* gene copy number. The association of IGF1R protein expression with histology was not described in three previous studies in NSCLC samples, including our own series.[22-24] Possible explanations for this discrepancy include different methodology of IGF1R protein detection or a smaller number of patients. We found that IGF1R protein or mRNA expression does not associate with prognosis in operable NSCLC. The lack of prognostic significance of IGF1R protein expression was also reported in the other surgical series of patients with NSCLC.[24] This finding is not surprising in light of studies with other receptors, including EGFR.[25] Indeed, biologically active, high affinity receptor pool on the cell surface may be much smaller than the overall receptor pool,[26] which has been detected with IHC in our study.

Increased gene copy number has prognostic value in several malignancies. Amplifications of *HER2* or high *EGFR* gene copy number are associated with poor survival of patients with breast or lung cancer,[27] respectively. Herein we show that high *IGF1R* gene copy number is increased in a substantial proportion of NSCLC samples (27% using criteria developed for *EGFR* gene copy number assessment), although *IGF1R* gene amplification is rare. High *IGF1R* gene copy number associates with IGF1R protein expression and has a positive prognostic impact in surgically resected NSCLC. The biologic mechanisms behind the prognostic association are unclear. In this study, we did not observe dramatic gain in *IGF1R* copy number as seen in *HER2* in breast or *EGFR* in lung cancer. One possibility is that the higher *IGF1R* gene copy numbers do not translate into more biologically active pool of IGF1R receptors with no further activation of PI3K-AKT and Ras/Raf-MAPK signaling. The other explanation is that activated, but possibly intact, IGF1R-related pathways result in more indolent course of the disease as compared with the course related to other molecular events observed in NSCLC, as for example *KRAS* mutation or cyclin D1 amplification. Better outcome of surgically treated patients with increased *IGF1R* gene copy number by FISH should be taken into account when looking at this marker in prospective clinical trials with IGF1R inhibitors, if the prognostic significance holds in advanced disease. The positive prognostic value of increased *IGF1R* copy number does not mean that this marker cannot be used for prediction of treatment efficacy. The prognostic and predictive value of the marker may be coordinated, as shown for activating *EGFR* mutations,[28] or opposite, as demonstrated for *EGFR* gene copy number[15] in NSCLC and EGFR inhibitor therapy.

Signaling interactions have been reported between ErbB/HER family members and IGF1R. The formation of EGFR/IGF1R heterodimers with active downstream signaling was described in NSCLC cell lines.[8] In vitro studies indicate that activation of IGF1R parallels trastuzumab resistance in breast cancer[29] and gefitinib resistance in NSCLC.[11] In this report, a significant correlation between IGF1R and EGFR protein expression was found, indicating that both receptors may be regulated through common mechanisms or may be coselected during cancer progression. Contrary to a report by Ludovini et al,[24] we did not find worse outcome of patients with tumors overexpressing both receptors.

In summary, our study shows that IGF1R protein expression and mRNA expression is higher in squamous cell carcinomas compared with other histologies. IGF1R protein and gene expression does not associate with survival, whereas high *IGF1R* gene copy number associates with better prognosis in surgically resected NSCLC. Future studies will determine if any of these tests will predict for sensitivity to IGF1R inhibitors and will elucidate the prognostic value of *IGF1R* gene copy number in NSCLC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** Rafal Dziadziuszko, Hoffmann-La Roche (C); Katarzyna Dziadziuszko, Hoffmann-La Roche (C); Jacek Jassem, Bristol-Myers Squibb (C), GlaxoSmithKline (C); Paul A. Bunn Jr, AstraZeneca (C); Fred R. Hirsch, AstraZeneca (C) **Stock Ownership:** None **Honoraria:** Rafal Dziadziuszko, Hoffmann-La Roche; Katarzyna Dziadziuszko, Hoffmann-La Roche **Research Funding:** Paul A. Bunn Jr, AstraZeneca, OSI Pharmaceuticals, Genentech, Biogen Idec; Fred R. Hirsch, AstraZeneca, OSI Pharmaceuticals, Genentech, Biogen Idec, Syndax Pharmaceuticals, Ventana Medical Systems **Expert Testimony:** None **Other Remuneration:** None

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